TITLE OF THE INVENTION
TELOMERASE REVERSE TRANSCRIPTASE FUSION PROTEIN, NUCLEOTIDES ENCODING IT, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/851,183, filed October 12, 2006, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates generally to the therapy of cancer. More specifically, the present invention relates to polynucleotides encoding fusion proteins wherein the fusion proteins comprise at least a portion of the tumor associated polypeptide telomerase reverse transcriptase (TERT). The present invention also provides recombinant vectors and hosts comprising said polynucleotides, purified fusion proteins and methods for eliciting or enhancing an immune response against the protein product of the TERT gene, using the compositions and molecules disclosed herein.

BACKGROUND OF THE INVENTION

Vaccination has become a standard procedure for the prevention of numerous infectious diseases. The application of vaccines to other diseases, such as cancer, is now an attractive possibility due to advances in molecular engineering and a better understanding of tumor immunology.

Cancer is one of the leading causes of mortality worldwide. Despite an abundance of cancer-related research, conventional therapies that combine surgery, radiation, and chemotherapy, often fail to effectively treat established cancers. Reliable methods of prevention also remain unavailable.

Cancer typically involves the malfunction of genes that contribute to the regulation of the cell cycle or cell proliferation, such as growth factors and their receptors, oncogenes, and tumor suppressor genes. The products of many of these genes are expressed on the surface of a wide variety of tumor cells and, hence, are designated tumor-associated antigens (TAAs). The introduction of genes encoding TAAs directly into a subject has been shown to generate a protective immune response against the TAA in many experimental models, making these molecules a target for vaccine therapy. However, because many of these gene products are also expressed in normal cells, albeit at lower levels, many immunological therapies targeting TAAs have proven ineffective due to self-tolerance.

Genes coding for several tumor-associated antigens (TAA) have been isolated, characterized and inserted in genetic vectors such as plasmid DNA and viral vectors. One tumor-associated antigen that has been implicated in the pathogenesis of cancer is telomerase (TERT).

Telomerase is a DNA polymerase that normally functions in maintaining telomere length at the ends of chromosomes. During normal cell growth, an RNA primer attaches to the 5' end of DNA and initiates replication. Upon removal of the RNA primer, a gap in length is introduced to the resulting daughter strand of DNA. Thus, replication of a linear strand of DNA with conventional polymerases leads to shortening of telomere length in each progressive round of replication. Such shortening of telomere length is responsible for cellular senescence or aging of normal human somatic cells.

Telomerase is a ribonucleoprotein comprising an RNA component and a catalytic protein component (telomerase reverse transcriptase). The catalytic component of human telomerase was described by Meyerson *et al.* (*Cell* 1197: 785-95 (1990) and Nakamura *et al.* (*Science* 277: 955-59 (1997)). The TERT enzyme uses its RNA component as a template for telomere DNA synthesis, thus allowing telomeres to maintain their length over successive generations of cell growth. Such maintenance of telomere length over numerous proliferative cycles allows a cell to escape normal senescence and become immortal, allowing a tumor to grow and metastasize over great lengths of time. Because telomerase confers replicative immortality to cells, telomerase activity has been detected in cancerous cell lines and a diverse range of tumor types (Kim *et al.* *Science* 266: 2011-15 (1994)). Conversely, telomerase is inactive or only transiently expressed at low levels in normal human tissues and normal somatic cell cultures. The combination of telomerase over-expression in most cancer types as well as low or absent expression in normal cells makes TERT a target for therapy and/or prophylaxis of diseases associated with aberrant cellular proliferation such as cancer.

The development of a telomerase-specific vaccine is now possible because the catalytic and RNA components of telomerase have been cloned and characterized (*see, e.g.*, U.S. Patent 6, 166,178). However, the development and commercialization of many vaccines have been hindered by difficulties associated with obtaining high expression levels of the desired immunogen in successfully transformed host organisms. The development of efficacious DNA-based vaccines has also been hindered by an inability to generate an immune response of sufficient magnitude in treated individuals to lead to tumor regression in a clinical setting. Therefore, despite the identification of the wild-type nucleotide sequences encoding telomerase proteins described above, it would be highly desirable to develop a vaccine which is capable of eliciting an enhanced TERT-specific immune response relative to a wild-type full-length TERT cDNA, when delivered to a mammal. It would also be desirable to develop methods for treating or preventing TERT-associated cancers which utilize nucleic acid molecules or proteins that safely and effectively potentiate a TERT-specific immune response.

SUMMARY

As stated above, expression of the tumor-associated antigen telomerase reverse transcriptase (TERT) gene is commonly associated with the development or presence of adenocarcinomas, including colorectal carcinomas. To this end, the disclosure relates to compositions and methods to elicit or enhance immunity to the protein products expressed by the human TERT (hTERT) gene. Specifically, the disclosure provides polynucleotides encoding fusion proteins wherein the fusion proteins comprise the hTERT protein, or variant thereof, fused to a substantial portion of the B subunit of *E. coli* heat labile enterotoxin (LTB). The disclosure also provides recombinant vectors, including but not limited to, adenovirus and plasmid vectors, comprising said polynucleotides and host cells comprising said recombinant vectors. Also provided herein are purified fusion proteins encoded by disclosed polynucleotides. The hTERT-LTB fusion proteins and polynucleotides which encode said fusion proteins are useful as vaccines for the prevention and/or treatment of telomerase-associated cancer. Said vaccines are useful as a monotherapy or as part of a therapeutic regime, said regime comprising administration of a second vaccine such as a polynucleotide, cell-based, protein, or peptide-based vaccine, or comprising radiotherapy or chemotherapy.

In a first aspect, there is provided a nucleic acid molecule comprising a sequence of nucleotides that encodes a human telomerase reverse transcriptase (hTERT) fusion protein, wherein the hTERT fusion protein comprises an hTERT variant , fused to a B subunit of *E. coli* heat labile enterotoxin (LTB) protein wherein said hTERT-LTB fusion protein comprises a sequence of amino acids as set forth in SEQ ID NO:6 and wherein the fusion protein is capable of producing an immune response in a mammal.

In a second aspect, there is provided a purified fusion protein comprising a human telomerase reverse transcriptase (hTERT) variant , fused to a B subunit of *E. coli* heat labile enterotoxin (LTB) protein wherein said hTERT-LTB fusion protein comprises a sequence of amino acids as set forth in SEQ ID NO:6.

In a third aspect, there is provided a vector comprising the nucleic acid molecule of the first aspect.

In a fourth aspect, there is provided a host cell comprising the vector of the third aspect.

In a fifth aspect, there is provided a vector comprising the nucleic acid molecule as defined above.

In a sixth aspect, there is provided a process for expressing an hTERT-LTB fusion protein in a recombinant host cell, comprising: (a) introducing a vector comprising the nucleic

acid molecule of the first aspect into a suitable host cell; and, (b) culturing the host cell under conditions which allow expression of said hTERT-LTB fusion protein.

In preferred embodiments of the disclosure, the sequence of nucleotides encoding hTERT and/or the sequence of nucleotides encoding LTB comprises codons that have been optimized for high levels of expression in a human host cell. In other words, in certain embodiments of the disclosure, the codon usage pattern of the polynucleotide sequence resembles that of highly expressed mammalian and/or human genes.

Another embodiment of this disclosure is an expression construct comprising nucleotides encoding hTERT-LTB. In preferred embodiments of this portion of the disclosure, the construct comprises a TPA leader sequence before the coding sequence for the TERT gene to ensure that the TERT-LTB fusion protein is secreted.

In additional preferred embodiments of the disclosure, telomerase catalytic activity of the telomerase antigen is inactivated so that the encoded TERT fusion protein is safer than wild-type TERT for vaccine purposes. The enzymatic activity of the TERT fusion protein can be inactivated by addition of mutations/deletions to the TERT-encoding nucleotide sequence. In specific exemplary embodiments of the disclosure, nucleotides have been mutated in order to change specific amino acids D712A and V713I in the human TERT protein sequence and D702A and V703I in the mouse TERT protein sequence.

The disclosure further provides adenoviral and plasmid vectors comprising a nucleotide sequence that encodes an hTERT-LTB fusion protein. This disclosure also describes the use of the adenovirus and plasmid vectors in immunogenic compositions and vaccines for the prevention and/or treatment of hTERT-associated cancer.

Also provided are adenovirus vectors comprising a sequence of nucleotides that encode a human TERT protein or TERT protein variant. Variants useful in this embodiment of the

disclosure comprise mutations that function to eliminate telomerase catalytic activity. In preferred embodiments of this portion of the disclosure, the adenovirus vector is an Ad6 vector. In other preferred embodiments the Ad vector is an Ad 5 vector.

The disclosure further provides methods for preventing the development of a cancer in a patient or treating a patient with a telomerase-associated tumor by eliciting an immune response to the TERT protein by administering a vaccine or pharmaceutical composition comprising the TERT fusions or TERT fusion proteins provided by the disclosure. In preferred embodiments of the methods herein, the immune response is enhanced relative to the response elicited by a wild-type TERT.

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

The term "promoter" refers to a recognition site on a DNA strand to which the RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences termed "enhancers" or inhibiting sequences termed "silencers".

The term "cassette" refers to a nucleotide or gene sequence that is to be expressed from a vector, for example, the nucleotide or gene sequence encoding the hTERT(AI)-LTB fusion. In general, a cassette comprises a gene sequence that can be inserted into a vector, which in some embodiments, provides regulatory sequences for expressing the nucleotide or gene sequence. In other embodiments, the nucleotide or gene sequence provides the regulatory sequences for its expression. In further embodiments, the vector provides some regulatory sequences and the nucleotide or gene sequence provides other regulatory sequences. For example, the vector can provide a promoter for transcribing the nucleotide or gene sequence and the nucleotide or gene sequence provides a transcription termination sequence. The regulatory sequences that can be provided by the vector include, but are not limited to, enhancers, transcription termination sequences, splice acceptor and donor sequences, introns, ribosome binding sequences, and poly(A) addition sequences.

The term "vector" refers to some means by which DNA fragments can be introduced into a host organism or host tissue. There are various types of vectors including plasmid, virus (including adenovirus), bacteriophages and cosmids.

The term "first generation," as used in reference to adenoviral vectors, describes adenoviral vectors that are replication-defective. First generation adenovirus vectors typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region.

The designation 'pV1JnsA/TPA-mTERT(AI)-LTBopt" refers to a plasmid construct, disclosed herein, comprising the CMV immediate-early (IE) promoter with intron A, a full-length codon-optimized murine TERT gene fused to a codon-optimized LTB gene, and bovine growth hormone-derived polyadenylation and transcriptional termination sequences (see EXAMPLE 2). Additionally, a leader sequence encoding the human tissue plasminogen activator (TPA) signal sequence is present 5' to the nucleotide sequence encoding mTERT-LTB. The designation "AI" indicates that mutations were added to the TERT sequence to functionally eliminate telomerase catalytic activity. The designation 'pV1JnsA/mTERT(AI)opt" refers to a construct essentially as described above, except the construct comprises a murine optimized TERT nucleotide sequence that was not fused to LTB or to TPA.

The designation 'pV1JnsA/TPA-hTERT(AI)-LTBopt" refers to a plasmid construct, disclosed herein, comprising the CMV immediate-early (IE) promoter with intron A, a full-length codon-optimized human TERT gene fused to a codon-optimized LTB gene, and bovine growth hormone-derived polyadenylation and transcriptional termination sequences (see EXAMPLE 2). Additionally, a leader sequence encoding the human tissue plasminogen activator (TPA) signal sequence is present 5' to the nucleotide sequence encoding hTERT-LTB. The hTERT sequence in this construct comprises mutations to functionally eliminate telomerase catalytic activity.

The designations "Ad6/TPAmTERT(AI)-LTBopt" and "Ad6/hTERT(AI)" refer to two constructs, disclosed herein, which comprise an Ad6 adenoviral genome deleted of the E1 and E3 regions. In the "Ad6/TPAmTERT(AI)-LTBopt" construct, the E1 region is replaced by a codon-optimized murine TERT-LTB gene in an E1 parallel orientation under the control of a human CMV promoter without intron A, followed by a bovine growth hormone polyadenylation signal. The TERT-encoding sequence comprises mutations to eliminate telomerase catalytic activity. The construct further comprises sequences encoding the human tissue plasminogen activator (TPA) signal sequence 5' to the TERT(AI)-LTB encoding nucleotide sequence. The "Ad6/hTERT(AI)" construct is essentially as described above, except the E1 region of the Ad6 genome is replaced with a TERT cDNA sequence, said sequence comprising mutations to abolish enzymatic activity.

The abbreviation 'LTB" refers generally to the B subunit of heat labile enterotoxin of *E. coli*, or substantial portion thereof, including subunits which are truncated on the C-terminal or N-terminal end but maintain adjuvant activity, as well as subunits that contain internal amino acid insertions, deletions, or substitutions but maintain adjuvant activity (Fingerut *et al. Vaccine* 23: 4685-4696 (2005)).

As used herein, a 'fusion protein" refers to a protein having at least two heterologous polypeptides covalently linked in which one polypeptide comes from one protein sequence or domain and the other polypeptide comes from a second protein sequence or domain. The fusion proteins of the present invention comprise a TERT polypeptide or fragment or variant

thereof, and a second polypeptide, which comprises a substantial portion of LTB. The TERT polypeptide, fragment or variant thereof may be a human TERT or TERT homolog from another species, e.g. mouse. The polypeptides that comprise the fusion protein are preferably linked N-terminus to C-terminus. The TERT polypeptide and the LTB polypeptide can be fused in any order. In some embodiments of this invention, the C-terminus of the TERT polypeptide is fused to the N-terminus of LTB, as exemplified in FIGUREs 1 and 2. However, fusion proteins in which the LTB subunit is fused to the N-terminus of the TERT polypeptide are also contemplated.

The term "TERT fusion protein" or "TERT-LTB fusion protein" are used interchangeably herein as a general term which refers to a fusion as described above, which comprises a TERT polypeptide or fragment or variant thereof fused to an LTB polypeptide. The term "TERT-LTB fusion" refers to a nucleic acid sequence in which at least a portion of the TERT gene is fused to a substantial portion of LTB subunit of *E. coli* heat labile enterotoxin. The term "TERT-LTB fusion protein" refers to a polypeptide encoded by a TERT-LTB fusion as described. The terms "TERT-LTB fusion" and "TERT-LTB fusion protein" are also understood to refer to fragments thereof, homologs thereof, and functional equivalents thereof (collectively referred to as "variants"), such as those in which one or more amino acids is inserted, deleted or replaced by other amino acid(s). The TERT-LTB fusions of the present invention, upon administration to a mammal such as a human being, can stimulate an immune response by helper T cells or cytotoxic T cells, at least as well as a "wild-type" hTERT sequence. In preferred embodiments of the invention, the TERT-LTB fusion can enhance the immune response as compared to a wild-type hTERT.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in which the disorder is to be prevented. As used herein, "treatment" also includes reduction of the likelihood of obtaining the disorder, as well as the reduction of the severity of the disorder in those already afflicted.

A "disorder" is any condition that would benefit from treatment with the molecules of the present invention, including the nucleic acid molecules described herein and the fusion proteins that are encoded by said nucleic acid molecules. Encompassed by the term "disorder" are chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. The molecules of the present invention are intended for use as treatments for disorders or conditions characterized by aberrant cell proliferation, including, but not limited to, breast cancer, colorectal cancer, and lung cancer.

The term "effective amount" means sufficient vaccine composition is introduced to produce the adequate levels of the polypeptide, so that an immune response results. One skilled in the art recognizes that this level may vary.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

"hTERT" refers to a human telomerase reverse transcriptase antigen or nucleotides encoding the human telomerase reverse transcriptase antigen. "hTERTopt" refers to a codon-optimized nucleotide sequence encoding human telomerase reverse transcriptase antigen.

"Substantially similar" means that a given nucleic acid or amino acid sequence shares at least 75%, preferably at least 85%, more preferably at least 90%, and even more preferably at least 95% identity with a reference sequence. In the present invention, the reference sequence can be relevant portions of the wild-type human TERT nucleotide or amino acid sequence, as set forth in SEQ ID NOs:11 and 12, respectively, or the nucleotide or amino acid sequence of LTB, as set forth in SEQ ID NOs:13 (opt sequence) and 14, as dictated by the context of the text. The reference sequence may also be, for example, a wild-type rhesus monkey or murine TERT sequence. Thus, a TERT protein sequence that is "substantially similar" to the wild-type human TERT protein or fragment thereof will share at least 75% identity with the relevant fragment of the wild-type human TERT, along the length of the fragment, preferably 85% identity, more preferably 90% identity and even more preferably 95% identity. Whether a given mTERT, hTERT, or LTB amino acid sequence or nucleotide sequence is "substantially similar" to a reference sequence can be determined for example, by comparing sequence information using sequence analysis software such as the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482, 1981).

A "substantial portion" of a gene, variant, fragment, or subunit thereof, means a portion of at least 50%, preferably 75%, more preferably 90%, and even more preferably 95% of a reference sequence.

A "gene" refers to a nucleic acid molecule whose nucleotide sequence codes for a polypeptide molecule. Genes may be uninterrupted sequences of nucleotides or they may include such intervening segments as introns, promoter regions, splicing sites and repetitive sequences. A gene can be either RNA or DNA. A preferred gene is one that encodes the invention peptide.

The term "nucleic acid" or "nucleic acid molecule" is intended for ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), probes, oligonucleotides, fragment or portions thereof, and primers.

"Wild-type TERT" or "wild-type protein" or "wt protein" refers to a protein comprising a naturally occurring sequence of amino acids or variant thereof. The amino acid sequence of wild-type human TERT is set forth in SEQ ID NO:12. The amino acid sequence of

the wild-type human TERT was previously described (U.S. Patent No. 6,166,178; U.S. Patent 6,261,836; U.S. Patent No. 6,927,285; U.S. Patent Application 2003-0096344; Meyerson *et al.*, *Cell* 90: 785-95 (1997); Nakamura et al., *Science* 277: 955-59 (1997)).

"Wild-type TERT gene" refers to a gene comprising a sequence of nucleotides that encodes a naturally occurring TERT protein, including proteins of human origin or proteins obtained from another organism, including, but not limited to, other mammals such as rat, mouse and rhesus monkey. The nucleotide sequence of the human TERT gene is available in the art (*supra*).

The designation "TERT(AI)" refers to a telomerase reverse transcriptase sequence that comprises mutations to eliminate or reduce telomerase catalytic activity.

The term "mammalian" refers to any mammal, including a human being.

The abbreviation "Ag" refers to an antigen.

The abbreviation "ORF" refers to the open reading frame of a gene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the nucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of an exemplary TPA-hTERT(AI)-LTBopt fusion.

FIGURE 2 shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of an exemplary TPA-mTERT(AI)-LTBopt fusion.

FIGURE 3 shows results of an IFNγ ELIspot assay on BALB/c mice vaccinated with repeated injections of plasmid pV1J/TPA-mTERT(AI)-LTBopt. 5 mice of each group were used to monitor immune response directed against mTERT or LTB using peptide pools. Data plotted are from 6 individual mice (empty circle). Geometric mean values are indicated (filled circles). No T cell response to mTERT was detected in mock treated mice (not shown). See EXAMPLE 7.

FIGURE 4 shows results from IFNγ intracellular staining on splenocytes from vaccinated BALB/c mice. $CD4^+$ and $CD8^+$ T cell response to mouse TERT was measured using pools of overlapping peptides that cover the entire protein. Immune response to LTB was also monitored. Data plotted are from 6 individual mice (filled triangles). Geometric mean values are indicated (straight line). No T cell response to TERT was detected in mock treated mice (not shown). See EXAMPLE 7.

FIGURE 5 shows the results of ^{51}Cr Release CTL killing by effector T cells of 4T1 target cells pulsed with $CD8^+$T cells specific peptide mTERTaa167 (AYQVCGSPL; SEQ ID NO:20). Effector cells were prepared from splenocytes of two immunized BALB/c mice and were re-stimulated *in vitro* with the specific peptide. Results are expressed as percentage of specific killing at different Effectors /Target ratio. See EXAMPLE 8.

FIGURE 6 shows the induction of an immune response to mouse TERT. C57BL/6 mice were immunized with 5 weekly injections of plasmid pV1J/TPA-mTERT(AI)-

LTBopt. Immune response was assessed on mouse splenocytes by IFNγ ELIspot assay using mouse TERT peptide pools. Antigen specific $CD4^+$ and $CD8^+$ T cell responses are shown as geometric mean values obtained from 6 immunized mice, standard deviation is also indicated. See EXAMPLE 7.

FIGURE 7 shows the induction of an mTERT specific immune response in BALB/c mice. Mice were immunized with 5 weekly injections of the constructs pV1J-mTERT(AI)opt or pV1J-TPAmTERT(AI)-LTBopt. Immune response was determined by IFNγ intracellular staining on PBMC using the CD8+ epitope corresponding to the peptide sequence mTERTaa167 (AYQVCGSPL; SEQ ID NO:20). Data plotted are from 8 individual mice (filled triangles). Geometric mean values are indicated (straight lines).

FIGURE 8 shows the TERT specific immune response is enhanced upon injection of adenovirus vectors expressing mTERT(AI)-LTBopt. Groups of 10 BALB/c mice were subjected to 5 weekly injections of pV1J/TPA-mTERT(AI)-LTBopt (50μg/inj.). Two weeks after the last injection of plasmid DNA, mice were either boosted with one additional injection of plasmid DNA (DNA-EP) or with 10^{10} vp of Ad6/TPA-mTERT(AI)-LTBopt (Ad). Immune responses to TERT were monitored by IFNγ ICS on PBMC. Data plotted are from 10 individual mice (filled diamonds). Geometric mean values of each group are also shown (straight lines). See EXAMPLE 10.

FIGURE 9 depicts the protocol utilized to analyze DMH-induced carcinogenesis and vaccination in BALB/c mice. DMH was administered i.p. on a weekly basis. Vaccination with pV1J/TPA-mTERT(AI)-LTBopt was carried out at the indicated time points. Analysis of intestinal lesions was carried out at week 12. See EXAMPLE 11.

FIGURE 10 shows the anti-tumor effect of pV1J/TPA-mTERT(AI)-LTBopt on DMH-induced carcinogenesis. BALB/c mice were treated with DMH and DNA-EP as indicated in FIGURE 9. Analysis of ACF and adenoma formation in the colon was carried out at week 12 from the beginning of the treatment. The number of ACF and adenoma in vaccinated mice was compared to that detected in non vaccinated controls. See EXAMPLE 11.

FIGURE 11 depicts the protocol utilized to analyze DMH-induced carcinogenesis and vaccination in BALB/c mice. DMH was administered i.p. on a weekly basis. Vaccination with pV1J/TPA-mTERT-LTB DNA and Ad were carried out at the indicated time points. Analysis of the intestinal lesions was carried out at week 30. See EXAMPLE 11.

FIGURE 12 demonstrates the anti-tumor effect of pV1J/TPA-mTERT(AI)-LTBopt DNA prime/Ad boost on the number of tumor lesions. BALB/c mice were treated DMH and DNA-EP as indicated in FIGURE 9. Analysis of ACF and adenoma formation in the colon was carried out at week 30 from the beginning of the treatment. The number of ACF and adenoma in vaccinated mice was compared to that detected in non vaccinated controls. See EXAMPLE 11.

FIGURE 13 demonstrates the anti-tumor effect of pV1J/TPA-mTERT-LTB(AI)opt DNA prime/Ad boost on the volume and differentiation stage of tumor lesions. BALB/c mice were treated DMH and DNA-EP as indicated. The volume (Panel A) and histological differentiation stage (Panel B) of colon adenomas present in vaccinated and control mice were analyzed 30 weeks from the beginning of the treatment. Tumors were classified as follows: G1: well-differentiated adenocarcinoma; G2: moderately-differentiated adenocarcinoma; G3: poorly-differentiated adenocarcinoma). See EXAMPLE 11.

FIGURE 14 shows the induction of an immune response to human TERT. HHD transgenic mice were immunized with two biweekly injections (DNA-DNA) of plasmid pV1J/TPA-hTERT(AI)-LTBopt or by one injection of plasmid pV1J/TPA-hTERT(AI)-LTBopt followed, after two weeks by one injection of Ad6hTERT(AI) 10^{10} pp (DNA/Ad). The immune response was assessed on mouse PBMC by IFNγ ICS assay. Mice PBMC were incubated with the CD8 immunodominant peptide for HLA-A2 allele hTERT865 (SEQ ID NO:22). Data plotted are from 10 individual mice (filled triangles). Geometric mean values are indicated (straight line). See EXAMPLEs 12 and 13.

FIGURE 15 shows the results of a CTL assay on Cr^{51} labeled target HeLa-HHD cells. CD8+ T cells obtained from one immunized mouse were tested in a CTL assay against HeLa-HHD cells, either exogenously loaded with the hTERT865 peptide or w/o peptide but in presence of the DMSO used for the peptide dilution. HeLa-HHD cells were also infected with an Ad vector coding for hTERT or an Ad coding for GFP as control. See EXAMPLE 14.

FIGURE 16 shows the induction of a cell-mediated immune response to hTERT in rhesus monkeys immunized by DNA-EP. ELIspot analysis was performed on PBMC from hTERT-immunized rhesus monkeys (pV1J/TPA-hTERT-LTB;DNA-EP/DNA-EP). The assay was performed in duplicate and the average value from the replicates is shown. Analysis performed after the second DNA-EP is shown (Panel A). Analysis performed after the fifth DNA-EP is also shown (Panel B). See EXAMPLE 15.

FIGURE 17 shows that the cell-mediated immune response to hTERT was amplified in rhesus monkeys that were vaccinated with DNA-EP and boosted with adenovirus expressing hTERT, compared to the CMI elicited in monkeys vaccinated by DNA-EP alone. ELIspot was performed on PBMC from hTERT-immunized rhesus monkeys at the end of the immunization program, which included five DNA-EP (pV1J/TPA-hTERT-LTB) injections followed by two Ad (Ad6-hTERT) injections. The assay was performed in duplicate and the average value from the replicates is shown. See EXAMPLE 15.

DETAILED DESCRIPTION OF THE INVENTION

Expression of the catalytic component of telomerase, known as telomerase reverse transcriptase (TERT) is commonly detected in a diverse range of tumor types. The combination of telomerase over-expression in most cancer types as well as low or absent expression in normal

cells makes TERT a target for therapy and/or prophylaxis of diseases associated with aberrant cellular proliferation such as cancer. To this end, the present invention relates to compositions and methods to elicit or enhance immunity to the protein product expressed by the TERT tumor-associated antigen, wherein aberrant TERT expression is associated with the carcinoma or its development. Association of aberrant TERT expression with a carcinoma does not require that the TERT protein be expressed in tumor tissue at all time points of its development, as abnormal TERT expression may be present at tumor initiation and not be detectable late into tumor progression or vice-versa.

Accordingly, the present invention provides polynucleotides, vectors, host cells, and encoded proteins comprising a TERT sequence or variant thereof for use in vaccines and pharmaceutical compositions for the treatment and/or prevention of a cancer. The polynucleotides of the present invention comprise a nucleotide sequence encoding a TERT protein or variant thereof, fused to a nucleotide sequence encoding the B subunit of *E. coli* heat labile enterotoxin (LTB), which is able to effectively adjuvant an immune response to the associated TERT antigen.

In some embodiments of the invention, the nucleotide sequences disclosed herein further comprise a TPA leader sequence before the coding sequence for the TERT gene to ensure that the TERT-LTB fusion protein is secreted. In preferred embodiments of this aspect of the invention, the TPA-hTERT(AI)-LTB fusion encodes an amino acid sequence as set forth in SEQ ID NO:2. A preferred nucleotide sequence is set forth in SEQ ID NO:1. In further preferred embodiments, the TPA-mTERT(AI)-LTB fusion encodes an amino acid sequence as set forth in SEQ ID NO:4. A preferred nucleotide sequence is set forth in SEQ ID NO:3.

The TERT nucleotide sequences of the present invention can be of human origin or can be a TERT homolog from another species, e.g. mouse. The wild-type human TERT nucleotide sequence is set forth in SEQ ID NO:12 and has been reported previously (*see, e.g.,* U.S. Patent No. 6,166,178; U.S. Patent 6,261,836; U.S. Patent No. 6,927,285; U.S. Patent Application 2003-0096344; Meyerson *et al.*, *Cell* 90: 785-95 (1997); Nakamura *et al.*, *Science* 277: 955-59 (1997)). The TERT portion of the TERT fusion may be full-length, or any variant sufficient to elicit a TERT-specific T-cell immune response in a mammal. TERT variants of the present invention include, but are not limited to sequences that are C- or N-terminally truncated, sequences with conservative substitutions, and sequences with internal deletions or insertions. Preferred TERT variants of the present invention comprise mutations that functionally eliminate telomerase catalytic activity. Encoded TERT fusion proteins of the present invention are capable of inducing a cell-mediated immune response when introduced into a vaccine recipient or patient in need thereof.

As stated above, in preferred embodiments of the present invention, telomerase catalytic activity of the telomerase antigen is inactivated (herein designated "TERT(AI)") so that

the encoded TERT fusion protein is safer than wild-type TERT for vaccine purposes. The enzymatic activity of the TERT fusion protein can be inactivated by addition of mutations/deletions to the TERT-encoding nucleotide sequence. Therefore, the present invention includes nucleic acid molecules that encode a TERT-LTB fusion protein, wherein the sequence of nucleotides encodes a variant hTERT protein, wherein said variant comprises one or more mutations relative to the wild-type hTERT amino acid sequence as set forth in SEQ ID NO: 12, and wherein said mutations function to eliminate telomerase catalytic activity of the encoded hTERT protein. In specific exemplary embodiments of the invention, nucleotides have been mutated in order to change specific amino acids D712A and V713I in the human TERT protein sequence (as set forth in SEQ ID NO:10) and D702V and V703I in the mouse TERT protein sequence. A nucleotide sequence encoding the mutated human TERT sequence set forth in SEQ ID NO:10 is disclosed in SEQ ID NO:9.

In preferred embodiments of the present invention, the TERT portion of the TERT-LTB fusion is human TERT or a variant thereof, such as hTERT(AI). In other preferred embodiments, the TERT portion is a murine TERT, or variant thereof, such as mTERT(AI). The TERT variants and TERT-encoding nucleotide sequence variants of the present invention comprise mutations that abolish telomerase enzymatic activity.

Accordingly, the present invention relates to synthetic polynucleotides comprising a sequence of nucleotides encoding a TERT-LTB fusion protein, said fusion protein comprising a TERT protein or a biologically inactive fragment or mutant form of a TERT protein fused to an LTB protein or variant thereof, which can effectively enhance the immune response to the TERT protein. Said mutant forms of the TERT protein include, but are not limited to: conservative amino acid substitutions, amino-terminal truncations, carboxy-terminal truncations, deletions, or additions. Any such TERT variant, fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the immunological properties of the TERT protein as set forth in SEQ ID NO:12. Preferred TERT variants are catalytically inactive, for example, the TERT variant set forth in SEQ ID NO:10.

The synthetic polynucleotides of the present invention encode mRNA molecules that express a TERT(AI)-LTB fusion protein that is capable of stimulating or enhancing the immune response to the associated TERT protein, so as to be useful in the development of a therapeutic or prophylactic cancer vaccine. The LTB portion of the TERT-LTB fusions of the present invention has been shown to strongly potentiate the immunogenicity of co-delivered antigens (*See, e.g.* Simmons et al. *Scand. J. Immunol.* 53:218-26 (2001)).

Also contemplated for use in the present invention are nucleotide sequences encoding LTB variants or mutants including but not necessarily limited to: nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations. In some cases, it may be advantageous to add specific point mutations to the nucleotide sequence encoding LTB to reduce or eliminate toxicity of the encoded protein. In preferred embodiments

of the present invention the LTB sequence fused to the TERT sequence is truncated of its signal sequence.

The LTB portion, or variant thereof, of the TERT-LTB fusions of the present invention may be fused to the amino terminus or the carboxy terminus of the TERT sequence. Further, the LTB sequence and the TERT sequence can be fused N-terminus to N-terminus, C-terminus to C-terminus, C-terminus to N-terminus or N-terminus to N-terminus. In preferred embodiments of the present invention, the C-terminus of the TERT polypeptide is fused to the N-terminus of LTB.

The present invention relates to a synthetic nucleic acid molecule (polynucleotide) comprising a sequence of nucleotides which encodes mRNA that expresses a novel TERT-LTB fusion protein; for example, nucleotide sequences encoding the fusion proteins as set forth in SEQ ID NOs:6 and 8. A particularly preferred TERT fusion of the present invention is the hTERT(AI)-LTB fusion sequence set forth in SEQ ID NO:5. The nucleic acid molecules of the present invention are substantially free from other nucleic acids.

One of skill in the art will recognize that other nucleotide sequences are useful for vaccine purposes, such as nucleotide sequences that encode the hTERT(AI)-LTB protein sequence set forth in SEQ ID NO:6. The present invention also includes nucleotide sequences that encode inactive hTERT-LTB fusion protein that are substantially similar, to SEQ ID NO:6, but not exactly the same, especially due to amino acid differences at the junction of the TERT(AI) sequence and the LTB sequence that were created by the cloning strategy.

The present invention also relates to recombinant vectors and recombinant host cells, both prokaryotic and eukaryotic, which contain the nucleic acid molecules disclosed throughout this specification. The synthetic DNA molecules, associated vectors, and hosts of the present invention are useful for the development of a cancer vaccine.

Exemplary nucleic acid molecules of the present invention comprise a nucleotide sequence selected from the group consisting of: SEQ ID NOs:5 and 7, as shown in FIGURES 1-2, which encode exemplary hTERT-LTB and mTERT-LTB fusion proteins of the present invention.

The present invention also includes biologically active fragments or mutants of SEQ ID NOs: 5 and 7, which encode mRNA expressing exemplary TERT-LTB fusion proteins. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the immunological properties of the hTERT protein, including but not limited to the hTERT protein as set forth in SEQ ID NO:12. Such polynucleotides include but are not necessarily limited to: nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations. Preferred mutated nucleotide sequences of the present invention encode mRNA molecules that express an enzymatically inactive TERT-LTB fusion protein in a eukaryotic cell, which is capable of eliciting a prophylactic or therapeutic immune response in a patient in need thereof so as to be useful in cancer vaccine development.

Also included within the scope of this invention are mutations in the DNA sequence that do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in the desired functionality of the polypeptide, such as the ability to elicit an immune response.

As stated above, the present invention further relates to recombinant vectors that comprise the nucleic acid molecules disclosed throughout this specification. These vectors may be comprised of DNA or RNA. For most cloning purposes, DNA vectors are preferred. Typical vectors include plasmids, modified viruses, baculovirus, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a TERT-LTB fusion protein. It is well within the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Also provided by the present invention are purified TERT-LTB fusion proteins encoded by the nucleic acids disclosed throughout this specification, especially TERT(AI)-LTB fusion proteins. In exemplary embodiments of this aspect of the invention, the TERT-LTB fusion protein comprises a sequence of amino acids selected from the group consisting of: SEQ ID NOs: 6 and 8.

Included in the present invention are DNA sequences that hybridize to the complement of SEQ ID NOs: 5 and 7 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows. Prehybridization of filters containing DNA is carried out for about 2 hours to overnight at about 65°C in buffer composed of 6x SSC, 5x Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for about 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for about 1 hour in a solution containing 2x SSC, 0.1% SDS. This is followed by a wash in 0.1x SSC, 0.1% SDS at 50°C for 45 minutes before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5x SSC, 5x Denhardt's solution, 50% formamide at about 42°C for about 12 to 48 hours or a washing step carried out in 0.2x SSPE, 0.2% SDS at about 65°C for about 30 to 60 minutes. Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd Edition; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, (1989) or Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition. Cold Spring Harbor Laboratory Press, Plainview, NY (2001). In addition to the foregoing, other conditions of high stringency which may be used are also well known in the art.

An expression vector containing a TERT-LTB fusion protein-encoding nucleic acid molecule may be used for high-level expression of TERT-LTB fusion protein in a

recombinant host cell. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Also, a variety of bacterial expression vectors may be used to express recombinant TERT-LTB fusion sequences in bacterial cells if desired. In addition, a variety of fungal cell expression vectors may be used to express recombinant TERT-LTB fusion sequences in fungal cells. Further, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells.

The present invention also relates to host cells transformed or transfected with vectors comprising the nucleic acid molecules of the present invention. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Such recombinant host cells can be cultured under suitable conditions to produce a TERT-LTB fusion protein or a biologically equivalent form. In a preferred embodiment of the present invention, the host cell is human. As defined herein, the term "host cell" is not intended to include a host cell in the body of a transgenic human being, human fetus, or human embryo.

As noted above, an expression vector containing DNA encoding a TERT-LTB fusion protein may be used for expression of TERT fusion protein in a recombinant host cell. Therefore, another aspect of this invention is a process for expressing a TERT-LTB fusion protein in a recombinant host cell, comprising: (a) introducing a vector comprising a nucleic acid comprising a sequence of nucleotides that encodes a TERT-LTB fusion protein into a suitable human host cell, wherein the TERT-LTB fusion protein comprises a TERT protein or inactive variant thereof, fused to a substantial portion of the LTB protein, and wherein the fusion protein is capable of producing an immune response in a mammal; and, (b) culturing the host cell under conditions which allow expression of said TERT-LTB fusion protein.

In preferred embodiments of the process for expressing a TERT-LTB fusion protein described above, the LTB sequence is deleted of its signal sequence.

Following expression of a TERT-LTB fusion in a host cell, TERT-LTB fusion protein may be recovered to provide purified TERT-LT fusion protein. Several protein purification procedures are available and suitable for use. Recombinant protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant TERT-LTB fusion protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for a TERT protein, or polypeptide fragments of a TERT protein.

The nucleic acid molecules comprising TERT-LTB fusions and the encoded fusion proteins of this invention were designed to enhance the TERT-specific immune response, relative to full-length wild-type cDNA encoding TERT, for use in vaccine development. To further enhance the immunogenic properties of the TERT-LTB fusion sequences of the present invention, in some embodiments described herein, the polynucleotides encoding TERT-LTB fusion proteins comprise optimized codons for further high level expression in a host cell, as described below. In these embodiments, at least a portion of the codons of the TERT-LTB fusions are designed so as to use the codons preferred by the projected host cell, which in preferred embodiments is a human cell. The optimized TERT-LTB fusions may be used for the development of recombinant adenovirus or plasmid-based DNA vaccines, which provide effective immunoprophylaxis against TERT-associated cancer through cell-mediated immunity. The synthetic molecules may be used as an immunogenic composition. This invention provides codon-optimized TERT-LTB fusion polynucleotides which, when directly introduced into a vertebrate *in vivo*, including mammals such as primates and humans, induce the expression of encoded proteins within the animal.

As stated above, in some embodiments of the present invention, the synthetic molecules comprise a sequence of nucleotides, wherein some of the nucleotides have been altered so as to use the codons preferred by a human cell, thus allowing for high-level fusion protein expression in a human host cell. The synthetic molecules may be used as a source of a TERT-LTB fusion protein, which may be used in a cancer vaccine to provide effective immunoprophylaxis against TERT-associated carcinomas through cell-mediated immunity. The nucleic acid molecules disclosed herein may also serve as the basis for a DNA-based cancer vaccine.

A "triplet" codon of four possible nucleotide bases can exist in over 60 variant forms. Because these codons provide the message for only 20 different amino acids (as well as transcription initiation and termination), some amino acids can be coded for by more than one codon, a phenomenon known as codon redundancy. For reasons not completely understood, alternative codons are not uniformly present in the endogenous DNA of differing types of cells. Indeed, there appears to exist a variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG. Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally believed that the likelihood of obtaining high levels of expression of a leucine-rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, it is likely that a gene rich in TTA codons will be poorly expressed in *E. coli*, whereas a CTG rich gene will probably be highly expressed in this host. Similarly, a preferred codon for expression of a leucine-rich polypeptide in yeast host cells would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are evident, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide an optimal form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is a TERT-LTB fusion gene that is codon-optimized for expression in a human cell. In a preferred embodiment of this invention, it has been found that the use of alternative codons encoding the same protein sequence may remove the constraints on expression of exogenous TERT-LTB fusion protein in human cells.

In accordance with some embodiments of the present invention, the nucleic acid molecules which encode the TERT-LTB fusion proteins are converted to a polynucleotide sequence having an identical translated sequence but with alternative codon usage as described by Lathe, "Synthetic Oligonucleotide Probes Deduced from Amino Acid Sequence Data: Theoretical and Practical Considerations" *J. Molec. Biol.* 183:1-12 (1985), which is hereby incorporated by reference. The methodology generally consists of identifying codons in the wild-type sequence that are not commonly associated with highly expressed human genes and replacing them with optimal codons for high expression in human cells. The new gene sequence is then inspected for undesired sequences generated by these codon replacements (e.g., "ATTTA" sequences, inadvertent creation of intron splice recognition sites, unwanted restriction enzyme sites, etc.). Undesirable sequences are eliminated by substitution of the existing codons with different codons coding for the same amino acid. The synthetic gene segments are then tested for improved expression.

It is understood that this procedure will not necessarily result in a polynucleotide sequence in which all of the codons are optimal codons according to the codon usage of highly expressed human and/or mammalian cells. However, it is preferred that, in embodiments of the invention wherein codon-optimized polynucleotide variants of TERT and/or LTB are contemplated, a substantial portion of the resulting codons resemble the codon usage of highly expressed human and/or mammalian genes.

The methods described above were used to create synthetic gene sequences which encode TERT-LTB fusion proteins, resulting in a gene comprising codons optimized for high level expression in human cells. While the above procedure provides a summary of a representative methodology for designing codon-optimized genes for use in cancer vaccines, it is understood by one skilled in the art that similar vaccine efficacy or increased expression of genes may be achieved by minor variations in the procedure or by minor variations in the sequence.

One of skill in the art will also recognize that additional nucleic acid molecules may be constructed that provide for high levels of TERT-LTB fusion expression in human cells,

wherein only a portion of the codons of the DNA molecules are codon-optimized. For example, in some embodiments of the present invention, codons comprising the TERT portion of the TERT-LTB fusion are optimized for high-level expression in human cells, and codons comprising the LTB portion of the TERT-LTB fusion are substantially similar to the wild-type LTB. In other embodiments of the present invention, codons comprising the LTB portion of the TERT-LTB fusion are optimized for high-level expression in human cells, and codons comprising the TERT portion of the TERT-LTB fusion are substantially similar to a wild-type TERT gene. In still other embodiments of the present invention, both the TERT and LTB portions of the TERT-LTB fusion are codon-optimized for high-level expression in human cells, for example, the hTERT-LTBopt sequence as set forth in SEQ ID NO:5. TERT-LTB fusions in which only a subset of codons are optimized within the TERT and/or the LTB portion of the TERT-LTB fusion are also contemplated by this invention.

The nucleic acids of the present invention may be assembled into an expression cassette which comprises sequences designed to provide for efficient expression of the protein in a human cell. The cassette preferably contains TERT-LTB fusion protein-encoding gene, with related transcriptional and translations control sequences operatively linked to it, such as a promoter, and termination sequences. In a preferred embodiment, the promoter is the cytomegalovirus promoter with intron A sequence (CMV), although those skilled in the art will recognize that any of a number of other known promoters such as a strong immunoglobulin or other eukaryotic gene promoter may be used. A preferred transcriptional terminator is the bovine growth hormone terminator, although other known transcriptional terminators may also be used. The combination of CMV-BGH terminator is particularly preferred.

In accordance with this invention, the TERT-LTB fusion expression cassette is inserted into a vector. The vector is preferably an adenoviral or plasmid vector, although linear DNA linked to a promoter, or other vectors, such as adeno-associated virus or a modified vaccinia virus, retroviral or lentiviral vector may also be used.

In preferred embodiment of the invention, the vector is an adenovirus vector (used interchangeably herein with "adenovector"). Adenovectors can be based on different adenovirus serotypes such as those found in humans or animals. Examples of animal adenoviruses include bovine, porcine, chimp, murine, canine, and avian (CELO). Preferred adenovectors are based on human serotypes, more preferably Group B, C, or D serotypes. Examples of human adenovirus Group B, C, D, or E serotypes include types 2 ("Ad2"), 4 ("Ad4"), 5 ("Ad5"), 6 ("Ad6"), 24 ("Ad24"), 26 ("Ad26"), 34 ("Ad34") and 35 ("Ad35"). In particularly preferred embodiments of the present invention, the expression vector is an adenovirus type 6 (Ad6) vector.

If the vector chosen is an adenovirus, it is preferred that the vector be a so-called first-generation adenoviral vector. These adenoviral vectors are characterized by having a non-functional E1 gene region, and preferably a deleted adenoviral E1 gene region. In addition, first generation vectors may have a non-functional or deleted E3 gene region (Danthinne *et al. Gene*

Therapy 7:1707-1714 (2000); F.L. Graham, *Immunology Today* 21(9): 426-428 (2000)). Adenovectors do not need to have their E1 and E3 regions completely removed. Rather, a sufficient amount the E1 region is removed to render the vector replication incompetent in the absence of the E1 proteins being supplied *in trans*; and the E1 deletion or the combination of the E1 and E3 deletions are sufficiently large enough to accommodate a gene expression cassette.

In some embodiments, the expression cassette is inserted in the position where the adenoviral E1 gene is normally located. In addition, these vectors optionally have a non-functional or deleted E3 region. It is preferred that the adenovirus genome used be deleted of both the E1 and E3 regions (ΔE1ΔE3). The adenoviruses can be multiplied in known cell lines which express the viral E1 gene, such as 293 cells, or PERC.6 cells, or in cell lines derived from 293 or PERC.6 cell which are transiently or stably transformed to express an extra protein. For examples, when using constructs that have a controlled gene expression, such as a tetracycline regulatable promoter system, the cell line may express components involved in the regulatory system. One example of such a cell line is T-Rex-293; others are known in the art.

For convenience in manipulating the adenoviral vector, the adenovirus may be in a shuttle plasmid form. This invention is also directed to a shuttle plasmid vector which comprises a plasmid portion and an adenovirus portion, the adenovirus portion comprising an adenoviral genome which has a deleted E1 and optional E3 deletion, and has an inserted expression cassette comprising a TERT-LTB fusion protein-encoding nucleotide sequence. In preferred embodiments, there is a restriction site flanking the adenoviral portion of the plasmid so that the adenoviral vector can easily be removed. The shuttle plasmid may be replicated in prokaryotic cells or eukaryotic cells.

In a preferred embodiment of the invention, the expression cassette is inserted into an Ad6 (ΔE1ΔE3) adenovirus plasmid (*See* Emini et al., US20040247615, which is hereby incorporated by reference). This vector comprises an Ad6 adenoviral genome deleted of the E1 and E3 regions. In other preferred embodiments of the invention, the expression cassette is inserted into the pMRKAd5-HV0 adenovirus plasmid (*See* Emini et al., US20030044421, which is hereby incorporated by reference). This plasmid comprises an Ad5 adenoviral genome deleted of the E1 and E3 regions. The design of the pMRKAd5-HV0 plasmid was improved over prior adenovectors by extending the 5' cis-acting packaging region further into the E1 gene to incorporate elements found to be important in optimizing viral packaging, resulting in enhanced virus amplification. Advantageously, these enhanced adenoviral vectors are capable of maintaining genetic stability following high passage propagation.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the adenoviruses, shuttle plasmids, and DNA immunogens of this invention.

It has been determined in accordance with the present invention that genetic vaccination with plasmid DNA coding for TPA-mTERT(AI)-LTBopt can break immune tolerance

in BALB/c and B6 mice (*See* EXAMPLE 7). It has also been shown herein that immunization with plasmid TPA-mTERT(AI)-LTBopt can elicit a cytotoxic immune response in BALB/c mice (*See* EXAMPLE 8). It has further been demonstrated that this construct is able to induce a stronger CD8+ immune response than mTERT(AI) alone (see EXAMPLE 9), and is able to control tumor growth (EXAMPLE 11). Thus, the data described herein demonstrate that fusion of the TERT coding sequence to the LTB cDNA results in an increase in the TERT-specific immune response.

It has also been shown in accordance with the present invention that hTERT(AI)-LTBopt can induce an HLA-A2 restricted CD8+ immune response (see EXAMPLE 12) and that this immune response can be amplified by boosting with Ad6-hTERT(AI).

Therefore, the vectors described above may be used in immunogenic compositions and vaccines for preventing the development of tumors associated with aberrant TERT expression and/or for treating existing cancers. The vectors of the present invention allow for vaccine development and commercialization by eliminating difficulties with obtaining high expression levels of exogenous TERT in successfully transformed host organisms and by providing a TERT-LTB fusion protein which can elicit an enhanced immune response when administered to a mammal such as a human being.

To this end, one aspect of the instant invention is a method of preventing or treating TERT-associated cancer comprising administering to a mammal a vaccine vector comprising a polynucleotide comprising a sequence of nucleotides that encodes a TERT-LTB fusion protein, wherein the TERT-LTB fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal.

In accordance with the method described above, the vaccine vector may be administered for the treatment or prevention of a cancer in any mammal, including but not limited to: lung cancer, breast cancer, and colorectal cancer. In a preferred embodiment of the invention, the mammal is a human.

Further, one of skill in the art may choose any type of vector for use in the treatment and prevention method described. Preferably, the vector is an adenovirus vector or a plasmid vector. In a preferred embodiment of the invention, the vector is an adenoviral vector comprising an adenoviral genome with a deletion in the adenovirus E1 region, and an insert in the adenovirus E1 region, wherein the insert comprises an expression cassette comprising: (a) a sequence of nucleotides that encodes a TERT-LTB fusion protein, wherein the TERT-LTB fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and (b) a promoter operably linked to the polynucleotide.

The instant invention further relates to an adenovirus vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising: (a) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and (b) a promoter operably linked to the polynucleotide.

In a preferred embodiment of this aspect of the invention, the adenovirus vector is an Ad 6 vector.

In another preferred embodiment of the invention, the adenovirus vector is an Ad 5 vector.

In yet another preferred embodiment, the adenovirus vector is an Ad 24 vector.

Also contemplated for use in the present invention is an adenovirus vaccine vector comprising an adenovirus genome that naturally infects a species other than human, including, but not limited to, chimpanzee adenoviral vectors. A preferred embodiment of this aspect of the invention is a chimp Ad 3 vaccine vector.

In another aspect, the invention relates to a vaccine plasmid comprising a plasmid portion and an expression cassette portion, the expression cassette portion comprising: (a) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and (b) a promoter operably linked to the polynucleotide. An example of a suitable plasmid would be the mammalian expression plasmid V1Jns as described (J. Shiver *et. al.* in *DNA Vaccines*, M. Liu et al. eds., N.Y. Acad. Sci., N.Y., 772:198-208 (1996), which is herein incorporated by reference).

In some embodiments of this invention, the recombinant adenovirus and plasmid-based polynucleotide vaccines disclosed herein are used in various prime/boost combinations in order to induce an enhanced immune response. In this case, the two vectors are administered in a "prime and boost" regimen. For example the first type of vector is administered one or more times, then after a predetermined amount of time, for example, 2 weeks, 1 month, 2 months, six months, or other appropriate interval, a second type of vector is administered one or more times. Preferably the vectors carry expression cassettes encoding the same polynucleotide or combination of polynucleotides.

In the embodiment where a plasmid DNA is also used, it is preferred that the vector contain one or more promoters recognized by mammalian or insect cells. In a preferred embodiment, the plasmid would contain a strong promoter such as, but not limited to, the CMV promoter. One of skill in the art will recognize that any of a number of other known promoters may be chosen for purposes of driving expression of the TERT-LTB nucleotide sequences of the present invention. Additional examples of promoters include naturally occurring promoters such

as the EF1 alpha promoter, Rous sarcoma virus promoter, and SV40 early/late promoters and the p-actin promoter; and artificial promoters such as a synthetic muscle specific promoter and a chimeric muscle- specific/CMV promoter (Li et al., *Nat. Biotechnol.* 17:241-245 (1999); Hagstrom et al., *Blood* 95:2536-2542 (2000)). The synthetic TERT-LTB fusion gene or other gene to be expressed would be linked to such a promoter.

As stated above, an adenoviral vector vaccine and a plasmid vaccine may be administered to a vertebrate as part of a single therapeutic regime to induce an immune response. To this end, the present invention relates to a method of protecting a mammal from a TERT-associated cancer comprising: (a) introducing into the mammal a first vector comprising: i) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and ii) a promoter operably linked to the polynucleotide; (b) allowing a predetermined amount of time to pass; and (c) introducing into the mammal a second vector comprising: i) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises an inactive TERT protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and ii) a promoter operably linked to the polynucleotide.

In one embodiment of the method of protection described above, the first vector is a plasmid and the second vector is an adenovirus vector. In an alternative embodiment, the first vector is an adenovirus vector and the second vector is a plasmid. In some embodiments of the present invention, the first vector is administered to the patient more than one time before the second vector is administered.

In the method described above, the first type of vector may be administered more than once, with each administration of the vector separated by a predetermined amount of time. Such a series of administration of the first type of vector may be followed by administration of a second type of vector one or more times, after a predetermined amount of time has passed. Similar to treatment with the first type of vector, the second type of vector may also be given one time or more than once, following predetermined intervals of time.

The instant invention further relates to a method of treating a patient suffering from a TERT-associated cancer comprising: (a) introducing into the mammal a first vector comprising: i) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises a TERT(AI) protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion protein is capable of producing an immune response in a mammal; and ii) a promoter operably linked to the polynucleotide; (b) allowing a predetermined amount of time to pass; and (c) introducing into the patient a second vector comprising: i) a sequence of nucleotides that encodes a TERT fusion protein, wherein the TERT fusion protein comprises a TERT(AI) protein or variant thereof, fused to a substantial portion of LTB and wherein the fusion

protein is capable of producing an immune response in a mammal; and ii) a promoter operably linked to the polynucleotide.

In one embodiment of the method of treatment described above, the first vector is a plasmid and the second vector is an adenovirus vector. In an alternative embodiment, the first vector is an adenovirus vector and the second vector is a plasmid. In further preferred embodiments of the method described above, the first vector is administered to the patient more than one time before the second vector is administered to the patient.

In preferred embodiments of the methods described above, the vectors comprise a sequence of nucleotides that encode a TERT(AI)-LTB fusion protein, wherein the TERT fusion protein comprises an inactive TERT protein, fused to a substantial portion of LTB.

The amount of expressible DNA or transcribed RNA to be introduced into a vaccine recipient will depend partially on the strength of the promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of about 1 ng to 100 mg, and preferably about 10 μg to 300 μg of a plasmid vaccine vector is administered directly into muscle tissue. An effective dose for recombinant adenovirus is approximately $10^6 - 10^{12}$ particles and preferably about 10^7—10^{11}particles. Subcutaneous injection, intradermal introduction, impression though the skin, and other modes of administration such as intraperitoneal, intravenous, intramuscular or inhalation delivery are also contemplated.

In preferred embodiments of the present invention, the vaccine vectors are introduced to the recipient through intramuscular injection.

The vaccine vectors of this invention may be naked, i.e., unassociated with any proteins, or other agents which impact on the recipient's immune system. In this case, it is desirable for the vaccine vectors to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, it may be advantageous to administer an agent which assists in the cellular uptake of DNA, such as, but not limited to calcium ion. These agents are generally referred to as transfection facilitating reagents and pharmaceutically acceptable carriers. Those of skill in the art will be able to determine the particular reagent or pharmaceutically acceptable carrier as well as the appropriate time and mode of administration.

All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing methodologies and materials that might be used in connection with the present invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in

the art without departing from the scope or spirit of the invention as defined in the appended claims.

The following examples illustrate, but do not limit the invention.

EXAMPLE 1

Construction of TERT Fusion Proteins

To determine if fusion of the telomerase reverse transcriptase (TERT) antigen to the LTB subunit of *E. coli* heat labile enterotoxin (Fingerut *et al.* *Vaccine* 23(38): 4685-96 (2005); Rigano *et al.* *Plant Cell Rep.* 22(7): 502-8 (2004)) could enhance the immunogenicity of TERT alone, vectors were constructed encoding the full-length telomerase reverse transcriptase, with modifications. First, the DNA sequence was codon-optimized to incorporate codon preferred by human host cells. Additionally, to ensure that the encoded antigen was safe for vaccine use, mutations were introduced to the TERT nucleotide sequence to inactivate telomerase catalytic activity of the encoded protein. Specifically, the mutations D712A and V713I were added to the human TERT sequence and the mutations D702A and V703I were added to the mouse TERT sequence (Arai *et al.* Two independent regions of human telomerase reverse transcriptase are important for its oligomerization and telomerase activity. *J. Biol. Chem.* 277 (10): 8538-44 (2002)).

TERT fusions were engineered by joining the C-terminal end of the modified TERT nucleotide sequences, described above, to a nucleotide sequence encoding a modified LTB (nt 64 to 375) in which the signal peptide coding sequence had been removed. Additionally, a leader sequence encoding the human tissue plasminogen activator (TPA) signal sequence was added 5' to the TERT-encoding nucleotide sequence to ensure the secretion of the TERT protein (Haddad *et al.* Comparative study of DNA-based immunization vectors: effect of secretion signals on the antibody responses in mice. *FEMS Immunol. Med. Microbiol.* 18(3): 193-202 (1997)). The entire sequence was codon-optimized to incorporate codons preferred by human host cells using computer algorithms. The synthetic codon optimized gene was assembled by synthetic oligonucleotides. In the final construct, two amino acids were added (S and R) between the TERT sequence and the LTB sequence due to the cloning strategy.

The nucleotide sequences encoding the TERT-fusions were cloned into the vector pV1JnsA under the control of the human cytomegalovirus (CMV)/intron A promoter plus the bovine growth hormone (BGH) polyadenylation signal. Plasmid pV1J/TPA-hTERT-LTBopt carries the codon-optimized, inactivated, cDNA of human TERT fused to the coding sequences of the TPA signal sequence on the N-terminal end and LTB on the C-terminal end. Similarly, plasmid pV1J/TPA-mTERT-LTBopt carries the codon-optimized, inactivated, cDNA of mouse TERT fused to TPA and LTB.

EXAMPLE 2

Plasmid and Adenovirus Constructs

pV1JnsA/TPA-mTERT (AI)-LTBopt: Plasmid 041046pucKana containing TPA-mTERT(AI)-LTB sequence was obtained from GENEART (Geneart GmbH, Regensburg, Germany). The plasmid was digested with *Bgl*II and *Sal*I and the resulting fragment was cloned into the *Bgl*II/*Sal*I site in the plasmid pV1JnsA (Montgomery *et al.*, *DNA Cell Biol.*, 12(9):777-83(1993)).

pV1JnsA/mTERT(AI)opt: pV1JnsA/TPA-mTERT(AI)-LTB was digested *Xba*I to remove LTB coding sequence (present between two *Xba*I sites) the resulting construct pV1JnsA/TPA-mTERT(AI) had 3 aa (S;R;N) after the last mTERT aa and before the stop codon. Removal of the TPA coding sequence was performed on pV1JnsA/TPA-mTERT(AI) by *BamH*I and *Eco*RV digestion. The TPA coding sequence was replaced by a PCR product obtained amplifying mTERT sequence with the sense primer (5'- G A T C T G A T G A T A T C G C C A C C A T G A C C A G A G C C C C C A G A T G -3; SEQ ID NO: 15) and anti-sense primer (5'-A G G G G G G A T C C G C A C A C C T G G T A G G C G C A G C T G G G G -3'; SEQ ID NO:16) and cloned back into the *BamH*I and *Eco*RV digested vector.

pV1JnsA/TPA-hTERT(AI)-LTBopt: The synthetic fragment corresponding to TPA-hTERT(AI)-LTB obtained by GENEART was cloned into pV1JnsA using *Bgl*II /*Sal*I restriction sites.

Ad6-TPAmTERT(AI)-LTBopt: Plasmid 041046pucKana containing TPA-mTERT(AI)-LTBopt sequence was obtained from GENEART. The plasmid was digested with *Bgl*II and *Sal*I and the fragment cloned *Bgl*II /*Sal*I into the plasmid pNEBAd6-CMVpA shuttle vector. The plasmid pNEBAd6-CMVpA-TPA-mTERT(AI)-LTB digested with *Eco*RI /*Hind*III was recombined to *Cla*I-linearized plasmid pMRK Ad6 ΔE1 ΔE3. The plasmid was cut with *Pac*I to release the Ad ITRs and transfected in Perc-6 cells. Ad6 vector amplification was carried out by serial passages and purified trough standard CsCL gradient purification.

Ad6-hTERT(AI): Plasmid pCRsript containing wild type sequence of hTERT (hTERT wild type sequence was rescued by reverse transcription of mRNA of human tumor cells) was digested *Bgl*II/ *Xba*I filled with Klenow enzyme and cloned in pV1JnsA digested with *Eco*RV and *Bgl*II. pV1JnsA-hTERT was mutagenized by using an oligonucleotide (5'- C T G T A C T T T G T C A A G G T G G C T A T C A C G G G C G C G T A C G -3'; SEQ ID NO:17) and the Stratagene quikchange multi site-directed mutagenesis kit (Stratagene, LA Jolla, CA; Cat: 200513) to obtain pV1JnsA-hTERT(AI). The plasmid was digested with *Bgl*II *and Sal*I and the fragment cloned *Bgl*II /*Sal*I into the plasmid pNEBAd6-CMVpA shuttle vector. The plasmid pNEBAd6-CMVpA-hTERT(AI) was digested *Pac*I/*Pme*I and recombined to *Cla*I-linearized plasmid pMRK Ad6 ΔE1 ΔE3. The plasmid was cut with *Pac*I to release the Ad ITRs and transfected in Perc-6 cells. Ad6 vector amplification was carried out by serial passages and purified trough standard CsCL gradient purification.

EXAMPLE 3

IFN-γ ELISPOT Assay

Mouse splenocytes secreting IFN-γ in an antigen-specific manner were detected using a standard enzyme-linked immunospot (ELISPOT) assay (Miyahira *et al. J Immunol Methods* 181(1): 45-54 (1995)). Ninety-six wells MAIP plates (Millipore Corp., Billerica, MA) were coated with 100 µl/ well of purified rat anti-mouse IFN-γ (IgG1, clone R4-6A2, Pharmingen) diluted to 2.5 µg/ml in sterile PBS. After washing with PBS, blocking of plates was carried out with 200 µl/well of R10 medium for 2 hrs at 37°C.

Splenocytes were obtained by removing the spleen from the euthanized mice in a sterile manner, followed by spleen disruption through grating on a metal grid. Red blood cells were removed by osmotic lysis by adding 1 ml of 0.1X PBS to the cell pellet and vortexing for approximately 15s. One ml of 2x PBS was then added and the volume was brought to 4 ml with 1x PBS. Cells were pelleted by centrifugation at 1200 rpm for 10 min at RT, and the pellet was resuspended in 1 ml R10 medium. Viable cells were counted using Türks staining.

Splenocytes were plated at $5x10^5$ and $2.5x10^5$ cells/well in duplicate and incubated for 20h at 37°C with 1 µg/ml suspension of each peptide. Concanavalin A (ConA) was used as positive internal control for each mouse at 5 µg/ml. After washing with PBS, 0.05% Tween 20, plates were incubated O/N at 4°C with 50 µl/well of biotin-conjugated rat anti-mouse IFNγ (RatIgG1, clone XMG 1.2, PharMingen) diluted to 1:2500 in assay buffer. After extensive washing, plates were developed by adding 50 µl/well NBT/B-CIP (Pierce) until development of spots was clearly visible. The reaction was stopped by washing plates thoroughly with distilled water. Plates were air dried and spots were then counted using an automated ELISPOT reader.

EXAMPLE 4

Intracellular Cytokine Staining.

One to two millions mouse splenocytes or PBMC obtained by retroorbital bleeding in EDTA were resuspended in 1ml RPMI 10% FCS were incubated with pool of peptides (5-6 µg/ml final concentration of each peptide) and brefeldin A (1 µg/ml; BD Pharmingen cat #555028/2300kk) at 37°C and 5% CO_2 for 12-16 hours. Cells were then washed with FACS buffer (PBS 1% FBS, 0.01% NaN_3) and incubated with purified anti-mouse CD16/CD32 Fc block (BD Pharmingen cat # 553142) for 15 min at 4°C. Cells were then washed and stained with surface antibodies: CD4-PE conjugated anti-mouse (BD Pharmingen, cat.# 553049), PercP CD8 conjugated anti mouse (BD Pharmingen cat# 553036) and APC- conjugated anti-mouse CD3e (BD Pharmingen cat# 553066) for 30 minutes at room temperature in the dark. After the washing cells were fixed and permeabilized with Cytofix-Cytoperm Solution (BD Pharmingen cat #555028/2300kk) for 20 min at 4°C in the dark. After washing with PermWash Solution (BD Pharmingen cat #555028/2300kk) cells were incubated with the IFNγ-FITC antibodies (BD

Pharmingen). Cells were then washed, fixed with formaldehyde 1% in PBS and analyzed on a FACS-Calibur flow cytometer, using CellQuest software (Becton Dickinson, San Jose, CA).

EXAMPLE 5

Cytotoxic T Lymphocyte (CTL) Assay

Splenocytes were obtained by removing the spleen from the euthanized mice in a sterile manner, followed by spleen disruption through grating on a metal grid. Red blood cells were removed by osmotic lysis by adding 1 ml of 0.1X PBS to the cell pellet and vortexing for approximately 15s. Spleen cells at 2 x 10^6 cells/ml in R10 were plated in a 24 well cell culture plate (2 ml of cells/well) in presence of the immunogenic peptide at the final concentration of 10μg/ml. Plates were incubated at 37°C, 95% humidity, 5% CO_2 for 6 days. At day 3 of culture, recombinant human IL-2 at a final concentration of 10U/ml was added.

Target cells growing at exponential phase were harvested and brought to 1 x 10^6 cells/ml/tube in the presence of the immunogenic peptide at 10μg/ml final concentration. Cells were labeled with 50-100μCi ^{51}Cr/tube at 37° for 2 hours. Target cells were washed three times with 10 ml medium by centrifuging at 250 g, room temperature for 5 min, and brought at 1 x 10^5 cells/ml.

CTL assay was performed by plating Effector/Target cells with E/T ratio at 100:1, 50:1, 25:1 and 12.5:1. After 4 hours of incubation, plates were centrifuged at 1200 rpm for 5 min and the supernatant was harvested (30 μl/well). Plates were dried overnight and counted using a Beta-plate counter.

EXAMPLE 6

Mice Immunization

Female C57BL/6 mice (H-2^b) were purchased from Charles River (Lecco, Italy). HLA-A2.1 mice (HHD) were kindly provided by F. Lemmonier (Institute Pasteur, Paris, France). BALB/c mice (H-2^d) were purchased from Charles River (Lecco, Italy). Mice were immunized at 8 weeks of age. Fifty micrograms of plasmid DNA were electroinjected in a 50 μl volume in mice quadriceps as previously described (Rizzuto *et al. Proc. Natl. Acad. Sci. U.S.A.* 96(11): 6417-22 (1999)). Electroporation (EP) was performed as previously described (Zucchelli *et al. J. Virol.* 74(24): 11598-607 (2000); Rizzuto et al., *supra*). Briefly, electrical shock consisted of 10 trains with 1000 square bipolar pulses (90V/cm, 75mA, 200μs/phase). Ad injections were carried out in mice quadriceps in 50 μl volume. Cell mediated immune response was analyzed at the indicated time.

EXAMPLE 7

Genetic Vaccination with Plasmid DNA Coding for TPA- mTERT(AI)-LTB Breaks Immune Tolerance.

To determine if vaccination of mice with TPA- mTERT(AI)-LTB could break immune tolerance, groups of 20 BALB/c mice were immunized with 5 weekly injections of plasmid pV1J/TPA-mTERT(AI)-LTB followed by electroporation, as described in EXAMPLE 6. Eleven days after the last injection, splenocytes were obtained from individual mice, which were used to analyze the cell-mediated immune response by IFN-γ ELISPOT assay and by intracellular cytokine staining (ICS).

Antigen-specific IFNγ secretion from stimulated splenocytes was measured using three pools of 15mer mTERT peptides overlapping by 11 aa and encompassing the entire mTERT protein. The mTERT-1 pool was composed of 94 individual peptides covering the mTERT region from aa 1 to 388. The mTERT-2 pool was composed of 106 individual peptides covering the mTERT region from aa 377 to 811. The mTERT-3 pool was composed of 78 individual peptides covering the mTERT region from aa 801 to 1122. As a negative control, cytokine production was also measured upon stimulation of the splenocytes with DMSO at the same concentration utilized to solubilize the TERT peptides. The immune response induced against the LTB adjuvant was also detected by using a pool of 24 individual 15-mer peptides overlapping by 11 aa and covering the entire LTB sequence. ELIspot results indicate that vaccination of mice with TPA-mTERT(AI)-LTBopt elicited a cell mediated immune response (CMI) against the N-terminal and central regions of the TERT protein, contained within the TERT-1 and TERT-2 peptide pools (FIGURE 3). CMI against LTB was also detected by ELIspot (FIGURE 3).

To better characterize the T cell response elicited by the plasmid pV1J/TPA-mTERT(AI)-LTBopt and to identify the CD8+ and CD4+ T cell subset responsible for IFN-γ production, IFN-γ intracellular staining was carried out on vaccinated mice splenocytes and analyzed by FACS. Data obtained by ICS demonstrate that genetic immunization of BALB/c mice vaccinated with TPA-mTERT(AI)-LTBopt elicited a significant $CD8^+$ T cell response against the N-terminal region of the TERT protein (contained within mTERT-1 peptide pool, *see* FIGURE 4). The $CD4^+$ T cell response was mapped in the central region of TERT (mTERT-2 peptide pool). In contrast, no CMI could be detected in these mice against the C-terminal region of the protein (mTERT-3 peptide pool). A $CD4^+$ T cell response against LTB was also detected (FIGURE 4).

To further map the CD8+ T-cell response, a midi pool of mTERT 15mer peptides was used. Each midi pool contained ten 15mers, which were each individually contained in a separate midi-pool. Therefore, a positive reaction of 2 midi pools would unequivocally identify one individual 15mer as being immunogenic. Because CD8+ epitopes are always 9aa long, any CD8+ peptide is contained in two adjacent 11mer peptides. Results demonstrated that the CD8+ T-cell immune response was localized to the TERT region included in the following 15mer mTERT peptides: mTERT-41 (TERTaa161; LVPPSCAYQVCGSPL (SEQ ID NO:18) and mTERT-42 (TERTaa165; SCAYQVCGSPLYQIC; SEQ ID NO:19). By overlapping the two 15mer peptides (mTERT41 and mTERT-42), three possible reactive nonamers were synthesized

and tested in ICS. Finally, the immunodominant $CD8^+$ epitope in BALB/c mice was identified in the following sequence (mTERTaa167; AYQVCGSPL; SEQ ID NO:20). These results demonstrate that vaccination of BALB/c mice with pV1J/TPA- mTERT-LTB could break immune tolerance to the mTERT antigen.

To confirm data obtained in BALB/c mice, C57BL/6 (B6) mice were also immunized with 5 weekly injections of plasmid pV1J/TPA-mTERT(AI)-LTBopt. IFN-γ intracellular staining was carried out on vaccinated mice splenocytes and analyzed by FACS. Similar to results obtained with BALB/c mice, tolerance against mTERT was broken in all B6 mice. Additionally, both $CD4^+$ and $CD8^+$ T cells producing IFNγ were detected by ICS upon stimulation with TERT peptides (see FIGURE 6).

The CD8+ immune response elicited by DNA vaccination in C57BL/6 mice was primarily biased towards the central region of the TERT protein (peptide pool mTERT-2). A weaker CD8+ immune response was also detectable in the mTERT peptide pool-1. The CD8+ immune response was mapped in B6 mice using midi pools as described above. Two CD8+ epitopes were identified in B6. The first immunogenic sequence was mapped to the aa sequence mTERT198 (VGRNFTNL; SEQ ID NO:21) and the second CD8+ epitope was mapped to the sequence mTERT486 (SLGKYGKL; SEQ ID NO:22) Additionally, the CD4+ immune response was localized to the N-terminal region of TERT (peptide pool mTERT-1). No immune response was detected against the C-terminal region of mTERT.

Similar to the results obtained with BALB/c mice described above, immunization and analysis of the subsequent immune response of B6 mice confirm that TPA-mTERT(AI)-LTBopt immunization can break immune tolerance to the mTERT antigen. Therefore, taken together, results obtained with BALB/c and B6 mice indicate that the TPA-mTERT(AI)-LTBopt fusion is a potent immunogen in the self context independently from the mice strain used.

EXAMPLE 8

Cytolytic Activity of TERT-Specific $CD8^+$ T Cells.

A cytotoxic T-lymphocyte (CTL) assay was used to detect the cytolytic activity of TERT-specific $CD8^+$ T cells elicited by immunization of BALB/c mice with pV1J/TPA-mTERT(AI)-LTBopt. Mice were immunized with 5 weekly injections of plasmid pV1J/TPA-mTERT(AI)-LTBopt followed by electroporation. Mice splenocytes obtained from 2 individual mice were collected 10 days after the last immunization. These splenocytes were stimulated for one week with the immunogenic peptide mTERTaa167 (AYQVCGSPL; SEQ ID NO:20) to obtain *in vitro* activated T cells. The BALB/c syngenic tumor cell line 4T1 (Aslakson *et al.* *Cancer Res.* 52(6): 1399-405 (1992)) was used as a target. 4T1 cells were loaded with the immunoreactive peptide and labeled with Cr^{51} to be used as target for the CTL assay. The activated T cells were co-incubated with the target 4T1 cells at different effectors/target ratio. Activated T cells obtained both from mouse# 1 and mouse #2 showed cytolytic activity on target

cells when loaded with the immunogenic peptide mTERTaa167 (AYQVCGSPL; SEQ ID NO:20). The two mice showed different degrees of cytotoxic activity ranging from 80% (mouse #1) to 25% (mouse #2) at effectors /target ratio of 50/1.

Results indicate that a cytotoxic immune response was induced upon DNA immunization with TPA-mTERT(AI)-LTBopt as self antigen in BALB/c mice (FIGURE 5).

EXAMPLE 9

Comparative Immunogenicity of TPA-mTERT(AI)-LTBopt Construct.

To compare the immunogenicity of the secreted mTERT(AI) protein fused to LTB encoded by pV1J/TPA-mTERT(AI)-LTBopt, to a non-secreted version of mTERT(AI) without fusion to LTB, a plasmid pV1J derivative carrying mTERT(AI) was constructed, as described in EXAMPLE 2.

Groups of 6 BALB/c mice were immunized with 5 weekly injections of plasmids pV1J/TPA-mTERT(AI)-LTBopt or pV1J/mTERT(AI)opt. The immune response was monitored by IFNγ intracellular staining using the mTERTaa167 CD8+ immunodominant epitope sequence previously identified for BALB/c mice (AYQVCGSPL (SEQ ID NO:20).

Results indicate that TPA-mTERT(AI)-LTBopt is a better construct for the induction of a CD8+ immune response against murine telomerase than mTERT(AI) (FIGURE 7). The difference observed in $CD8^+$ T cell response induced in the group immunized by TPA-mTERT-LTB(AI)opt is statistically different from that obtained using the mTERT(AI)opt in the context of DNA immunization ($p=0.04$ student t test).

EXAMPLE 10

Comparison of Immunization Regimens

The anti-mTERT cell-mediated immune response elicited by immunization of BALB/c mice with DNA TPA-mTERT(AI)-LTBopt plus electrical stimulation alone was compared to the immune response induced by a diversified prime/ boost immunization regimen using DNA TPA-mTERT(AI)-LTBopt plus electrical stimulation as prime and Ad6 TPA-mTERT(AI)-LTBopt as final booster of the immune response.

Groups of 10 mice were immunized with 5 weekly injections of plasmid pV1J/TPA-mTERT(AI)-LTBopt. One week later, a booster injection was given consisting of either one additional injection of 50μg of DNA plus electroporation (EP) or with 10^{10} vp of the Ad6/TPA-mTERT (AI)-LTBopt. IFNγ intracellular staining was performed on mouse PBMCs to assess the resulting cell-mediated immune response. A peptide encompassing a $CD8^+$ T cell specific epitope mTERT 167 (AYQVCGSPL; SEQ ID NO:20) which was previously mapped to the N-terminal region of TERT, was used as antigen. Results indicate that the amplitude of the immune response elicited by the DNA-EP/Ad combination was 4.6 fold greater than that which was observed in mice that had received only a DNA-EP regimen (see FIGURE 8).

EXAMPLE 11

Vaccination with TPA-mTERT-LTB Controls Tumor Growth.

To determine if the mTERT-specific immune response could generate an anti-tumor effect, BALB/c mice were treated with the chemical carcinogen dimethyl hydrazine by intraperitoneal (i.p.) injection prior to vaccination. Treatment of mice with this carcinogen leads to progressive tumor development in the colon characterized by aberrant crypt formation, adenoma, and finally carcinoma. Chemical treatment of these mice did not render them immunocompromized, as immunization with TPA-mTERT(AI)-LTBopt elicited an immune response comparable to that previously found in non-treated mice (data not shown).

To assess the impact of genetic immunization on early stages of DMH induced carcinogenesis, BALB/c mice were treated with DMH and immunized by DNA followed by electroporation (DNA-EP) with pV1J/TPA-mTERT(AI)-LTBopt according to the schedule described in FIGURE 9.

Twenty mice were euthanized 12 weeks after the beginning of DMH treatment to count the number of ACF (aberrant crypt foci) and the number of adenoma which had formed. A significant reduction of both the number of ACF and the number of adenoma present was observed in vaccinated mice compared to non-vaccinated mice (FIGURE 10).

The efficacy of mTERT vaccination on a later stage of tumor development was also monitored. To enhance and sustain the immune response to mTERT, mice were subjected to repeated DNA-EP TPA-mTERT(AI)-LTBopt and Ad6TPA-mTERT(AI)-LTBopt (FIGURE 11). The immune response elicited in these mice was comparable to that detected in BALB/c mice vaccinated with the same DNA/Ad regimen and not treated with DMH.

Twenty mice were euthanized 30 weeks after the beginning of DMH treatment to count the number of ACF (aberrant crypt foci) and of adenoma formed. Like results seen with early stage tumor development, a significant reduction in the number of ACF and adenomas was observed in vaccinated mice compared to non-vaccinated mice (FIGURE 12).

Finally, tumors found in vaccinated mice were smaller in volume compared to those found in non-vaccinated mice (FIGURE 13A). Moreover, histological analysis showed that the tumors present in vaccinated mice were at a less advanced stage compared to tumors in non-vaccinated animals (FIGURE 13B).

EXAMPLE 12

Characterization of pV1JTPA-hTERT(AI)-LTBopt.

The immunogenic potential of pV1J/TPA-hTERT(AI)-LTBopt was evaluated in a non–self context since mice transgenic for hTERT are not available. C57BL/6 mice transgenic for the human HLA-A2 (HHD transgenic mice) were utilized so that TERT immunogenic peptides would be presented in the context of human HLA class I. HHD transgenic mice were vaccinated

by DNA-EP with 2 biweekly injections of 50 μg of plasmid pV1J/TPA-hTERT(AI)-LTBopt. The induced immune response was evaluated by ICS on mice PBMC. The hTERT immunogenic peptide corresponding to the hTERT 865 epitope RLVDDFLLV (SEQ ID NO:23) was used as antigen. This epitope sequence was previously described as a strong binder for HLA-A2 and inducer of CTL activity in T cells *in vitro* priming experiments (Dupont et al. *Cancer Research* 65(12): 5417-27 (2005)).

Results obtained from 10 mice showed that on average, 12% of CD8+ cells produced IFN-γ when stimulated with the immunogenic hTERT 865 peptide (FIGURE 14). Results indicate that the pV1JTPA-hTERT(AI)-LTBopt vaccine candidate is immunogenic and induced a HLA-A2 restricted CD8 immune response.

EXAMPLE 13

Characterization of Immune Response in Vaccinated Mice after Ad6-hTERT(AI) Boost.

An Ad6 hTERT(AI) construct was assembled as described in EXAMPLE 2. This construct comprises wild-type codons, with the exception of two mutations that were added to abrogate telomerase catalytic activity. An Ad6 hTERT-LTB fusion construct was also made, but could not be rescued, so further testing was performed only with Ad6hTERT(AI).

The Ad6 hTERT(AI) construct was tested for its boosting capability on the immune response induced by DNA–EP in HHD transgenic mice. Groups of 10 mice were vaccinated by DNA-EP with 1 injection of 50 μg of plasmid pV1J/TPA-hTERT(AI)-LTBopt and boosted either with a second DNA injection or with Ad6 hTERT 10^{10} pp two weeks after the first DNA injection. The induced immune response was measured by using the immunodominant peptide for HLA-A2 allele hTERT865 previously identified. Reactive CD8+ T cells producing IFN-γ were detected with high frequencies. As shown in FIGURE 14, the amplitude of immune response elicited by the DNA-EP/Ad combination was 3 fold greater than what observed in mice that had received only a DNA-EP regimen.

The results demonstrated that hTERT specific immune response primed by injection of pV1J/TPA-hTERT(AI)-LTBopt DNA was amplified by boosting it with Ad6-hTERT(AI).

EXAMPLE 14

Characterization of CTL Activity Induced by Immunization with hTERT.

The $CD8^+$ T cell response to hTERT elicited in vaccinated mice was further characterized by testing the cytotoxic activity (CTL) of activated T cells against tumor target cells. Splenocytes from immunized mice were stimulated in culture for 6 days in the presence of the hTERT865 peptide (SEQ ID NO:23; see EXAMPLE 12) and IL-2. Thereafter, activated CD8+ T cells were tested against target cells for their lytic activity in a Cr^{51} release assay. HeLa cells stably transfected to express the chimeric MHC class I molecule present on HHD mice (HeLa-HHD) were used as target cells. HeLa–HHD target cells were either loaded exogenously with the immunogenic peptide hTERT865 or infected 24 hours before with an Ad6 vector coding for hTERT, in order to endogenously over-express the TERT antigen. As a control, target cells were infected with an Ad6 vector coding for GFP. The results indicate that CD8+ T cells induced by the vaccination described were able to kill target cell over-expressing the hTERT antigen (FIGURE 15).

EXAMPLE 15

Characterization of Immunogenicity of Human-TERT Vaccine in Rhesus Monkeys.

The immunogenicity of the genetic vaccination platform based on DNA prime, Ad boost, with hTERT was evaluated in rhesus monkeys. Because the homology of human TERT and rhesus TERT sequence is 96%, tolerance was expected to play a major role in determining vaccination efficacy.

The vaccination protocol consisted of five injections of DNA-EP (pV1J/TPA-hTERT-LTB, 5mg/injection), given every two weeks. Four weeks after the last DNA-EP treatment, monkeys were boosted with an Ad vector expressing hTERT (Ad6-hTERT, 10^{11} vp) 2 times with a two week interval. Data on body weight and clinical signs were collected every week. Negative effects on animal body weight or occurrence of clinical signs were not observed.

The induced immune response was monitored by IFN-γ ELISPOT assay of PBMC using hTERT peptide pools similar to those described in EXAMPLE 7 and a peptide pool covering the LTB sequence. Immune responses were scored positive if the signal obtained was at least four times higher than the background reactivity (DMSO). ELIspot analysis of the induced CMI indicated that there was a detectable immune response after the first 2 DNA-EP treatments in four out of four vaccinated monkeys (FIGURE 16A). A slight increase of the response was observed after the fifth DNA-EP (FIGURE 16B). The immune response was also measured at the end of the complete immunization protocol, after the two Ad boosts. Results indicate that the Ad boosts induced a consistent increase in the amplitude of CMI (FIGURE 17). Therefore, the power of a heterologous prime/boost modality was further confirmed by this experiment.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a sequence of nucleotides that encodes a human telomerase reverse transcriptase (hTERT) fusion protein, wherein the hTERT fusion protein comprises an hTERT variant , fused to a B subunit of *E. coli* heat labile enterotoxin (LTB) protein wherein said hTERT-LTB fusion protein comprises a sequence of amino acids as set forth in SEQ ID NO:6 and wherein the fusion protein is capable of producing an immune response in a mammal.

2. The nucleic acid molecule of claim 1, wherein the sequence of nucleotides encoding hTERT has been codon-optimized for high-level expression in a human cell.

3. The nucleic acid molecule of claim 1, wherein the sequence of nucleotides encoding LTB has been codon-optimized for high-level expression in a human cell.

4. The nucleic acid molecule of claim 3, wherein the sequence of nucleotides encoding LTB and the sequence of nucleotides encoding hTERT have been codon-optimized for high-level expression in a human cell.

5. The nucleic acid molecule of claim 4, wherein the sequence of nucleotides comprises a sequence as set forth in SEQ ID NO:5.

6. A purified fusion protein comprising a human telomerase reverse transcriptase (hTERT) variant , fused to a B subunit of *E. coli* heat labile enterotoxin (LTB) protein wherein said hTERT-LTB fusion protein comprises a sequence of amino acids as set forth in SEQ ID NO:6.

7. A vector comprising the nucleic acid molecule of claim 1 .

8. The vector of claim 7, wherein the vector is a plasmid vector or an adenovirus vector.

9. The vector of claim 8, wherein the vector is a plasmid pV1J.

10. A host cell comprising the vector of claim 7.

11. The nucleic acid molecule of claim 4, wherein the sequence of nucleotides further comprises a leader sequence encoding the human tissue plasminogen activator (TPA) signal sequence.

12. The nucleic acid molecule of claim 11, wherein the sequence of nucleotides comprises a sequence as set forth in SEQ ID NO:1

13. A vector comprising the nucleic acid molecule of claim 12.

14. A process for expressing an hTERT-LTB fusion protein in a recombinant host cell, comprising:

(a) introducing a vector comprising the nucleic acid molecule of claim 1 into a suitable host cell; and,

(b) culturing the host cell under conditions which allow expression of said hTERT-LTB fusion protein.

SEQUENCE LISTING

<110> Istituto di Ricerche di Biologia Molecolare P. Angeletti S.p.A.

<120> TELOMERASE REVERSE TRANSCRIPTASE FUSION PROTEIN, NUCLEOTIDES ENCODING IT, AND USES THEREOF

<130> ITR0119Y PCT

<150> 60/851,183
<151> 2006-10-12

<160> 23

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 3783
<212> DNA
<213> Artificial Sequence

<220>
<223> TPA-hTERT(AI)-LTBopt

<400> 1
atggatgcaa tgaagagggg cctgtgctgc gtgctgctgc tgtgtggcgc cgtgtttgtg 60
agccctagcg agatccccag agcccccaga tgcagagccg tgcggagcct gctgcggagc 120
cactaccggg aagtgctgcc cctggccacc ttcgtgcggc ggctgggccc ccagggctgg 180
cggctggtgc agcggggcga ccctgccgcc ttccgggccc tggtggctca gtgcctggtg 240
tgcgtgccct gggacgccag acccccccca gccgccccta gcttccggca ggtgagctgc 300
ctgaaggaac tggtggccag agtgctgcag cggctgtgcg agagaggcgc caagaacgtg 360
ctggccttcg gcttcgccct gctggacggc gccagaggcg gccctcccga ggccttcacc 420
acaagcgtgc ggagctacct gcccaacacc gtgaccgacg ccctgcgggg cagcggcgcc 480
tggggcctgc tgctgagaag agtgggcgac gacgtgctgg tgcacctgct ggcccggtgc 540
gccctgttcg tgctggtggc ccccagctgc gcctaccagg tgtgcggccc acccctgtac 600
cagctgggag ccgccaccca ggccaggccc ccaccccacg ccagcggccc cagacggaga 660
ctgggctgcg agcgggcctg gaaccacagc gtgagagagg ccggcgtgcc cctgggcctg 720
ccagcccctg gcgccagaag aagaggcggc agcgccagcc ggagcctgcc cctgcccaag 780
cggcccagaa gaggcgctgc ccccgagccc gagcggaccc ccgtgggcca gggcagctgg 840
gcccaccccg gcagaaccag aggccccagc gaccggggct tctgcgtggt gagccccgcc 900
agacccgccg aggaggccac aagcctggag ggcgccctga gcggcacccg gcacagccac 960
cccagcgtgg gccggcagca ccacgccgga ccccccagca ccagcagacc ccccagaccc 1020
tgggacaccc cctgcccccc tgtgtacgcc gagaccaagc acttcctgta cagcagcggc 1080
gacaaggagc agctgcggcc cagcttcctg ctgagcagcc tgagacccag cctgaccggc 1140
gccaggagac tggtggagac catcttcctg ggcagccggc cctggatgcc cggcaccccc 1200
cggagactgc cccggctgcc ccagcggtac tggcagatgc ggcccctgtt cctggagctg 1260
ctgggcaacc acgcccagtg ccctacggc gtgctgctga aaacccactg ccccctgaga 1320
gccgccgtga cccccgctgc cggcgtgtgc gccagagaga agccccaggg cagcgtggcc 1380
gctcccgagg aggaggacac cgaccccaga cgcctggtgc agctgctgcg gcagcacagc 1440
agcccttggc aggtgtacgg cttcgtgcgg gcctgcctga gaaggctggt gcccccctggc 1500
ctgtggggca gcagacacaa cgagcggcgg ttcctgcgga acaccaagaa gttcatcagc 1560
ctggggaagc acgccaagct gagcctgcag gaactgacct ggaagatgag cgtgcgggac 1620
tgcgcctggc tgcggcggag ccctggcgtg ggctgcgtgc cagccgccga gcaccggctg 1680
cgggaggaga tcctggccaa gttcctgcac tggctgatga gcgtgtacgt ggtggaactg 1740
ctgcggtcct tcttctacgt gaccgaaacc accttccaga agaaccggct gttcttctac 1800
cggaagagcg tgtggagcaa gctgcagagc atcggcatca ggcagcacct gaagagagtg 1860
cagctgcggg agctgagcga ggccgaagtg agacagcacc gggaggccag acctgccctg 1920
ctgaccagcc ggctgcggtt catccccaag cccgacggcc tgcggcccat cgtgaacatg 1980
gactacgtgg tgggcgccag aaccttccgg cgggagaagc gggccgagcg gctgaccagc 2040
agagtgaagg ccctgttcag cgtgctgaac tacgagcggg ccaggagacc cggcctgctg 2100
ggcgccagcg tgctgggcct ggacgacatc caccgggcct ggcggacctt cgtgctgaga 2160
gtgcgggccc aggacccccc acccgagctg tacttcgtga aagtggccat caccggcgcc 2220
tacgacacca tcccccagga ccggctgacc gaagtgatcg ccagcatcat caagccccag 2280
aacacctact gcgtgcggcg gtacgccgtg gtgcagaagg ccgcccacgg ccacgtgcgg 2340
aaggccttca agagccacgt gagcaccctg accgacctgc agccctacat gcggcagttc 2400
gtggcccacc tgcaggagac cagccccctg cgggatgccg tggtgatcga gcagagcagc 2460
agcctgaacg aggccagcag cggcctgttc gacgtgttcc tgcgcttcat gtgccaccac 2520
gccgtgcgga tccggggcaa gagctacgtg cagtgccagg gcatccctca gggcagcatc 2580
ctgagcacac tgctgtgctc tctgtgctac ggcgacatgg agaacaagct gttcgccggc 2640

```
atccggcggg acggactgct gctgcgcctg gtggacgact tcctgctggt gacccctcac 2700
ctgacccacg ccaagacctt cctgcggacc ctggtgcggg gcgtgcccga gtacggctgt 2760
gtggtgaacc tgcgcaagac cgtggtgaac ttccccgtgg aggacgaggc cctgggcggc 2820
acagccttcg tgcagatgcc cgcccatggc ctgttccctt ggtgcgggct gctgctggac 2880
acccggaccc tggaagtgca gagcgactac agcagctacg cccggaccag catccgggcc 2940
agcctgacat tcaaccgcgg cttcaaggcc ggcagaaaca tgcggcggaa gctgtttggc 3000
gtgctgcggc tgaagtgcca cagcctgttt ctggacctgc aggtgaacag cctgcagacc 3060
gtgtgcacca acatctacaa gatcctgctg ctgcaggcct accggttcca cgcctgcgtg 3120
ctgcagctgc ccttccatca gcaggtgtgg aagaacccca ccttcttcct gcgcgtgatc 3180
tctgacaccg ccagcctgtg ctacagcatt ctgaaggcca agaacgccgg catgagcctg 3240
ggcgccaagg gcgctgccgg acccctgccc agcgaggccg tgcagtggct gtgtcaccag 3300
gcctttctgc tgaagctgac ccggcaccgc gtgacctacg tgcccctgct gggaagcctg 3360
cggaccgccc agacccagct gagccggaag ctgcctggca ccaccctgac agccctggag 3420
gccgctgcca accccgccct gcctagcgac ttcaagacca tcctggactc tagagccccc 3480
cagagcatca ccgagctgtg cagcgagtac cggaacaccc agatttacac catcaacgac 3540
aagatcctga gctacaccga gtctatggcc ggcaagcggg agatggtgat catcaccttc 3600
aagagcggcg ccacctttca ggtggaagtg cctggcagcc agcacatcga cagccagaag 3660
aaggccatcg agcggatgaa ggacaccctg cggatcacct acctgaccga gaccaagatc 3720
gacaagctgt gtgtgtggaa caacaagacc cccaacagca tcgccgccat ctctatggag 3780
aac                                                               3783
```

<210> 2
<211> 1267
<212> PRT
<213> Artificial Sequence

<220>
<223> -hTERT(AI)-LTBopt

<400> 2

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1               5                  10                  15
Ala Val Phe Val Ser Pro Ser Glu Ile Pro Arg Ala Pro Arg Cys Arg
            20                  25                  30
Ala Val Arg Ser Leu Leu Arg Ser His Tyr Arg Glu Val Leu Pro Leu
        35                  40                  45
Ala Thr Phe Val Arg Arg Leu Gly Pro Gln Gly Trp Arg Leu Val Gln
    50                  55                  60
Arg Gly Asp Pro Ala Ala Phe Arg Ala Leu Val Ala Gln Cys Leu Val
65                  70                  75                  80

Cys Val Pro Trp Asp Ala Arg Pro Pro Pro Ala Ala Pro Ser Phe Arg
                85                  90                  95
Gln Val Ser Cys Leu Lys Glu Leu Val Ala Arg Val Leu Gln Arg Leu
            100                 105                 110
Cys Glu Arg Gly Ala Lys Asn Val Leu Ala Phe Gly Phe Ala Leu Leu
        115                 120                 125
Asp Gly Ala Arg Gly Gly Pro Pro Glu Ala Phe Thr Thr Ser Val Arg
    130                 135                 140
Ser Tyr Leu Pro Asn Thr Val Thr Asp Ala Leu Arg Gly Ser Gly Ala
145                 150                 155                 160
Trp Gly Leu Leu Leu Arg Arg Val Gly Asp Asp Val Leu Val His Leu
                165                 170                 175
Leu Ala Arg Cys Ala Leu Phe Val Leu Val Ala Pro Ser Cys Ala Tyr
            180                 185                 190
Gln Val Cys Gly Pro Pro Leu Tyr Gln Leu Gly Ala Ala Thr Gln Ala
        195                 200                 205
Arg Pro Pro Pro His Ala Ser Gly Pro Arg Arg Arg Leu Gly Cys Glu
    210                 215                 220
Arg Ala Trp Asn His Ser Val Arg Glu Ala Gly Val Pro Leu Gly Leu
225                 230                 235                 240
Pro Ala Pro Gly Ala Arg Arg Arg Gly Gly Ser Ala Ser Arg Ser Leu
                245                 250                 255
Pro Leu Pro Lys Arg Pro Arg Arg Gly Ala Ala Pro Glu Pro Glu Arg
            260                 265                 270
Thr Pro Val Gly Gln Gly Ser Trp Ala His Pro Gly Arg Thr Arg Gly
        275                 280                 285
Pro Ser Asp Arg Gly Phe Cys Val Val Ser Pro Ala Arg Pro Ala Glu
    290                 295                 300
```

```
Glu Ala Thr Ser Leu Glu Gly Ala Leu Ser Gly Thr Arg His Ser His
305                 310                 315                 320
Pro Ser Val Gly Arg Gln His His Ala Gly Pro Pro Ser Thr Ser Arg
                325                 330                 335
Pro Pro Arg Pro Trp Asp Thr Pro Cys Pro Pro Val Tyr Ala Glu Thr
            340                 345                 350
Lys His Phe Leu Tyr Ser Ser Gly Asp Lys Glu Gln Leu Arg Pro Ser
        355                 360                 365
Phe Leu Leu Ser Ser Leu Arg Pro Ser Leu Thr Gly Ala Arg Arg Leu
    370                 375                 380
Val Glu Thr Ile Phe Leu Gly Ser Arg Pro Trp Met Pro Gly Thr Pro
385                 390                 395                 400
Arg Arg Leu Pro Arg Leu Pro Gln Arg Tyr Trp Gln Met Arg Pro Leu
                405                 410                 415
Phe Leu Glu Leu Leu Gly Asn His Ala Gln Cys Pro Tyr Gly Val Leu
            420                 425                 430
Leu Lys Thr His Cys Pro Leu Arg Ala Ala Val Thr Pro Ala Ala Gly
        435                 440                 445
Val Cys Ala Arg Glu Lys Pro Gln Gly Ser Val Ala Ala Pro Glu Glu
    450                 455                 460
Glu Asp Thr Asp Pro Arg Arg Leu Val Gln Leu Leu Arg Gln His Ser
465                 470                 475                 480
Ser Pro Trp Gln Val Tyr Gly Phe Val Arg Ala Cys Leu Arg Arg Leu
                485                 490                 495
Val Pro Pro Gly Leu Trp Gly Ser Arg His Asn Glu Arg Arg Phe Leu
            500                 505                 510

Arg Asn Thr Lys Lys Phe Ile Ser Leu Gly Lys His Ala Lys Leu Ser
        515                 520                 525
Leu Gln Glu Leu Thr Trp Lys Met Ser Val Arg Asp Cys Ala Trp Leu
    530                 535                 540
Arg Arg Ser Pro Gly Val Gly Cys Val Pro Ala Ala Glu His Arg Leu
545                 550                 555                 560
Arg Glu Glu Ile Leu Ala Lys Phe Leu His Trp Leu Met Ser Val Tyr
                565                 570                 575
Val Val Glu Leu Leu Arg Ser Phe Phe Tyr Val Thr Glu Thr Thr Phe
            580                 585                 590
Gln Lys Asn Arg Leu Phe Phe Tyr Arg Lys Ser Val Trp Ser Lys Leu
        595                 600                 605
Gln Ser Ile Gly Ile Arg Gln His Leu Lys Arg Val Gln Leu Arg Glu
    610                 615                 620
Leu Ser Glu Ala Glu Val Arg Gln His Arg Glu Ala Arg Pro Ala Leu
625                 630                 635                 640
Leu Thr Ser Arg Leu Arg Phe Ile Pro Lys Pro Asp Gly Leu Arg Pro
                645                 650                 655
Ile Val Asn Met Asp Tyr Val Val Gly Ala Arg Thr Phe Arg Arg Glu
            660                 665                 670
Lys Arg Ala Glu Arg Leu Thr Ser Arg Val Lys Ala Leu Phe Ser Val
        675                 680                 685
Leu Asn Tyr Glu Arg Ala Arg Arg Pro Gly Leu Leu Gly Ala Ser Val
    690                 695                 700
Leu Gly Leu Asp Asp Ile His Arg Ala Trp Arg Thr Phe Val Leu Arg
705                 710                 715                 720
Val Arg Ala Gln Asp Pro Pro Pro Glu Leu Tyr Phe Val Lys Val Ala
                725                 730                 735
Ile Thr Gly Ala Tyr Asp Thr Ile Pro Gln Asp Arg Leu Thr Glu Val
            740                 745                 750
Ile Ala Ser Ile Ile Lys Pro Gln Asn Thr Tyr Cys Val Arg Arg Tyr
        755                 760                 765
Ala Val Val Gln Lys Ala Ala His Gly His Val Arg Lys Ala Phe Lys
    770                 775                 780
Ser His Val Ser Thr Leu Thr Asp Leu Gln Pro Tyr Met Arg Gln Phe
785                 790                 795                 800
Val Ala His Leu Gln Glu Thr Ser Pro Leu Arg Asp Ala Val Val Ile
                805                 810                 815
Glu Gln Ser Ser Ser Leu Asn Glu Ala Ser Ser Gly Leu Phe Asp Val
            820                 825                 830
Phe Leu Arg Phe Met Cys His His Ala Val Arg Ile Arg Gly Lys Ser
        835                 840                 845
```

```
Tyr Val Gln Cys Gln Gly Ile Pro Gln Gly Ser Ile Leu Ser Thr Leu
    850                 855                 860
Leu Cys Ser Leu Cys Tyr Gly Asp Met Glu Asn Lys Leu Phe Ala Gly
865                 870                 875                 880
Ile Arg Arg Asp Gly Leu Leu Leu Arg Leu Val Asp Asp Phe Leu Leu
                885                 890                 895
Val Thr Pro His Leu Thr His Ala Lys Thr Phe Leu Arg Thr Leu Val
            900                 905                 910
Arg Gly Val Pro Glu Tyr Gly Cys Val Val Asn Leu Arg Lys Thr Val
        915                 920                 925
Val Asn Phe Pro Val Glu Asp Glu Ala Leu Gly Gly Thr Ala Phe Val
    930                 935                 940

Gln Met Pro Ala His Gly Leu Phe Pro Trp Cys Gly Leu Leu Leu Asp
945                 950                 955                 960
Thr Arg Thr Leu Glu Val Gln Ser Asp Tyr Ser Ser Tyr Ala Arg Thr
                965                 970                 975
Ser Ile Arg Ala Ser Leu Thr Phe Asn Arg Gly Phe Lys Ala Gly Arg
            980                 985                 990
Asn Met Arg Arg Lys Leu Phe Gly Val Leu Arg Leu Lys Cys His Ser
        995                 1000                1005
Leu Phe Leu Asp Leu Gln Val Asn Ser Leu Gln Thr Val Cys Thr Asn
    1010                1015                1020
Ile Tyr Lys Ile Leu Leu Leu Gln Ala Tyr Arg Phe His Ala Cys Val
1025                1030                1035                1040
Leu Gln Leu Pro Phe His Gln Gln Val Trp Lys Asn Pro Thr Phe Phe
                1045                1050                1055
Leu Arg Val Ile Ser Asp Thr Ala Ser Leu Cys Tyr Ser Ile Leu Lys
            1060                1065                1070
Ala Lys Asn Ala Gly Met Ser Leu Gly Ala Lys Gly Ala Ala Gly Pro
        1075                1080                1085
Leu Pro Ser Glu Ala Val Gln Trp Leu Cys His Gln Ala Phe Leu Leu
    1090                1095                1100
Lys Leu Thr Arg His Arg Val Thr Tyr Val Pro Leu Leu Gly Ser Leu
1105                1110                1115                1120
Arg Thr Ala Gln Thr Gln Leu Ser Arg Lys Leu Pro Gly Thr Thr Leu
                1125                1130                1135
Thr Ala Leu Glu Ala Ala Ala Asn Pro Ala Leu Pro Ser Asp Phe Lys
            1140                1145                1150
Thr Ile Leu Asp Ser Arg Ala Pro Gln Ser Ile Thr Glu Leu Cys Ser
        1155                1160                1165
Glu Tyr Arg Asn Thr Gln Ile Tyr Thr Ile Asn Asp Lys Ile Leu Ser
    1170                1175                1180
Tyr Thr Glu Ser Met Ala Gly Lys Arg Glu Met Val Ile Ile Thr Phe
1185                1190                1195                1200
Lys Ser Gly Ala Thr Phe Gln Val Glu Val Pro Gly Ser Gln His Ile
                1205                1210                1215
Asp Ser Gln Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile
            1220                1225                1230
Thr Tyr Leu Thr Glu Thr Lys Ile Asp Lys Leu Cys Val Trp Asn Asn
        1235                1240                1245
Lys Thr Pro Asn Ser Ile Ala Ala Ile Ser Met Glu Asn Ser Glu Gln
    1250                1255                1260
Ile Asp Asn
1265

<210> 3
<211> 3753
<212> DNA
<213> Artificial Sequence

<220>
<223> TPA-mTERT(AI)-LTBopt

<400> 3
atggatgcaa tgaagagggg cctgtgctgc gtgctgctgc tgtgtggcgc cgtgtttgtg 60
agccctagcg agatcaccag agcccccaga tgccctgccg tgagaagcct gctgcggagc 120
cggtacagag aagtgtggcc cctggccacc tttgtgagga gactgggccc tgagggcagg 180
agactggtgc agcctggcga ccccaaaatc tacaggaccc tggtggccca gtgtctggtg 240
```

tgtatgcact ggggcagcca gccccctccc gccgacctga gcttccacca ggtgtccagc 300
ctgaaggaac tggtggccag agtggtgcag agactgtgcg agcggaacga gagaaacgtg 360
ctggccttcg gcttcgagct gctgaacgag gccagaggcg gccctcccat ggccttcacc 420
agctctgtga ggagctacct gcccaacacc gtgatcgaga ccctgagagt gagcggcgcc 480
tggatgctgc tgctgagcag agtgggcgat gacctgctgg tgtacctgct ggcccactgc 540
gccctgtatc tgctggtgcc ccccagctgc gcctaccagg tgtgcggatc ccccctgtac 600
cagatttgcg ccaccaccga catctggccc agcgtgtctg ccagctacag acccaccaga 660
cctgtgggcc ggaacttcac caacctgcgg ttcctgcagc agatcaagag cagcagcaga 720
caggaggccc ccaagccccc ggccctgccc agcagaggca ccaagagaca cctgagcctg 780
accagcacca gcgtgcccag cgccaagaaa gccagatgct accccgtgcc tagagtggag 840
gagggccctc acagacaggt gctgcccacc cccagcggca agagctgggt gcccagcccc 900
gccagaagcc ccgaagtgcc caccgccgag aaggacctga gcagcaaggg caaagtgagc 960
gacctgtctc tgagcggcag cgtgtgttgc aagcacaagc ccagcagcac cagcctgctg 1020
agcccccccа gacagaacgc cttccagctg aggcctttca tcgagacccg gcacttcctg 1080
tacagcagag gcgatggcca ggagagactg aaccccagct tcctgctgag caacctgcag 1140
cctaacctga ccggcgccag acgcctggtg gagatcatct tcctgggcag cagacccaga 1200
accagcggcc ctctgtgcag aacccaccgg ctgagcaggc ggtactggca gatgagaccc 1260
ctgttccagc agctgctggt gaaccacgcc gagtgccagt atgtgcggct gctgaggagc 1320
cactgcagat tcaggaccgc caaccagcag gtgaccgacg ccctgaacac cagccccct 1380
cacctgatgg atctgctgag gctgcacagc agcccctggc aggtgtacgg cttcctgaga 1440
gcctgcctgt gcaaagtggt gtccgccagc ctgtggggca ccagacacaa cgagcggcgg 1500
ttcttcaaga atctgaagaa gttcatcagc ctgggcaagt acggcaagct gagcctgcag 1560
gaactgatgt ggaagatgaa agtggaggac tgccactggc tgagaagcag ccccggcaag 1620
gacagagtgc ctgccgccga gcacagactg agggagagaa tcctggccac attcctgttc 1680
tggctgatgg acacctacgt ggtgcagctg ctgcggtcct tcttctacat caccgagagc 1740
accttccaga agaaccggct gttcttctac cggaagtctg tgtggagcaa gctgcagagc 1800
atcggagtga gacagcacct ggagagagtg aggctgagag agctgagcca ggaggaagtg 1860
agacaccacc aggatacctg gctggccatg cccatctgcc ggctgagatt catccccaag 1920
cccaacggcc tgagacccat cgtgaacatg agctacagca tgggcacaag agccctgggc 1980
agaagaaagc aggcccagca cttcacccag cggctgaaaa ccctgttctc catgctgaac 2040
tacgagcgga ccaagcaccc acacctgatg ggcagcagcg tgctgggcat gaacgacatc 2100
taccggacct ggagagcctt cgtgctgaga gtgcgggccc tggaccagac ccctcggatg 2160
tacttcgtga aggccgccat caccggcgcc tacgacgcca tcccccaggg caaactggtg 2220
gaagtggtgg ccaacatgat caggcacagc gagtccacct actgcatcag gcagtacgcc 2280
gtggtgagaa gagacagcca gggccaggtg cacaagagct tccggagaca ggtgaccacc 2340
ctgagcgatc tgcagcctta catgggccag ttcctgaagc acctgcagga tagcgacgcc 2400
agcgccctga gaaatagcgt ggtgatcgag cagagcatca gcatgaacga gtccagcagc 2460
agcctgttcg acttcttcct gcacttcctg aggcacagcg tggtgaagat cggcgacaga 2520
tgctacaccc agtgtcaggg catccctcag ggctctagcc tgagcaccct gctgtgtagc 2580
ctgtgcttcg gcgacatgga gaataagctg ttcgccgaag tgcagagaga tggcctgctg 2640
ctgcgcttcg tggacgattt cctgctggtg accccacacc tggaccaggc caagaccttc 2700
ctgagcacac tggtgcacgg cgtgcccgag tacggctgca tgatcaatct gcagaaaacc 2760
gtggtgaact tccctgtgga gcccggcacc ctgggcggag ccgcccctta ccagctgccc 2820
gcccactgcc tgttcccctg gtgcggactg ctgctggata cccagaccct ggaagtgttc 2880
tgcgactaca gcggctacgc ccagaccagc atcaagacca gcctgacctt ccagagcgtg 2940
ttcaaggccg gcaagaccat gaggaacaag ctgctgagcg tgctgagact gaagtgccac 3000
ggcctgttcc tggatctgca ggtgaacagc ctgcagaccg tgtgtatcaa catctacaag 3060
attttcctgc tgcaggccta cagattccac gcctgcgtga tccagctgcc cttcgaccag 3120
agagtgcgga agaacctgac cttcttcctg gggatcatca gcagccaggc cagctgctgc 3180
tacgccatcc tgaaagtgaa gaaccccggc atgaccctga aggccagcgg cagcttccct 3240
cccgaggccg cccactggct gtgctaccag gcctttctgc tgaagctggc cgcccacagc 3300
gtgatctaca agtgcctgct gggccctctg agaaccgccc agaagctgct gtgccggaag 3360
ctgcccgagg ccaccatgac cattctgaaa gccgccgccg acccccgccct gagcaccgac 3420
ttccagacca tcctggactc tagagccccct cagagcatca ccgagctgtg cagcgagtac 3480
cggaacaccc agatttacac catcaacgac aagatcctga gctacaccga gtctatggcc 3540
ggcaagcggg agatggtgat catcaccttc aagagcggcg ccacctttca ggtggaagtg 3600
cctggcagcc agcacatcga cagccagaag aaggccatcg agcggatgaa ggacaccctg 3660
cggatcacct acctgaccga gaccaagatc gacaagctgt gtgtgtggaa caacaagacc 3720
cccaacagca tcgccgccat ctctatggag aac 3753

<210> 4
<211> 1251
<212> PRT
<213> Artificial Sequence

<220>
<223> TPA-mTERT(AI)-LTBopt

<400> 4

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1               5                  10                  15
Ala Val Phe Val Ser Pro Ser Glu Ile Thr Arg Ala Pro Arg Cys Pro
            20                  25                  30
Ala Val Arg Ser Leu Leu Arg Ser Arg Tyr Arg Glu Val Trp Pro Leu
        35                  40                  45
Ala Thr Phe Val Arg Arg Leu Gly Pro Glu Gly Arg Arg Leu Val Gln
    50                  55                  60
Pro Gly Asp Pro Lys Ile Tyr Arg Thr Leu Val Ala Gln Cys Leu Val
65                  70                  75                  80
Cys Met His Trp Gly Ser Gln Pro Pro Pro Ala Asp Leu Ser Phe His
                85                  90                  95
Gln Val Ser Ser Leu Lys Glu Leu Val Ala Arg Val Val Gln Arg Leu
            100                 105                 110
Cys Glu Arg Asn Glu Arg Asn Val Leu Ala Phe Gly Phe Glu Leu Leu
        115                 120                 125
Asn Glu Ala Arg Gly Gly Pro Pro Met Ala Phe Thr Ser Ser Val Arg
    130                 135                 140
Ser Tyr Leu Pro Asn Thr Val Ile Glu Thr Leu Arg Val Ser Gly Ala
145                 150                 155                 160
Trp Met Leu Leu Leu Ser Arg Val Gly Asp Asp Leu Leu Val Tyr Leu
                165                 170                 175
Leu Ala His Cys Ala Leu Tyr Leu Leu Val Pro Pro Ser Cys Ala Tyr
            180                 185                 190
Gln Val Cys Gly Ser Pro Leu Tyr Gln Ile Cys Ala Thr Thr Asp Ile
        195                 200                 205
Trp Pro Ser Val Ser Ala Ser Tyr Arg Pro Thr Arg Pro Val Gly Arg
    210                 215                 220
Asn Phe Thr Asn Leu Arg Phe Leu Gln Gln Ile Lys Ser Ser Ser Arg
225                 230                 235                 240
Gln Glu Ala Pro Lys Pro Leu Ala Leu Pro Ser Arg Gly Thr Lys Arg
                245                 250                 255
His Leu Ser Leu Thr Ser Thr Ser Val Pro Ser Ala Lys Lys Ala Arg
            260                 265                 270
Cys Tyr Pro Val Pro Arg Val Glu Glu Gly Pro His Arg Gln Val Leu
        275                 280                 285
Pro Thr Pro Ser Gly Lys Ser Trp Val Pro Ser Pro Ala Arg Ser Pro
    290                 295                 300
Glu Val Pro Thr Ala Glu Lys Asp Leu Ser Ser Lys Gly Lys Val Ser
305                 310                 315                 320
Asp Leu Ser Leu Ser Gly Ser Val Cys Cys Lys His Lys Pro Ser Ser
                325                 330                 335
Thr Ser Leu Leu Ser Pro Pro Arg Gln Asn Ala Phe Gln Leu Arg Pro
            340                 345                 350
Phe Ile Glu Thr Arg His Phe Leu Tyr Ser Arg Gly Asp Gly Gln Glu
        355                 360                 365
Arg Leu Asn Pro Ser Phe Leu Leu Ser Asn Leu Gln Pro Asn Leu Thr
    370                 375                 380
Gly Ala Arg Arg Leu Val Glu Ile Ile Phe Leu Gly Ser Arg Pro Arg
385                 390                 395                 400
Thr Ser Gly Pro Leu Cys Arg Thr His Arg Leu Ser Arg Arg Tyr Trp
                405                 410                 415
Gln Met Arg Pro Leu Phe Gln Gln Leu Leu Val Asn His Ala Glu Cys
            420                 425                 430
Gln Tyr Val Arg Leu Leu Arg Ser His Cys Arg Phe Arg Thr Ala Asn
        435                 440                 445
Gln Gln Val Thr Asp Ala Leu Asn Thr Ser Pro Pro His Leu Met Asp
    450                 455                 460
Leu Leu Arg Leu His Ser Ser Pro Trp Gln Val Tyr Gly Phe Leu Arg
465                 470                 475                 480
Ala Cys Leu Cys Lys Val Val Ser Ala Ser Leu Trp Gly Thr Arg His
                485                 490                 495
Asn Glu Arg Arg Phe Phe Lys Asn Leu Lys Lys Phe Ile Ser Leu Gly
            500                 505                 510
Lys Tyr Gly Lys Leu Ser Leu Gln Glu Leu Met Trp Lys Met Lys Val
        515                 520                 525
Glu Asp Cys His Trp Leu Arg Ser Ser Pro Gly Lys Asp Arg Val Pro
    530                 535                 540
Ala Ala Glu His Arg Leu Arg Glu Arg Ile Leu Ala Thr Phe Leu Phe
```

545 550 555 560
Trp Leu Met Asp Thr Tyr Val Val Gln Leu Leu Arg Ser Phe Phe Tyr
565 570 575
Ile Thr Glu Ser Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr Arg Lys
580 585 590
Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Val Arg Gln His Leu Glu
595 600 605
Arg Val Arg Leu Arg Glu Leu Ser Gln Glu Glu Val Arg His His Gln
610 615 620
Asp Thr Trp Leu Ala Met Pro Ile Cys Arg Leu Arg Phe Ile Pro Lys
625 630 635 640
Pro Asn Gly Leu Arg Pro Ile Val Asn Met Ser Tyr Ser Met Gly Thr
645 650 655
Arg Ala Leu Gly Arg Arg Lys Gln Ala Gln His Phe Thr Gln Arg Leu
660 665 670
Lys Thr Leu Phe Ser Met Leu Asn Tyr Glu Arg Thr Lys His Pro His
675 680 685
Leu Met Gly Ser Ser Val Leu Gly Met Asn Asp Ile Tyr Arg Thr Trp
690 695 700
Arg Ala Phe Val Leu Arg Val Arg Ala Leu Asp Gln Thr Pro Arg Met
705 710 715 720
Tyr Phe Val Lys Ala Ala Ile Thr Gly Ala Tyr Asp Ala Ile Pro Gln
725 730 735
Gly Lys Leu Val Glu Val Val Ala Asn Met Ile Arg His Ser Glu Ser
740 745 750

Thr Tyr Cys Ile Arg Gln Tyr Ala Val Val Arg Arg Asp Ser Gln Gly
755 760 765
Gln Val His Lys Ser Phe Arg Arg Gln Val Thr Thr Leu Ser Asp Leu
770 775 780
Gln Pro Tyr Met Gly Gln Phe Leu Lys His Leu Gln Asp Ser Asp Ala
785 790 795 800
Ser Ala Leu Arg Asn Ser Val Val Ile Glu Gln Ser Ile Ser Met Asn
805 810 815
Glu Ser Ser Ser Ser Leu Phe Asp Phe Phe Leu His Phe Leu Arg His
820 825 830
Ser Val Val Lys Ile Gly Asp Arg Cys Tyr Thr Gln Cys Gln Gly Ile
835 840 845
Pro Gln Gly Ser Ser Leu Ser Thr Leu Leu Cys Ser Leu Cys Phe Gly
850 855 860
Asp Met Glu Asn Lys Leu Phe Ala Glu Val Gln Arg Asp Gly Leu Leu
865 870 875 880
Leu Arg Phe Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Asp Gln
885 890 895
Ala Lys Thr Phe Leu Ser Thr Leu Val His Gly Val Pro Glu Tyr Gly
900 905 910
Cys Met Ile Asn Leu Gln Lys Thr Val Val Asn Phe Pro Val Glu Pro
915 920 925
Gly Thr Leu Gly Gly Ala Ala Pro Tyr Gln Leu Pro Ala His Cys Leu
930 935 940
Phe Pro Trp Cys Gly Leu Leu Leu Asp Thr Gln Thr Leu Glu Val Phe
945 950 955 960
Cys Asp Tyr Ser Gly Tyr Ala Gln Thr Ser Ile Lys Thr Ser Leu Thr
965 970 975
Phe Gln Ser Val Phe Lys Ala Gly Lys Thr Met Arg Asn Lys Leu Leu
980 985 990
Ser Val Leu Arg Leu Lys Cys His Gly Leu Phe Leu Asp Leu Gln Val
995 1000 1005
Asn Ser Leu Gln Thr Val Cys Ile Asn Ile Tyr Lys Ile Phe Leu Leu
1010 1015 1020
Gln Ala Tyr Arg Phe His Ala Cys Val Ile Gln Leu Pro Phe Asp Gln
1025 1030 1035 1040
Arg Val Arg Lys Asn Leu Thr Phe Phe Leu Gly Ile Ile Ser Ser Gln
1045 1050 1055
Ala Ser Cys Cys Tyr Ala Ile Leu Lys Val Lys Asn Pro Gly Met Thr
1060 1065 1070
Leu Lys Ala Ser Gly Ser Phe Pro Pro Glu Ala Ala His Trp Leu Cys
1075 1080 1085
Tyr Gln Ala Phe Leu Leu Lys Leu Ala Ala His Ser Val Ile Tyr Lys

```
    1090                1095                1100
Cys Leu Leu Gly Pro Leu Arg Thr Ala Gln Lys Leu Leu Cys Arg Lys
1105                1110                1115                1120
Leu Pro Glu Ala Thr Met Thr Ile Leu Lys Ala Ala Ala Asp Pro Ala
                1125                1130                1135
Leu Ser Thr Asp Phe Gln Thr Ile Leu Asp Ser Arg Ala Pro Gln Ser
            1140                1145                1150
Ile Thr Glu Leu Cys Ser Glu Tyr Arg Asn Thr Gln Ile Tyr Thr Ile
        1155                1160                1165
Asn Asp Lys Ile Leu Ser Tyr Thr Glu Ser Met Ala Gly Lys Arg Glu
    1170                1175                1180

Met Val Ile Ile Thr Phe Lys Ser Gly Ala Thr Phe Gln Val Glu Val
1185                1190                1195                1200
Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala Ile Glu Arg Met
                1205                1210                1215
Lys Asp Thr Leu Arg Ile Thr Tyr Leu Thr Glu Thr Lys Ile Asp Lys
            1220                1225                1230
Leu Cys Val Trp Asn Asn Lys Thr Pro Asn Ser Ile Ala Ala Ile Ser
        1235                1240                1245
Met Glu Asn
    1250
```

<210> 5
<211> 3711
<212> DNA
<213> Artificial Sequence

<220>
<223> hTERT(AI)-LTBopt

<400> 5

```
cccagagccc ccagatgcag agccgtgcgg agcctgctgc ggagccacta ccgggaagtg 60
ctgcccctgg ccaccttcgt gcggcggctg ggcccccagg gctggcggct ggtgcagcgg 120
ggcgaccctg ccgccttccg ggccctggtg gctcagtgcc tggtgtgcgt gccctgggac 180
gccagacccc ccccagccgc ccctagcttc cggcaggtga gctgcctgaa ggaactggtg 240
gccagagtgc tgcagcggct gtgcgagaga ggcgccaaga acgtgctggc cttcggcttc 300
gccctgctgg acggcgccag aggcggccct cccgaggcct tcaccacaag cgtgcggagc 360
tacctgccca acaccgtgac cgacgccctg cggggcagcg gcgcctgggg cctgctgctg 420
agaagagtgg gcgacgacgt gctggtgcac ctgctggccc ggtgcgccct gttcgtgctg 480
gtggcccccа gctgcgccta ccaggtgtgc ggcccacccc tgtaccagct gggagccgcc 540
acccaggcca ggcccccacc ccacgccagc ggccccagac ggagactggg ctgcgagcgg 600
gcctggaacc acagcgtgag agaggccggc gtgcccctgg gcctgccagc ccctggcgcc 660
agaagaagag gcggcagcgc cagccggagc ctgcccctgc ccaagcggcc cagaagaggc 720
gctgcccccg agcccgagcg gacccccgtg ggccagggca gctgggccca ccccggcaga 780
accagaggcc ccagcgaccg gggcttctgc gtggtgagcc ccgccagacc cgccgaggag 840
gccacaagcc tggagggcgc cctgagcggc acccggcaca gccaccccag cgtgggccgg 900
cagcaccacg ccggaccccc cagcaccagc agacccccca gaccctggga cacccccтgc 960
ccccctgtgt acgccgagac caagcacttc ctgtacagca gcggcgacaa ggagcagctg 1020
cggcccagct tcctgctgag cagcctgaga cccagcctga ccggcgccag gagactggtg 1080
gagaccatct tcctgggcag ccggccctgg atgcccggca ccccccggag actgccccgg 1140
ctgccccagc ggtactggca gatgcggccc ctgttcctgg agctgctggg caaccacgcc 1200
cagtgccccт acggcgtgct gctgaaaacc cactgccccc tgagagccgc cgtgaccccc 1260
gctgccggcg tgtgcgccag agagaagccc cagggcagcg tggccgctcc cgaggaggag 1320
gacaccgacc ccagacgcct ggtgcagctg ctgcggcagc acagcagccc ttggcaggtg 1380
tacggcttcg tgcgggcctg cctgagaagg ctggtgcccc ctggcctgtg gggcagcaga 1440
cacaacgagc ggcggttcct gcggaacacc aagaagttca tcagcctggg gaagcacgcc 1500
aagctgagcc tgcaggaact gacctggaag atgagcgtgc gggactgcgc ctggctgcgg 1560
cggagccctg gcgtgggctg cgtgccagcc gccgagcacc ggctgcggga ggagatcctg 1620
gccaagttcc tgcactggct gatgagcgtg tacgtggtgg aactgctgcg gtccttcttc 1680
tacgtgaccg aaaccacctt ccagaagaac cggctgttct tctaccggaa gagcgtgtgg 1740
agcaagctgc agagcatcgg catcaggcag cacctgaaga gagtgcagct gcgggagctg 1800
agcgaggccg aagtgagaca gcaccgggag gccagacctg ccctgctgac cagccggctg 1860
cggttcatcc ccaagcccga cggcctgcgg cccatcgtga acatggacta cgtggtgggc 1920
gccagaacct tccggcggga gaagcgggcc gagcggctga ccagcagagt gaaggccctg 1980
ttcagcgtgc tgaactacga gcgggccagg agacccggcc tgctgggcgc cagcgtgctg 2040
ggcctggacg acatccaccg ggcctggcgg accttcgtgc tgagagtgcg ggcccaggac 2100
cccccacccg agctgtactt cgtgaaagtg gccatcaccg gcgcctacga caccatcccc 2160
caggaccggc tgaccgaagt gatcgccagc atcatcaagc cccagaacac ctactgcgtg 2220
```

```
cggcggtacg ccgtggtgca gaaggccgcc cacggccacg tgcggaaggc cttcaagagc 2280
cacgtgagca ccctgaccga cctgcagccc tacatgcggc agttcgtggc ccacctgcag 2340
gagaccagcc ccctgcggga tgccgtggtg atcgagcaga gcagcagcct gaacgaggcc 2400
agcagcggcc tgttcgacgt gttcctgcgc ttcatgtgcc accacgccgt gcggatccgg 2460
ggcaagagct acgtgcagtg ccagggcatc cctcagggca gcatcctgag cacactgctg 2520
tgctctctgt gctacggcga catggagaac aagctgttcg ccggcatccg gcgggacgga 2580
ctgctgctgc gcctggtgga cgacttcctg ctggtgaccc ctcacctgac ccacgccaag 2640
accttcctgc ggaccctggt gcggggcgtg cccgagtacg gctgtgtggt gaacctgcgc 2700
aagaccgtgg tgaacttccc cgtggaggac gaggccctgg gcggcacagc cttcgtgcag 2760
atgcccgccc atggcctgtt cccttggtgc gggctgctgc tggacacccg gaccctggaa 2820
gtgcagagcg actacagcag ctacgcccgg accagcatcc gggccagcct gacattcaac 2880
cgcggcttca aggccggcag aaacatgcgg cggaagctgt ttggcgtgct gcggctgaag 2940
tgccacagcc tgtttctgga cctgcaggtg aacagcctgc agaccgtgtg caccaacatc 3000
tacaagatcc tgctgctgca ggcctaccgg ttccacgcct gcgtgctgca gctgcccttc 3060
catcagcagg tgtggaagaa ccccaccttc ttcctgcgcg tgatctctga caccgccagc 3120
ctgtgctaca gcattctgaa ggccaagaac gccggcatga gcctgggcgc caagggcgct 3180
gccggacccc tgcccagcga ggccgtgcag tggctgtgtc accaggcctt tctgctgaag 3240
ctgacccggc accgcgtgac ctacgtgccc ctgctgggaa gcctgcggac cgcccagacc 3300
cagctgagcc ggaagctgcc tggcaccacc ctgacagccc tggaggccgc tgccaacccc 3360
gccctgccta gcgacttcaa gaccatcctg gactctagag cccctcagag catcaccgag 3420
ctgtgcagcg agtaccggaa cacccagatt tacaccatca acgacaagat cctgagctac 3480
accgagtcta tggccggcaa gcgggagatg gtgatcatca ccttcaagag cggcgccacc 3540
tttcaggtgg aagtgcctgg cagccagcac atcgacagcc agaagaaggc catcgagcgg 3600
atgaaggaca ccctgcggat cacctacctg accgagacca agatcgacaa gctgtgtgtg 3660
tggaacaaca agacccccaa cagcatcgcc gccatctcta tggagaactg a          3711
```

<210> 6
<211> 1236
<212> PRT
<213> Artificial Sequence

<220>
<223> hTERT(AI)-LTBopt

<400> 6

```
Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser His
 1               5                  10                  15
Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly Pro
            20                  25                  30
Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg Ala
        35                  40                  45
Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro Pro
    50                  55                  60
Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu Val
65                  70                  75                  80
Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val Leu
                85                  90                  95
Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro Glu
            100                 105                 110
Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr Asp
        115                 120                 125
Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val Gly
    130                 135                 140
Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val Leu
145                 150                 155                 160
Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr Gln
                165                 170                 175
Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly Pro
            180                 185                 190
Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg Glu
        195                 200                 205
Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg Gly
    210                 215                 220
Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg Gly
225                 230                 235                 240
Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp Ala
                245                 250                 255
His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val Val
            260                 265                 270
```

```
Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala Leu
        275                 280                 285
Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His Ala
    290                 295                 300
Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro Cys
305                 310                 315                 320
Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly Asp
                325                 330                 335
Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro Ser
            340                 345                 350
Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser Arg
        355                 360                 365
Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln Arg
    370                 375                 380
Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His Ala
385                 390                 395                 400
Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg Ala
                405                 410                 415
Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln Gly
            420                 425                 430
Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu Val
        435                 440                 445
Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe Val
    450                 455                 460
Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser Arg
465                 470                 475                 480
His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser Leu
                485                 490                 495
Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met Ser
            500                 505                 510
Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys Val
        515                 520                 525
Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe Leu
    530                 535                 540
His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe Phe
545                 550                 555                 560
Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr Arg
                565                 570                 575

Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His Leu
            580                 585                 590
Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln His
        595                 600                 605
Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile Pro
    610                 615                 620
Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val Gly
625                 630                 635                 640
Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser Arg
                645                 650                 655
Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg Pro
            660                 665                 670
Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg Ala
        675                 680                 685
Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro Glu
    690                 695                 700
Leu Tyr Phe Val Lys Val Ala Ile Thr Gly Ala Tyr Asp Thr Ile Pro
705                 710                 715                 720
Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln Asn
                725                 730                 735
Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His Gly
            740                 745                 750
His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp Leu
        755                 760                 765
Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser Pro
    770                 775                 780
Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu Ala
785                 790                 795                 800
Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His Ala
                805                 810                 815
```

Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro Gln
820 825 830
Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp Met
835 840 845
Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu Arg
850 855 860
Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala Lys
865 870 875 880
Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys Val
885 890 895
Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu Ala
900 905 910
Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe Pro
915 920 925
Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser Asp
930 935 940
Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe Asn
945 950 955 960
Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly Val
965 970 975
Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn Ser
980 985 990
Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln Ala
995 1000 1005

Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln Val
1010 1015 1020
Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala Ser
1025 1030 1035 1040
Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu Gly
1045 1050 1055
Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp Leu
1060 1065 1070
Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr Tyr
1075 1080 1085
Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser Arg
1090 1095 1100
Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn Pro
1105 1110 1115 1120
Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp Ser Arg Ala Pro Gln
1125 1130 1135
Ser Ile Thr Glu Leu Cys Ser Glu Tyr Arg Asn Thr Gln Ile Tyr Thr
1140 1145 1150
Ile Asn Asp Lys Ile Leu Ser Tyr Thr Glu Ser Met Ala Gly Lys Arg
1155 1160 1165
Glu Met Val Ile Ile Thr Phe Lys Ser Gly Ala Thr Phe Gln Val Glu
1170 1175 1180
Val Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala Ile Glu Arg
1185 1190 1195 1200
Met Lys Asp Thr Leu Arg Ile Thr Tyr Leu Thr Glu Thr Lys Ile Asp
1205 1210 1215
Lys Leu Cys Val Trp Asn Asn Lys Thr Pro Asn Ser Ile Ala Ala Ile
1220 1225 1230
Ser Met Glu Asn
1235

<210> 7
<211> 3681
<212> DNA
<213> Artificial Sequence

<220>
<223> mTERT(AI)-LTBopt

<400> 7
accagagccc ccagatgccc tgccgtgaga agcctgctgc ggagccggta cagagaagtg 60
tggcccctgg ccacctttgt gaggagactg ggccctgagg gcaggagact ggtgcagcct 120
ggcgacccca aaatctacag gaccctggtg gcccagtgtc tggtgtgtat gcactggggc 180
agccagcccc ctcccgccga cctgagcttc caccaggtgt ccagcctgaa ggaactggtg 240

gccagagtgg tgcagagact gtgcgagcgg aacgagagaa acgtgctggc cttcggcttc 300
gagctgctga acgaggccag aggcggccct cccatggcct tcaccagctc tgtgaggagc 360
tacctgccca acaccgtgat cgagaccctg agagtgagcg gcgcctggat gctgctgctg 420
agcagagtgg gcgatgacct gctggtgtac ctgctggccc actgcgccct gtatctgctg 480
gtgccccccа gctgcgccta ccaggtgtgc ggatcccccc tgtaccagat ttgcgccacc 540
accgacatct ggcccagcgt gtctgccagc tacagaccca ccagacctgt gggccggaac 600
ttcaccaacc tgcggttcct gcagcagatc aagagcagca gcagacagga ggcccccaag 660
cccctggccc tgcccagcag aggcaccaag agacacctga gcctgaccag caccagcgtg 720
cccagcgcca agaaagccag atgctacccc gtgcctagag tggaggaggg ccctcacaga 780
caggtgctgc ccacccccag cggcaagagc tgggtgccca gccccgccag aagccccgaa 840
gtgcccaccg ccgagaagga cctgagcagc aagggcaaag tgagcgacct gtctctgagc 900
ggcagcgtgt gttgcaagca caagcccagc agcaccagcc tgctgagccc ccccagacag 960
aacgccttcc agctgaggcc tttcatcgag acccggcact tcctgtacag cagaggcgat 1020
ggccaggaga gactgaaccc cagcttcctg ctgagcaacc tgcagcctaa cctgaccggc 1080
gccagacgcc tggtggagat catcttcctg ggcagcagac ccagaaccag cggccctctg 1140
tgcagaaccc accggctgag caggcggtac tggcagatga gacccctgtt ccagcagctg 1200
ctggtgaacc acgccgagtg ccagtatgtg cggctgctga ggagccactg cagattcagg 1260
accgccaacc agcaggtgac cgacgccctg aacaccagcc cccctcacct gatggatctg 1320
ctgaggctgc acagcagccc ctggcaggtg tacggcttcc tgagagcctg cctgtgcaaa 1380
gtggtgtccg ccagcctgtg gggcaccaga cacaacgagc ggcggttctt caagaatctg 1440
aagaagttca tcagcctggg caagtacggc aagctgagcc tgcaggaact gatgtggaag 1500
atgaaagtgg aggactgcca ctggctgaga agcagccccg gcaaggacag agtgcctgcc 1560
gccgagcaca gactgaggga gagaatcctg gccacattcc tgttctggct gatggacacc 1620
tacgtggtgc agctgctgcg gtccttcttc tacatcaccg agagcacctt ccagaagaac 1680
cggctgttct tctaccggaa gtctgtgtgg agcaagctgc agagcatcgg agtgagacag 1740
cacctggaga gagtgaggct gagagagctg agccaggagg aagtgagaca ccaccaggat 1800
acctggctgg ccatgcccat ctgccggctg agattcatcc ccaagcccaa cggcctgaga 1860
cccatcgtga acatgagcta cagcatgggc acaagagccc tgggcagaag aaagcaggcc 1920
cagcacttca cccagcggct gaaaaccctg ttctccatgc tgaactacga gcggaccaag 1980
cacccacacc tgatgggcag cagcgtgctg ggcatgaacg acatctaccg gacctggaga 2040
gccttcgtgc tgagagtgcg ggccctggac cagacccctc ggatgtactt cgtgaaggcc 2100
gccatcaccg gcgcctacga cgccatcccc cagggcaaac tggtggaagt ggtggccaac 2160
atgatcaggc acagcgagtc cacctactgc atcaggcagt acgccgtggt gagaagagac 2220
agccagggcc aggtgcacaa gagcttccgg agacaggtga ccaccctgag cgatctgcag 2280
ccttacatgg gccagttcct gaagcacctg caggatagcg acgccagcgc cctgagaaat 2340
agcgtggtga tcgagcagag catcagcatg aacgagtcca gcagcagcct gttcgacttc 2400
ttcctgcact tcctgaggca cagcgtggtg aagatcggcg acagatgcta cacccagtgt 2460
cagggcatcc ctcagggctc tagcctgagc accctgctgt gtagcctgtg cttcggcgac 2520
atggagaata agctgttcgc cgaagtgcag agagatggcc tgctgctgcg cttcgtggac 2580
gatttcctgc tggtgacccc acacctggac caggccaaga ccttcctgag cacactggtg 2640
cacggcgtgc ccgagtacgg ctgcatgatc aatctgcaga aaaccgtggt gaacttccct 2700
gtggagcccg gcaccctggg cggagccgcc ccttaccagc tgcccgccca ctgcctgttc 2760
ccctggtgcg gactgctgct ggatacccag accctggaag tgttctgcga ctacagcggc 2820
tacgcccaga ccagcatcaa gaccagcctg accttccaga gcgtgttcaa ggccggcaag 2880
accatgagga acaagctgct gagcgtgctg agactgaagt gccacggcct gttcctggat 2940
ctgcaggtga acagcctgca gaccgtgtgt atcaacatct acaagatttt cctgctgcag 3000
gcctacagat tccacgcctg cgtgatccag ctgcccttcg accagagagt gcggaagaac 3060
ctgaccttct tcctggggat catcagcagc caggccagct gctgctacgc catcctgaaa 3120
gtgaagaacc ccggcatgac cctgaaggcc agcggcagct tccctcccga ggccgcccac 3180
tggctgtgct accaggcctt tctgctgaag ctggccgccc acagcgtgat ctacaagtgc 3240
ctgctgggcc ctctgagaac cgcccagaag ctgctgtgcc ggaagctgcc cgaggccacc 3300
atgaccattc tgaaagccgc cgccgacccc gccctgagca ccgacttcca gaccatcctg 3360
gactctagag cccctcagag catcaccgag ctgtgcagcg agtaccggaa cacccagatt 3420
tacaccatca acgacaagat cctgagctac accgagtcta tggccggcaa gcgggagatg 3480
gtgatcatca ccttcaagag cggcgccacc tttcaggtgg aagtgcctgg cagccagcac 3540
atcgacagcc agaagaaggc catcgagcgg atgaaggaca ccctgcggat cacctacctg 3600
accgagacca agatcgacaa gctgtgtgtg tggaacaaca agacccccaa cagcatcgcc 3660
gccatctcta tggagaactg a 3681

<210> 8
<211> 1232
<212> PRT
<213> Artificial Sequence

<220>
<223> mTERT(AI)-LTBopt

<400> 8
Thr Arg Ala Pro Arg Cys Pro Ala Val Arg Ser Leu Leu Arg Ser Arg

```
 1               5                  10                  15
Tyr Arg Glu Val Trp Pro Leu Ala Thr Phe Val Arg Arg Leu Gly Pro
            20                  25                  30
Glu Gly Arg Arg Leu Val Gln Pro Gly Asp Pro Lys Ile Tyr Arg Thr
        35                  40                  45
Leu Val Ala Gln Cys Leu Val Cys Met His Trp Gly Ser Gln Pro Pro
    50                  55                  60
Pro Ala Asp Leu Ser Phe His Gln Val Ser Ser Leu Lys Glu Leu Val
65                  70                  75                  80
Ala Arg Val Val Gln Arg Leu Cys Glu Arg Asn Glu Arg Asn Val Leu
                85                  90                  95
Ala Phe Gly Phe Glu Leu Leu Asn Glu Ala Arg Gly Gly Pro Pro Met
            100                 105                 110
Ala Phe Thr Ser Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Ile Glu
        115                 120                 125
Thr Leu Arg Val Ser Gly Ala Trp Met Leu Leu Leu Ser Arg Val Gly
    130                 135                 140
Asp Asp Leu Leu Val Tyr Leu Leu Ala His Cys Ala Leu Tyr Leu Leu
145                 150                 155                 160
Val Pro Pro Ser Cys Ala Tyr Gln Val Cys Gly Ser Pro Leu Tyr Gln
                165                 170                 175
Ile Cys Ala Thr Thr Asp Ile Trp Pro Ser Val Ser Ala Ser Tyr Arg
            180                 185                 190
Pro Thr Arg Pro Val Gly Arg Asn Phe Thr Asn Leu Arg Phe Leu Gln
        195                 200                 205
Gln Ile Lys Ser Ser Ser Arg Gln Glu Ala Pro Lys Pro Leu Ala Leu
    210                 215                 220
Pro Ser Arg Gly Thr Lys Arg His Leu Ser Leu Thr Ser Thr Ser Val
225                 230                 235                 240
Pro Ser Ala Lys Lys Ala Arg Cys Tyr Pro Val Pro Arg Val Glu Glu
                245                 250                 255
Gly Pro His Arg Gln Val Leu Pro Thr Pro Ser Gly Lys Ser Trp Val
            260                 265                 270
Pro Ser Pro Ala Arg Ser Pro Glu Val Pro Thr Ala Glu Lys Asp Leu
        275                 280                 285
Ser Ser Lys Gly Lys Val Ser Asp Leu Ser Leu Ser Gly Ser Val Cys
    290                 295                 300
Cys Lys His Lys Pro Ser Ser Thr Ser Leu Leu Ser Pro Pro Arg Gln
305                 310                 315                 320
Asn Ala Phe Gln Leu Arg Pro Phe Ile Glu Thr Arg His Phe Leu Tyr
                325                 330                 335
Ser Arg Gly Asp Gly Gln Glu Arg Leu Asn Pro Ser Phe Leu Leu Ser
            340                 345                 350
Asn Leu Gln Pro Asn Leu Thr Gly Ala Arg Arg Leu Val Glu Ile Ile
        355                 360                 365
Phe Leu Gly Ser Arg Pro Arg Thr Ser Gly Pro Leu Cys Arg Thr His
    370                 375                 380
Arg Leu Ser Arg Arg Tyr Trp Gln Met Arg Pro Leu Phe Gln Gln Leu
385                 390                 395                 400
Leu Val Asn His Ala Glu Cys Gln Tyr Val Arg Leu Leu Arg Ser His
                405                 410                 415

Cys Arg Phe Arg Thr Ala Asn Gln Gln Val Thr Asp Ala Leu Asn Thr
            420                 425                 430
Ser Pro Pro His Leu Met Asp Leu Leu Arg Leu His Ser Ser Pro Trp
        435                 440                 445
Gln Val Tyr Gly Phe Leu Arg Ala Cys Leu Cys Lys Val Val Ser Ala
    450                 455                 460
Ser Leu Trp Gly Thr Arg His Asn Glu Arg Arg Phe Phe Lys Asn Leu
465                 470                 475                 480
Lys Lys Phe Ile Ser Leu Gly Lys Tyr Gly Lys Leu Ser Leu Gln Glu
                485                 490                 495
Leu Met Trp Lys Met Lys Val Glu Asp Cys His Trp Leu Arg Ser Ser
            500                 505                 510
Pro Gly Lys Asp Arg Val Pro Ala Ala Glu His Arg Leu Arg Glu Arg
        515                 520                 525
Ile Leu Ala Thr Phe Leu Phe Trp Leu Met Asp Thr Tyr Val Val Gln
    530                 535                 540
Leu Leu Arg Ser Phe Phe Tyr Ile Thr Glu Ser Thr Phe Gln Lys Asn
```

545 550 555 560
Arg Leu Phe Phe Tyr Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile
565 570 575
Gly Val Arg Gln His Leu Glu Arg Val Arg Leu Arg Glu Leu Ser Gln
580 585 590
Glu Glu Val Arg His His Gln Asp Thr Trp Leu Ala Met Pro Ile Cys
595 600 605
Arg Leu Arg Phe Ile Pro Lys Pro Asn Gly Leu Arg Pro Ile Val Asn
610 615 620
Met Ser Tyr Ser Met Gly Thr Arg Ala Leu Gly Arg Arg Lys Gln Ala
625 630 635 640
Gln His Phe Thr Gln Arg Leu Lys Thr Leu Phe Ser Met Leu Asn Tyr
645 650 655
Glu Arg Thr Lys His Pro His Leu Met Gly Ser Ser Val Leu Gly Met
660 665 670
Asn Asp Ile Tyr Arg Thr Trp Arg Ala Phe Val Leu Arg Val Arg Ala
675 680 685
Leu Asp Gln Thr Pro Arg Met Tyr Phe Val Lys Ala Ala Ile Thr Gly
690 695 700
Ala Tyr Asp Ala Ile Pro Gln Gly Lys Leu Val Glu Val Val Ala Asn
705 710 715 720
Met Ile Arg His Ser Glu Ser Thr Tyr Cys Ile Arg Gln Tyr Ala Val
725 730 735
Val Arg Arg Asp Ser Gln Gly Gln Val His Lys Ser Phe Arg Arg Gln
740 745 750
Val Thr Thr Leu Ser Asp Leu Gln Pro Tyr Met Gly Gln Phe Leu Lys
755 760 765
His Leu Gln Asp Ser Asp Ala Ser Ala Leu Arg Asn Ser Val Val Ile
770 775 780
Glu Gln Ser Ile Ser Met Asn Glu Ser Ser Ser Ser Leu Phe Asp Phe
785 790 795 800
Phe Leu His Phe Leu Arg His Ser Val Val Lys Ile Gly Asp Arg Cys
805 810 815
Tyr Thr Gln Cys Gln Gly Ile Pro Gln Gly Ser Ser Leu Ser Thr Leu
820 825 830
Leu Cys Ser Leu Cys Phe Gly Asp Met Glu Asn Lys Leu Phe Ala Glu
835 840 845

Val Gln Arg Asp Gly Leu Leu Leu Arg Phe Val Asp Asp Phe Leu Leu
850 855 860
Val Thr Pro His Leu Asp Gln Ala Lys Thr Phe Leu Ser Thr Leu Val
865 870 875 880
His Gly Val Pro Glu Tyr Gly Cys Met Ile Asn Leu Gln Lys Thr Val
885 890 895
Val Asn Phe Pro Val Glu Pro Gly Thr Leu Gly Gly Ala Ala Pro Tyr
900 905 910
Gln Leu Pro Ala His Cys Leu Phe Pro Trp Cys Gly Leu Leu Leu Asp
915 920 925
Thr Gln Thr Leu Glu Val Phe Cys Asp Tyr Ser Gly Tyr Ala Gln Thr
930 935 940
Ser Ile Lys Thr Ser Leu Thr Phe Gln Ser Val Phe Lys Ala Gly Lys
945 950 955 960
Thr Met Arg Asn Lys Leu Leu Ser Val Leu Arg Leu Lys Cys His Gly
965 970 975
Leu Phe Leu Asp Leu Gln Val Asn Ser Leu Gln Thr Val Cys Ile Asn
980 985 990
Ile Tyr Lys Ile Phe Leu Leu Gln Ala Tyr Arg Phe His Ala Cys Val
995 1000 1005
Ile Gln Leu Pro Phe Asp Gln Arg Val Arg Lys Asn Leu Thr Phe Phe
1010 1015 1020
Leu Gly Ile Ile Ser Ser Gln Ala Ser Cys Cys Tyr Ala Ile Leu Lys
1025 1030 1035 1040
Val Lys Asn Pro Gly Met Thr Leu Lys Ala Ser Gly Ser Phe Pro Pro
1045 1050 1055
Glu Ala Ala His Trp Leu Cys Tyr Gln Ala Phe Leu Leu Lys Leu Ala
1060 1065 1070
Ala His Ser Val Ile Tyr Lys Cys Leu Leu Gly Pro Leu Arg Thr Ala
1075 1080 1085
Gln Lys Leu Leu Cys Arg Lys Leu Pro Glu Ala Thr Met Thr Ile Leu

```
    1090                1095                1100
Lys Ala Ala Ala Asp Pro Ala Leu Ser Thr Asp Phe Gln Thr Ile Leu
1105                1110                1115                1120
Asp Ser Arg Ala Pro Gln Ser Ile Thr Glu Leu Cys Ser Glu Tyr Arg
                1125                1130                1135
Asn Thr Gln Ile Tyr Thr Ile Asn Asp Lys Ile Leu Ser Tyr Thr Glu
            1140                1145                1150
Ser Met Ala Gly Lys Arg Glu Met Val Ile Ile Thr Phe Lys Ser Gly
        1155                1160                1165
Ala Thr Phe Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln
    1170                1175                1180
Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Thr Tyr Leu
1185                1190                1195                1200
Thr Glu Thr Lys Ile Asp Lys Leu Cys Val Trp Asn Asn Lys Thr Pro
                1205                1210                1215
Asn Ser Ile Ala Ala Ile Ser Met Glu Asn Ser Glu Gln Ile Asp Asn
            1220                1225                1230
```

<210> 9
<211> 3393
<212> DNA
<213> Artificial Sequence

<220>
<223> hTERT(AI)

<400> 9

```
cccagagccc ccagatgcag agccgtgcgg agcctgctgc ggagccacta ccgggaagtg 60
ctgcccctgg ccaccttcgt gcggcggctg ggccccgagg gctggcggct ggtgcagcgg 120
ggcgaccctg ccgccttccg ggccctggtg gctcagtgcc tggtgtgcgt gccctgggac 180
gccagacccc ccccagccgc ccctagcttc cggcaggtga gctgcctgaa ggaactggtg 240
gccagagtgc tgcagcggct gtgcgagaga ggcgccaaga acgtgctggc cttcggcttc 300
gccctgctgg acggcgccag aggcggccct cccgaggcct tcaccacaag cgtgcggagc 360
tacctgccca acaccgtgac cgacgccctg cggggcagcg gcgcctgggg cctgctgctg 420
agaagagtgg gcgacgacgt gctggtgcac ctgctggccc ggtgcgccct gttcgtgctg 480
gtggccccca gctgcgccta ccaggtgtgc ggcccacccc tgtaccagct gggagccgcc 540
acccaggcca ggccccccacc ccacgccagc ggccccagac ggagactggg ctgcgagcgg 600
gcctggaacc acagcgtgag agaggccggc gtgcccctgg gcctgccagc ccctggcgcc 660
agaagaagag gcggcagcgc cagccggagc ctgcccctgc ccaagcggcc cagaagaggc 720
gctgcccccg agcccgagcg gaccccgtg ggccagggca gctgggccca ccccggcaga 780
accagaggcc ccagcgaccg gggcttctgc gtggtgagcc ccgccagacc cgccgaggag 840
gccacaagcc tggagggcgc cctgagcggc acccggcaca gccaccccag cgtgggccgg 900
cagcaccacg ccggaccccc cagcaccagc agacccccca gaccctggga cacccccctgc 960
ccccctgtgt acgccgagac caagcacttc ctgtacagca gcggcgacaa ggagcagctg 1020
cggcccagct tcctgctgag cagcctgaga cccagcctga ccggcgccag gagactggtg 1080
gagaccatct tcctgggcag ccggccctgg atgcccggca ccccccggag actgccccgg 1140
ctgccccagc ggtactggca gatgcggccc ctgttcctgg agctgctggg caaccacgcc 1200
cagtgccccct acggcgtgct gctgaaaacc cactgccccc tgagagccgc cgtgacccccc 1260
gctgccggcg tgtgcgccag agagaagccc cagggcagcg tggccgctcc cgaggaggag 1320
gacaccgacc ccagacgcct ggtgcagctg ctgcggcagc acagcagccc ttggcaggtg 1380
tacggcttcg tgcgggcctg cctgagaagg ctggtgcccc ctggcctgtg gggcagcaga 1440
cacaacgagc ggcggttcct gcggaacacc aagaagttca tcagcctggg gaagcacgcc 1500
aagctgagcc tgcaggaact gacctggaag atgagcgtgc gggactgcgc ctggctgcgg 1560
cggagccctg gcgtgggctg cgtgccagcc gccgagcacc ggctgcggga ggagatcctg 1620
gccaagttcc tgcactggct gatgagcgtg tacgtggtgg aactgctgcg gtccttcttc 1680
tacgtgaccg aaaccacctt ccagaagaac cggctgttct tctaccggaa gagcgtgtgg 1740
agcaagctgc agagcatcgg catcaggcag cacctgaaga gagtgcagct gcgggagctg 1800
agcgaggccg aagtgagaca gcaccgggag gccagacctg ccctgctgac cagccggctg 1860
cggttcatcc ccaagcccga cggcctgcgg cccatcgtga acatggacta cgtggtgggc 1920
gccagaacct tccggcggga gaagcgggcc gagcggctga ccagcagagt gaaggccctg 1980
ttcagcgtgc tgaactacga gcgggccagg agacccggcc tgctgggcgc cagcgtgctg 2040
ggcctggacg acatccaccg ggcctggcgg accttcgtgc tgagagtgcg ggcccaggac 2100
cccccacccg agctgtactt cgtgaaagtg gccatcaccg gcgcctacga caccatcccc 2160
caggaccggc tgaccgaagt gatcgccagc atcatcaagc cccagaacac ctactgcgtg 2220
cggcggtacg ccgtggtgca gaaggccgcc cacggccacg tgcggaaggc cttcaagagc 2280
cacgtgagca ccctgaccga cctgcagccc tacatgcggc agttcgtggc ccacctgcag 2340
gagaccagcc ccctgcggga tgccgtggtg atcgagcaga gcagcagcct gaacgaggcc 2400
agcagcggcc tgttcgacgt gttcctgcgc ttcatgtgcc accacgccgt gcggatccgg 2460
ggcaagagct acgtgcagtg ccagggcatc cctcagggca gcatcctgag cacactgctg 2520
```

tgctctctgt gctacggcga catggagaac aagctgttcg ccggcatccg gcgggacgga 2580
ctgctgctgc gcctggtgga cgacttcctg ctggtgaccc ctcacctgac ccacgccaag 2640
accttcctgc ggaccctggt gcggggcgtg cccgagtacg gctgtgtggt gaacctgcgc 2700
aagaccgtgg tgaacttccc cgtggaggac gaggccctgg gcggcacagc cttcgtgcag 2760
atgcccgccc atggcctgtt cccttggtgc gggctgctgc tggacacccg gaccctggaa 2820
gtgcagagcg actacagcag ctacgcccgg accagcatcc gggccagcct gacattcaac 2880
cgcggcttca aggccggcag aaacatgcgg cggaagctgt ttggcgtgct gcggctgaag 2940
tgccacagcc tgtttctgga cctgcaggtg aacagcctgc agaccgtgtg caccaacatc 3000
tacaagatcc tgctgctgca ggcctaccgg ttccacgcct gcgtgctgca gctgcccttc 3060
catcagcagg tgtggaagaa ccccaccttc ttcctgcgcg tgatctctga caccgccagc 3120
ctgtgctaca gcattctgaa ggccaagaac gccggcatga gcctgggcgc caagggcgct 3180
gccggacccc tgcccagcga ggccgtgcag tggctgtgtc accaggcctt tctgctgaag 3240
ctgacccggc accgcgtgac ctacgtgccc ctgctgggaa gcctgcggac cgcccagacc 3300
cagctgagcc ggaagctgcc tggcaccacc ctgacagccc tggaggccgc tgccaacccc 3360
gccctgccta gcgacttcaa gaccatcctg gac 3393

<210> 10
<211> 1137
<212> PRT
<213> Artificial Sequence

<220>
<223> hTERT(AI)

<400> 10
Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser His
 1   5   10   15
Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly Pro
            20   25   30
Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg Ala
        35   40   45
Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro Pro
    50   55   60
Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu Val
65   70   75   80
Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val Leu
                85   90   95
Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro Glu
            100   105   110
Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr Asp
        115   120   125
Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val Gly
    130   135   140
Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val Leu
145   150   155   160
Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr Gln
                165   170   175
Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly Pro
            180   185   190
Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg Glu
        195   200   205
Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg Gly
    210   215   220
Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg Gly
225   230   235   240
Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp Ala
                245   250   255
His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val Val
            260   265   270
Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala Leu
        275   280   285
Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His Ala
    290   295   300
Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro Cys
305   310   315   320
Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly Asp
                325   330   335
Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro Ser
            340   345   350

```
Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser Arg
        355                 360                 365
Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln Arg
    370                 375                 380
Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His Ala
385                 390                 395                 400
Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg Ala
                405                 410                 415
Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln Gly
            420                 425                 430
Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu Val
        435                 440                 445
Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe Val
    450                 455                 460
Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser Arg
465                 470                 475                 480
His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser Leu
                485                 490                 495
Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met Ser
            500                 505                 510
Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys Val
        515                 520                 525
Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe Leu
    530                 535                 540
His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe Phe
545                 550                 555                 560
Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr Arg
                565                 570                 575
Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His Leu
            580                 585                 590
Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln His
        595                 600                 605
Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile Pro
    610                 615                 620
Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val Gly
625                 630                 635                 640
Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser Arg
                645                 650                 655
Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg Pro
            660                 665                 670
Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg Ala
        675                 680                 685
Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro Glu
    690                 695                 700
Leu Tyr Phe Val Lys Val Ala Ile Thr Gly Ala Tyr Asp Thr Ile Pro
705                 710                 715                 720
Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln Asn
                725                 730                 735
Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His Gly
            740                 745                 750

His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp Leu
        755                 760                 765
Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser Pro
    770                 775                 780
Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu Ala
785                 790                 795                 800
Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His Ala
                805                 810                 815
Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro Gln
            820                 825                 830
Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp Met
        835                 840                 845
Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu Arg
    850                 855                 860
Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala Lys
865                 870                 875                 880
Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys Val
                885                 890                 895
```

```
Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu Ala
            900                 905                 910
Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe Pro
        915                 920                 925
Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser Asp
    930                 935                 940
Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe Asn
945                 950                 955                 960
Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly Val
                965                 970                 975
Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn Ser
            980                 985                 990
Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln Ala
        995                 1000                1005
Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln Val
    1010                1015                1020
Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala Ser
1025                1030                1035                1040
Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu Gly
                1045                1050                1055
Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp Leu
            1060                1065                1070
Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr Tyr
        1075                1080                1085
Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser Arg
    1090                1095                1100
Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn Pro
1105                1110                1115                1120
Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp Ser Glu Gln Ile Asp
                1125                1130                1135
Asn
```

<210> 11
<211> 3399
<212> DNA
<213> Homo Sapiens

<400> 11

```
atgccgcgcg ctccccgctg ccgagccgtg cgctccctgc tgcgcagcca ctaccgcgag 60
gtgctgccgc tggccacgtt cgtgcggcgc ctggggcccc agggctggcg gctggtgcag 120
cgcggggacc cggcggcttt ccgcgcgctg gtggcccagt gcctggtgtg cgtgccctgg 180
gacgcacggc cgccccccgc cgcccctctcc ttccgccagg tgtcctgcct gaaggagctg 240
gtggcccgag tgctgcagag gctgtgcgag cgcggcgcga agaacgtgct ggccttcggc 300
ttcgcgctgc tggacggggc ccgcgggggc ccccccgagg ccttcaccac cagcgtgcgc 360
agctacctgc ccaacacggt gaccgacgca ctgcgggggа gcggggcgtg ggggctgctg 420
ctgcgccgcg tgggcgacga cgtgctggtt cacctgctgg cacgctgcgc gctctttgtg 480
ctggtggctc ccagctgcgc ctaccaggtg tgcgggccgc cgctgtacca gctcggcgct 540
gccactcagg cccggccccc gccacacgct agtggacccc gaaggcgtct gggatgcgaa 600
cgggcctgga accatagcgt cagggaggcc ggggtccccc tgggcctgcc agccccgggt 660
gcgaggaggc gcgggggcag tgccagccga agtctgccgt tgcccaagag gcccaggcgt 720
ggcgctgccc ctgagccgga gcggacgccc gttgggcagg ggtcctgggc ccacccgggc 780
aggacgcgtg gaccgagtga ccgtggtttc tgtgtggtgt cacctgccag acccgccgaa 840
gaagccacct ctttggaggg tgcgctctct ggcacgcgcc actcccaccc atccgtgggc 900
cgccagcacc acgcgggccc cccatccaca tcgcggccac cacgtccctg ggacacgcct 960
tgtcccccgg tgtacgccga gaccaagcac ttcctctact cctcaggcga caaggagcag 1020
ctgcggccct ccttcctact cagctctctg aggcccagcc tgactggcgc tcggaggctc 1080
gtggagacca tctttctggg ttccaggccc tggatgccag ggactccccg caggttgccc 1140
cgcctgcccc agcgctactg gcaaatgcgg cccctgtttc tggagctgct tgggaaccac 1200
gcgcagtgcc cctacggggt gctcctcaag acgcactgcc cgctgcgagc tgcggtcacc 1260
ccagcagccg gtgtctgtgc ccgggagaag ccccagggct ctgtggcggc ccccgaggag 1320
gaggacacag acccccgtcg cctggtgcag ctgctccgcc agcacagcag cccctggcag 1380
gtgtacggct tcgtgcgggc ctgcctgcgc cggctggtgc ccccaggcct ctggggctcc 1440
aggcacaacg aacgccgctt cctcaggaac accaagaagt tcatctccct ggggaagcat 1500
gccaagctct cgctgcagga gctgacgtgg aagatgagcg tgcgggactg cgcttggctg 1560
cgcaggagcc caggggttgg ctgtgttccg gccgcagagc accgtctgcg tgaggagatc 1620
ctggccaagt tcctgcactg gctgatgagt gtgtacgtcg tcgagctgct caggtctttc 1680
ttttatgtca cggagaccac gtttcaaaag aacaggctct ttttctaccg gaagagtgtc 1740
tggagcaagt tgcaaagcat tggaatcaga cagcacttga agagggtgca gctgcgggag 1800
```

```
ctgtcggaag cagaggtcag gcagcatcgg gaagccaggc ccgccctgct gacgtccaga 1860
ctccgcttca tccccaagcc tgacgggctg cggccgattg tgaacatgga ctacgtcgtg 1920
ggagccagaa cgttccgcag agaaaagagg gccgagcgtc tcacctcgag ggtgaaggca 1980
ctgttcagcg tgctcaacta cgagcgggcg cggcgccccg gcctcctggg cgcctctgtg 2040
ctgggcctgg acgatatcca cagggcctgg cgcaccttcg tgctgcgtgt gcgggcccag 2100
gacccgccgc ctgagctgta ctttgtcaag gtggatgtga cgggcgcgta cgacaccatc 2160
ccccaggaca ggctcacgga ggtcatcgcc agcatcatca aaccccagaa cacgtactgc 2220
gtgcgtcggt atgccgtggt ccagaaggcc gcccatgggc acgtccgcaa ggccttcaag 2280
agccacgtct ctaccttgac agacctccag ccgtacatgc gacagttcgt ggctcacctg 2340
caggagacca gcccgctgag ggatgccgtc gtcatcgagc agagctcctc cctgaatgag 2400
gccagcagtg gcctcttcga cgtcttccta cgcttcatgt gccaccacgc cgtgcgcatc 2460
aggggcaagt cctacgtcca gtgccagggg atcccgcagg gctccatcct ctccacgctg 2520
ctctgcagcc tgtgctacgg cgacatggag aacaagctgt ttgcggggat tcggcgggac 2580
gggctgctcc tgcgtttggt ggatgatttc ttgttggtga cacctcacct cacccacgcg 2640
aaaaccttcc tcaggaccct ggtccgaggt gtccctgagt atggctgcgt ggtgaacttg 2700
cggaagacag tggtgaactt ccctgtagaa gacgaggccc tgggtggcac ggcttttgtt 2760
cagatgccgg cccacggcct attcccctgg tgcggcctgc tgctggatac ccggaccctg 2820
gaggtgcaga gcgactactc cagctatgcc cggacctcca tcagagccag tctcaccttc 2880
aaccgcggct tcaaggctgg gaggaacatg cgtcgcaaac tctttggggt cttgcggctg 2940
aagtgtcaca gcctgtttct ggatttgcag gtgaacagcc tccagacggt gtgcaccaac 3000
atctacaaga tcctcctgct gcaggcgtac aggtttcacg catgtgtgct gcagctccca 3060
tttcatcagc aagtttggaa gaaccccaca tttttcctgc gcgtcatctc tgacacggcc 3120
tccctctgct actccatcct gaaagccaag aacgcaggga tgtcgctggg ggccaagggc 3180
gccgccggcc ctctgccctc cgaggccgtg cagtggctgt gccaccaagc attcctgctc 3240
aagctgactc gacaccgtgt cacctacgtg ccactcctgg ggtcactcag gacagcccag 3300
acgcagctga gtcggaagct cccggggacg acgctgactg ccctggaggc cgcagccaac 3360
ccggcactgc cctcagactt caagaccatc ctggactga                         3399
```

<210> 12
<211> 1132
<212> PRT
<213> Homo Sapiens

<400> 12

```
Met Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser
 1               5                  10                  15
His Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly
            20                  25                  30
Pro Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg
        35                  40                  45
Ala Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro
    50                  55                  60
Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu
65                  70                  75                  80
Val Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val
                85                  90                  95
Leu Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro
            100                 105                 110
Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr
        115                 120                 125
Asp Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val
    130                 135                 140
Gly Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val
145                 150                 155                 160
Leu Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr
                165                 170                 175
Gln Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly
            180                 185                 190
Pro Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg
        195                 200                 205
Glu Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg
    210                 215                 220
Gly Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg
225                 230                 235                 240
Gly Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp
                245                 250                 255
Ala His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val
            260                 265                 270
Val Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala
```

```
        275                 280                 285
Leu Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His
    290                 295                 300
Ala Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro
305                 310                 315                 320
Cys Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly
                325                 330                 335
Asp Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro
            340                 345                 350
Ser Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser
        355                 360                 365
Arg Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln
    370                 375                 380
Arg Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His
385                 390                 395                 400
Ala Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg
                405                 410                 415
Ala Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln
            420                 425                 430
Gly Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu
        435                 440                 445
Val Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe
    450                 455                 460
Val Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser
465                 470                 475                 480
Arg His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser
                485                 490                 495
Leu Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met
            500                 505                 510
Ser Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys
        515                 520                 525
Val Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe
    530                 535                 540
Leu His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe
545                 550                 555                 560
Phe Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr
                565                 570                 575
Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His
            580                 585                 590
Leu Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln
        595                 600                 605
His Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile
    610                 615                 620
Pro Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val
625                 630                 635                 640
Gly Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser
                645                 650                 655
Arg Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg
            660                 665                 670
Pro Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg
        675                 680                 685
Ala Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro
    690                 695                 700
Glu Leu Tyr Phe Val Lys Val Asp Val Thr Gly Ala Tyr Asp Thr Ile
705                 710                 715                 720
Pro Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln
                725                 730                 735
Asn Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His
            740                 745                 750
Gly His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp
        755                 760                 765
Leu Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser
    770                 775                 780

Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu
785                 790                 795                 800
Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His
                805                 810                 815
Ala Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro
```

820 825 830
Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp
835 840 845
Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu
850 855 860
Arg Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala
865 870 875 880
Lys Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys
885 890 895
Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
900 905 910
Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
915 920 925
Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
930 935 940
Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945 950 955 960
Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
965 970 975
Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
980 985 990
Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
995 1000 1005
Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
1010 1015 1020
Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025 1030 1035 1040
Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
1045 1050 1055
Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
1060 1065 1070
Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
1075 1080 1085
Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
1090 1095 1100
Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105 1110 1115 1120
Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
1125 1130

<210> 13
<211> 309
<212> DNA
<213> E. coli

<400> 13
gcccctcaga gcatcaccga gctgtgcagc gagtaccgga acacccagat ttacaccatc 60
aacgacaaga tcctgagcta caccgagtct atggccggca agcgggagat ggtgatcatc 120
accttcaaga gcggcgccac ctttcaggtg gaagtgcctg gcagccagca catcgacagc 180
cagaagaagg ccatcgagcg gatgaaggac accctgcgga tcacctacct gaccgagacc 240
aagatcgaca agctgtgtgt gtggaacaac aagaccccca acagcatcgc cgccatctct 300
atggagaac 309

<210> 14
<211> 109
<212> PRT
<213> E. coli

<400> 14
Ala Pro Gln Ser Ile Thr Glu Leu Cys Ser Glu Tyr Arg Asn Thr Gln
1 5 10 15
Ile Tyr Thr Ile Asn Asp Lys Ile Leu Ser Tyr Thr Glu Ser Met Ala
20 25 30
Gly Lys Arg Glu Met Val Ile Ile Thr Phe Lys Ser Gly Ala Thr Phe
35 40 45
Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala
50 55 60
Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Thr Tyr Leu Thr Glu Thr
65 70 75 80

Lys Ile Asp Lys Leu Cys Val Trp Asn Asn Lys Thr Pro Asn Ser Ile
85 90 95
Ala Ala Ile Ser Met Glu Asn Ser Glu Gln Ile Asp Asn
100 105

<210> 15
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 15
gatctgatga tatcgccacc atgaccagag cccccagatg 40

<210> 16
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 16
agggggatc cgcacacctg gtaggcgcag ctgggg 36

<210> 17
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 17
ctgtactttg tcaaggtggc tatcacgggc gcgtacg 37

<210> 18
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 18
Leu Val Pro Pro Ser Cys Ala Tyr Gln Val Cys Gly Ser Pro Leu
1 5 10 15

<210> 19
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 19
Ser Cys Ala Tyr Gln Val Cys Gly Ser Pro Leu Tyr Gln Ile Cys
1 5 10 15

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 20
Ala Tyr Gln Val Cys Gly Ser Pro Leu
1 5

<210> 21
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 21
Val Gly Arg Asn Phe Thr Asn Leu
1 5

<210> 22
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 22
Ser Leu Gly Lys Tyr Gly Lys Leu
1 5

<210> 23
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide

<400> 23
Arg Leu Val Asp Asp Phe Leu Leu Val
1 5

??

??

??

??

ITR0119Y