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(54) Title: TUMOR-SPECIFIC EXPRESSION CASSETTE AND USES THEREOF

(57) Abstract: Provided herein is an expression cassette comprising: 1) a chimeric promoter, comprising an hTERT promoter and a minimal promoter; 2) a coding sequence (CDS); and 3) a poly (A) sequence or a polyadenylation signal sequence. The CDS sequence in the expression cassette is expressed only in tumor or cancer cells or is expressed at a much higher level in tumor or cancer cells than that in normal cells, and thus the expression cassette can be used in the treatment of cancer.

# TUMOR-SPECIFIC EXPRESSION CASSETTE AND USES THEREOF

## CROSS REFERENCE TO RELATED APPLICATION

This application claims priority from PCT international application PCT/CN2023/100812 filed on June 16, 2023, which is incorporated herein by reference in its entirety.

## TECHNICAL FIELD

The present invention relates to a tumor-specific expression vector, particularly to hybrid promoters to drive gene expression efficiently and specifically in cancer cells. The invention further relates to expression cassettes and vectors containing the hybrid promoter and other transcriptional cis-elements. The present invention also relates to the use of the vector in detection, diagnosis or treatment of various cancers.

## BACKGROUND AND INVENTION

One strategy of gene therapy against cancer involves new treatment modality that introduces genes into cancerous cell or surrounding tissue to cause cell death or slow the growth of the cancer. For identifying cancer specific gene profiling, transcriptomes of various cancer cells have been studied in these decades. Alteration in transcriptional gene pattern is well recognized essential for uncontrolled proliferation and escaping programmed cell death in cancer cells. Dysregulated transcriptional machine usually consists of overexpression, defection of degeneration and hyperactivity of pro-oncogenic transcription factors and mutation of tumor suppressive ones. Ideally, cancer-specific killing could be achieved by delivering therapeutic genes under the control of cis elements that can be interacted and activated by overexpressed or constitutively activated tumoral transcription factors. In topical medication or systemic delivery of anti-cancer gene molecules, it is a major problem whether the expression of therapeutic genes is controlled by tumor-specific promoters, which determines the success of cancer gene therapy.

Tumor specific promoters are considered promising genetic tools for cancer treatment. Well used tumor specific promoters including alpha-fetoprotein promoter (AFP), thyroid transcription factor 1 (TTF-1), glypican-3 protein (GPC3), human

secretory leukocyte protease inhibitor (hSLPI), ERBB2, Mucin 1 (MUC1), L-plastin, α lactalbumin (LALBA), cyclooxygenase 2 (COX2), epithelial glycoprotein (EPG2), A33, uPAR, carcinoembryonic antigen (CEA), breast cancer 1 (BRCA1), BRCA2 and survivin. To a large extent, the good tumor selectivity of these promoters ensures that gene expression in malignant processes, but not specificity for certain types of tissues. Nevertheless, these naive tumor-specific promoters are active in a limited class of cancer cells or the transcriptional activity varies widely in different tumors. For example, the activity of the human survivin promoter ranges from 0.3% to 16% of that of the CMV promoter. How to efficiently express therapeutic gene in cancer cells is the bottleneck for tumor targeting gene therapy.

Increased studies indicated that efficiency of tumor-specific promoters can be improved by engineering promoter transcription factor binding sites and hybrid with cis elements containing binding motif of other transcription factors. Hybrid promoters may include combinations of known promoters with each other or with certain heterologous regulatory elements to increase the strength and specificity of expression in cancer cells. Typically, researchers creating hybrid promoters prefer to maximize the efficiency and specificity of expression in a particular type of cancer cell. The advantage of using promoters and other regulatory elements that are strictly specific for a certain type of tumor is the minimization of side effects that occur due to reduced expression of the therapeutic gene in normal tissues. However, the disadvantage of such approaches is their non-universal nature.

Limitless self-renewal is one of the hallmarks of cancer and is attained by telomere maintenance, for which the activity of telomerase consisted of non-coding RNA component hTR and the catalytic subunit hTERT is essential. Consistently, hTERT expression is identified in almost 90% of human primary cancers. However, in normal tissues, hTERT was only detected in spermatogonia, colon cryptic stem or progenitor cells, and some transitional epithelial basal cells, but not in major functional organs, such as liver kidney muscle, or placenta. Similar with most naïve tumor specific promoters, hTERT shows varied expression activity in different types of tumors. In general, advanced cancer cells frequently present higher telomerase activity and expression level. Some cancers in early stages only show weak transcriptional activity of hTERT, which may be resulted from lower level of oncogenic transcriptional factors, such as c-Myc and ETS in these cells.

Reactive oxygen species (ROS) has been studied widely in past half century and considered playing an important role in oncogenesis. In normal cell, excess cellular levels of ROS cause damage to proteins, nucleic acids, lipids, membranes and organelles, which can lead to activation of cell death processes such as apoptosis. Interestingly, high level ROS and limited apoptosis is observed in various cancer cell, suggesting tumor specific molecular mechanism protects cancer cells from ROS-induced apoptosis. The transcription factor NRF2 is well studied master regulator of the cellular antioxidant response. NRF2 expression is often enhanced in various tumor types, including lung, breast, colon, and ovarian cancer, high NRF2 level plays a critical role in tumor cell growth and chemoresistance by elevating reactive oxygen species (ROS) inhibiting enzymes, detoxifying factors, anti-apoptotic proteins and drug transporters. NRF2 regulates these genes via binding on cis-elements named anti-oxidative response element, AREs. Identically, these genes with AREs are upregulated in cancers and involve to oncogenesis.

## SUMMARY OF THE INVENTION

To overcome bottleneck of expression efficiency and specificity for cancer therapy, we engineered and generated series hybrid promoters, consisting of anti-oxidative response elements, trimmed hTERT and other tumor specific core promoters and post-poly (A) enhancers.

In one aspect, the present disclosure provides an expression cassette, comprising:

1) a chimeric promoter, comprising an hTERT promoter and a minimal promoter;
2) a coding sequence (CDS); and
3) a poly(A) sequence or a polyadenylation signal sequence.

In some embodiments, the hTERT promoter is an hTERT core promoter, preferably comprising at least one of the sequences of SEQ ID NOs: 1-7 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the minimal promoter is a minimal viral promoter or a minimal TATA box promoter.

In some embodiments, the minimal promoter is a minimal CMV promoter, preferably comprising the sequence of SEQ ID NO: 16 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the minimal TATA box promoter is an E1F TATA box promoter, preferably comprising the sequence of SEQ ID NO: 17 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette further comprises one or more anti-oxidative response elements.

In some embodiments, the anti-oxidative response element is from NQO1 or GCLM.

In some embodiments, the anti-oxidative response element comprises the sequence of SEQ ID NO: 8 or a sequence having at least 90% sequence identity thereto, or the anti-oxidative response element comprises the sequence of SEQ ID NO: 10 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette further comprises one or more enhancers.

In some embodiments, the enhancer is a SV40 enhancer, preferably comprising the sequence of SEQ ID NO: 12 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the coding sequence encodes a protein that is capable of inducing death of a cell once the protein is expressed by the cell.

In some embodiments, the protein is selected from the group consisting of a cytotoxin, a tumor-associated antigen, a tumor specific antigen, a cytokine, a membrane penetrator, a checkpoint protein and an antibody.

In some embodiments, the protein is neutrophile elastase, diphtheria toxin, IL-2 or granzyme B.

In some embodiments, when introduced into a non-tumor cell, the expression cassette is not expressed in the non-tumor cell, or is expressed at a level lower than that in a tumor cell.

In another aspect, the present disclosure provides a nucleic acid molecule comprising the expression cassette.

In another aspect, the present disclosure provides a vector comprising the expression cassette or the nucleic acid molecule.

In some embodiments, the vector is a viral vector, such as a lentivirus vector, a retrovirus vector, an adeno-associated virus (AAV) vector, or an adenovirus vector.

In another aspect, the present disclosure provides a composition comprising

1) the nucleic acid molecule or the vector; and

2) a pharmaceutically acceptable carrier and/or a deliver agent.

In some embodiments, the deliver agent is a biodegradable polymer, preferably, a in vivo jetPEI (Polyethylenimine), hyperbranched Poly(β-amino ester)s (HPAEs), lipid nanoparticle (LNP), liposomes or exosome.

In some embodiments, the composition further comprises one or more anti-cancer agents.

In another aspect, the present disclosure provides uses of the expression cassette, the nucleic acid molecule or the vector in the manufacture of a medicament for the treatment of a cancer.

In another aspect, the present disclosure provides a method of treating cancer, comprising administrating to a subject in need thereof a therapeutically effective amount of the nucleic acid molecule, the vector, or the composition.

In some embodiments, the cancer is a solid tumor, preferably, a breast cancer, endometrial cancer, ovarian cancer, cervical cancer, lung cancer, gastric cancer, esophageal cancer, colorectal cancer, anal cancer, urothelial cancer, pancreatic cancer, salivary gland cancer, melanoma, skin cancer, head and neck cancer, sarcoma or brain cancer.

In another embodiment, the cancer is a hematological malignancy, preferably, a multiple myeloma (MM), lymphoma, chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), or acute myelogenous leukemia (AML), diffuse large B cell lymphoma (DLBCL), non-Hodgkin lymphoma (NHL), extranodal NK/T cell lymphoma (ENKL), Hodgkin Lymphoma (HL), plasmablastic lymphoma, Burkitt's lymphoma, marginal zone lymphoma (MZL), or mantle cell lymphoma (MCL).

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically illustrates structures of the tumor specific regulatory elements. In each insert, upper panel depicts vector name/ID and its composition; lower panel shows brief map of individual regulatory elements.

FIG. 2 shows the relative transcription activity of vector comprised with hTERT and relative hybrid promoters in HEK293 cells. Vectors containing hTERT elements showed stronger transcriptional activity in HEK293 cells than the ones with tissue specific elements.

FIGs. 3A-3B show the relative transcription activity of vector comprised with hTERT and relative hybrid promoters in MC38 cells (A) or HCT116 cells (B). Vectors containing ARE-hTERT hybrid promoters displayed comparable transcriptional activity with strong CMV promoters.

FIGs. 4A-4C show the relative transcription activity of vector comprised tumor specific promoters in A375 cells (A), G361 cells (B) or G361 cells (C). Vectors containing ARE-hTERT hybrid promoters displayed comparable transcriptional activity with strong CMV promoters. In contrast, vectors containing tumor specific promoters used in clinical trials showed weak transcriptional activity, which is much lower than ARE-hTERT ones.

FIG. 5 shows the relative transcription activity of vector comprised tumor specific promoters in Pan02 cells. Vectors containing ARE-hTERT hybrid promoters displayed comparable transcriptional activity with strong CMV promoters. In contrast, vectors containing tumor specific promoters used in clinical trials showed weak transcriptional activity, which is much lower than ARE-hTERT ones.

FIGs. 6A-6D show the relative transcription activity of vector comprised tumor specific promoters in Bel 7402 cells (A, C) or HepG2 cells (B, D). Vectors containing ARE-hTERT hybrid promoters displayed comparable transcriptional activity with strong CMV promoters. In contrast, vectors containing tumor specific promoters used in clinical trials showed weak transcriptional activity, which is much lower than ARE-hTERT ones.

FIGs. 7A-7B show the relative transcription activity of vector comprised tumor specific promoters in HUVECs cells (A) or smooth muscle cells (B). Vectors containing ARE-hTERT hybrid promoters displayed little or poor transcriptional activity in human normal cells.

## DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Current Protocols in Molecular Biology or Current Protocols in Immunology, John Wiley & Sons, New York, N.Y.(2009); Ausubel et al, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995;

Sambrook and Russell, Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Maniatis et al. Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984) and other like references.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and "comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term "comprising" can be substituted with the term "containing" or "including" or sometimes when used herein with the term "having."

When used herein "consisting of" excludes any element, step, or ingredient not specified in the claim element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any of the aforementioned terms of "comprising," "containing," "including," and "having," whenever used herein in the context of an aspect or embodiment of the application can be replaced with the term "consisting of" or "consisting essentially of" to vary scopes of the disclosure.

As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

Unless otherwise stated, any numerical value, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1 mg/mL to 10 mg/mL includes 0.9 mg/mL to 11 mg/mL. As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

The term "expression cassette" or "gene expression cassette" refers to a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to a termination signal. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA, in the sense or antisense direction. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter sequence or of an inducible promoter sequence that initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development. Generally, a gene expression cassette for expressing a protein of interest comprises at least a promoter sequence, a coding sequence (CDS) of the protein of interest, and a poly A addition sequence.

The term "promoter" or "promoter sequence" is a DNA sequence that RNA polymerase recognizes, binds and starts transcription. It generally contains conserved sequence required for RNA polymerase binding and transcription initiation, most of which are located upstream of the transcription start point, and the promoter itself is not transcribed. Examples of promoters include but are not limited to CMV, EF1A, CAG, CBh, and SFFV promoters.

The term “chimeric promoter”, also known as “composite promoter” or “hybrid promoter”, refers to a promoter that comprises at least two parts from different natural promoters or artificially synthetic promoters. The two parts are not naturally adjacent to each other in one nucleic acid molecule. In some embodiments of the present invention, a chimeric promoter comprises at least a first sequence from a tissue selective promoter sequence a tumor-selective promoter sequence and a second sequence from a minimal promoter. A tumor-selective promoter sequence is defined herein as a promoter sequence that is capable of driving transcription of a gene in tumor cell while remaining largely "silent" or expressed at low or relatively low levels in other tissue types. For example, the tumor-selective promoter sequence may be an hTR promoter sequence, hTERT promoter sequence, CEA promoter sequence, a PSA promoter sequence, a probasin promoter sequence, a ARR2PB promoter sequence, or an AFP promoter sequence. Telomerase is a ribonucleoprotein complex that is responsible for the complete replication of chromosomal ends. Many studies have demonstrated that a majority of malignant tumors express telomerase activity, whereas most normal cells do not. Three major components associated with telomerase activity in humans have been identified: (a) the RNA component (hTER); (b) the telomerase-associated protein (hTEP1); and (c) the telomerase catalytic unit or human telomerase reverse transcriptase (hTERT). Only hTER and hTERT, however, are required for the reconstitution of telomerase activity *in vitro* and therefore represent the minimal catalytic core of telomerase in humans. The promoter region of hTERT has been cloned and characterized previously. Telomerase-specific expression of cytotoxic or proapoptotic genes such as the diphtheria toxin A-chain, FADD, caspases, and Bax by the hTERT promoter has been achieved and reported in various gene transfer systems, such as plasmid and adenovirus. However, the transcription promoting strength of the hTERT promoter, like most other intrinsic mammalian promoters, is usually much weaker than commonly used viral promoters such as the CMV promoter and the SV40 early promoter. Consequently, it uses for gene therapy is hampered by the problem of low transgene expression. The present inventors have developed a novel chimeric promoter composed of an hTERT promoter sequence fused to a minimal promoter sequence. In some embodiments of the present invention, the hTERT promoter sequence comprises any one of the sequences of SEQ ID NOs: 1-7. In a particular embodiment, the hTERT promoter sequence comprises the sequence of SEQ ID NO: 1. A “minimal promoter sequence” as used herein to refer to

a portion of a promoter that includes a nucleotide sequence that maintains the ability to bind and locate a transactivator or a component of a transcription complex to a particular location in a nucleic acid (e.g., a DNA molecule). Promoter elements, particularly a TATA element or the like, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation are referred to as minimal promoters. In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription. A minimal promoter thus consists only of all basal elements needed for transcription initiation, e.g., a TATA box and/or an initiator. Any minimal promoter sequence known to those of ordinary skill in the art is contemplated for inclusion in the promoter sequences of the present invention. The minimal promoter sequence, for example, can be a minimal viral promoter, such as an adenoviral promoter sequence, a baculoviral promoter sequence, a CMV promoter sequence, a parvovirus promoter sequence, a herpesvirus promoter sequence, a poxvirus promoter sequence, an adeno-associated virus promoter sequence, a semiliki forest virus promoter sequence, an SV40 promoter sequence, a vaccinia virus promoter sequence, or a retrovirus promoter sequence. In some embodiments, the minimal promoter sequence is from a CMV promoter sequence, and thus referred to herein as "CMVmini promoter". In some embodiments, the CMVmini promoter comprises the sequence of SEQ ID NO: 16. Therefore, in some embodiments, the chimeric promoter comprises an hTERT promoter sequence of any one of the sequences of SEQ ID NOs: 1-7 operatively coupled to a CMVmini promoter of the sequence of SEQ ID NO: 16. In some embodiments, the minimal promoter sequence is a minimal TATA box promoter. "minimal TATA box promoter" used herein refers to the TATA region of a promoter, exclusive of other cis-acting elements. In some embodiments, the minimal TATA box promoter is a E1F TATA box promoter. In some embodiments, the E1F TATA box promoter comprises the sequence of SEQ ID NO: 17. Therefore, in some embodiments, the chimeric promoter comprises an hTERT promoter sequence of any one of the sequences of SEQ ID NOs: 1-7 operatively coupled to an E1F TATA box promoter of the sequence of SEQ ID NO: 17.

As used herein, the term "operably linked" refers to a linkage of two or more polynucleotides or two or more nucleic acid sequences in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked

to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, since enhancers generally function when separated from the promoter by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not contiguous.

A "nucleic acid" as used herein will generally refer to a molecule (i.e., a strand) of DNA, RNA or a derivative or analog thereof, comprising nucleobases. Any of the tumor-selective promoter sequences and minimal promoter sequences set forth above can be incorporated into the nucleic acid sequences of the present invention. In certain particular embodiments, the tissue-selective promoter sequence is an hTERT promoter sequence such as, for example, the sequence set forth in SEQ ID NO:1. Further, in some embodiments, the nucleic acids include one or more additional promoter sequences that may or may not be operatively coupled to the tumor-selective promoter sequence.

The term "coding sequence (CDS)" is a region or nucleic acid sequence that codes a protein or RNA (e.g., siRNA) of interest. The region or sequence usually begins at the 5' end by a start codon and ends at the 3' end with a stop codon. In some embodiments, the protein of interest is a cytotoxic (or cytostatic) agent which directly or indirectly stimulates cell death. The cytotoxic agent can be a cytotoxic protein or peptide or an apoptotic triggering protein or peptide. The cytotoxic protein or peptide can be a bacterial cytotoxin such as an alpha-pore forming toxin (e.g., cytolysin A from *E. coli*), a beta-pore-forming toxin (e.g., α-Hemolysin, PVL-panton Valentine leukocidin, aerolysin, clostridial Epsilon-toxin, *Clostridium perfringens* enterotoxin), binary toxins (anthrax toxin, edema toxin, *C. botulinum* C2 toxin, C spirofome toxin, *C. perfringens* iota toxin, *C. difficile* cyto-lethal toxins (A and B)), prion, parasporin, a cholesterol-dependent cytolysins (e.g., pneumolysin), a small pore-forming toxin (e.g., Gramicidin A), a cyanotoxin (e.g., microcystins, nodularins), a hemotoxin, a neurotoxin (e.g., botulinum neurotoxin), a cytotoxin, cholera toxin, diphtheria toxin, pseudomonas exotoxin A, tetanus toxin, or an immunotoxin (idarubicin, ricin A, CRM9, Pokeweed antiviral protein, DT). Exemplary apoptotic triggering proteins or peptides include apoptotic protease activating factor-1 (Apaf-1), cytochrome-c, caspase initiator proteins (CASP2, CASP8, CASP9, CASP10), apoptosis inducing factor (AIF), p53, p73, p63, Bcl-2,

Bax, granzyme B, poly-ADP ribose polymerase (PARP), and P 21-activated kinase 2 (PAK2). The protein of interest can be any tumoristatic, i.e. of greater toxicity to tumor cells relative to non-tumor cells.

The term "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3′ end. The 3′ poly(A) tail is a long sequence of adenine nucleotides (e.g., 50, 60, 70, 100, 200, 500, 1000, 2000, 3000, 4000, or 5000) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal sequence or "poly(A) sequence", for triggering the endonuclease cleavage of an mRNA and the additional of a series of adenosines to the 3′ end of the cleaved mRNA. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3′ end at the cleavage site. The term "poly A addition sequence" as used herein refers to a sequence downstream of a coding sequence for the addition of poly(A) sequence to a RNA transcript. The poly A addition sequence may be a poly(A) sequence itself or a polyadenylation signal sequence described above. In some embodiments, the polyadenylation signal sequence is from a growth hormone gene-derived polyadenylation signal sequence, such as a bovine growth hormone gene-derived polyadenylation signal sequence and a human growth hormone gene-derived polyadenylation signal sequence, an SV40 virus-derived polyadenylation signal sequence, and a human or rabbit β-globin gene-derived polyadenylation signal sequence. In some embodiments, the polyadenylation signal sequence comprises the sequence of SEQ ID NO: . The incorporation of the poly A addition sequence into the gene expression cassette may increases transcription efficiency.

The term "enhancer" refers to a sequence having a promoting effect on the action of a promoter. An enhancer can promote transcription irrespective of the direction of a sequence. The enhancer to be used in the present invention may include one kind of enhancer, but a plurality of enhancers including two or more enhancers identical to each other may be used or a plurality of enhancers different from each other may be used in combination. Their order is not specifically limited. For example, a CMV enhancer, an SV40 enhancer, an hTERT (telomerase reverse transcriptase) enhancer, or the like may be used. As an example, the hTERT enhancer, the SV40 enhancer, and the CMV enhancer may be linked in the stated order. The enhancer can be arranged downstream of the poly A addition sequence.

The term "anti-oxidative response element (ARE)" is a cis-acting enhancer sequence located in the regulatory regions of some antioxidant and detoxifying genes. The ARE can be activated by redox-cycling phenols and electrophiles. Some studies have shown that a protein called nuclear factor erythroid 2-related factor 2 (Nrf2) may be the principal transcription factor necessary for ARE activation, even though many other transcription factors also bind to the ARE sequence. Upon toxic insult, glutathione depletion or chemical activation, the transcription factor Nrf2 translocates to the nucleus and dimerizes with small Maf proteins to form a trans-activation complex that binds to the ARE. Consequently, Nrf2-induced ARE activation coordinates the expression of many genes involved in combating oxidative stress and toxicity in a wide variety of tissues and cell types. In some embodiments, the ARE is from NQO1 gene. In some embodiments, the ARE comprises the sequence of SEQ ID NO: 8. In some embodiments, the ARE comprises the sequence of SEQ ID NO: 10.

The term "vector" is intended to refer to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked.

Such vectors are referred to herein as “recombinant expression vectors” (or simply, “expression vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However, the disclosure is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

As used herein, the term "subject" refers to a human or any non-human animal (e g, mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

The phrase "pharmaceutically acceptable carrier " as used herein refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, carrier, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), solvent or encapsulating material, involved in carrying or transporting a therapeutic compound for administration to the subject. Each excipient should be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject.

The term “delivery agent” as used herein refers to a transporter that carries a therapeutic agent (e.g., a nucleic acid molecule or a vector of the present invention), whether attached to some element on the surface, attached directed to the surface, embedded onto the surface, or contained partially or completely within the surface, or within the interior of the agent. Non-limiting examples of delivery agents that can be used include a lipidoid, a liposome, a lipoplex, a lipid nanoparticle, a microsphere, a nanosphere, a microcapsules, a nanocapsule, a peptide, a protein, a cell, a nanoparticle mimic, a nanotube, a micelle, or a polymeric compound, such as, poly-glycolic acid, poly-lactic acid, hyaluronic acid, modified polysaccharides, chitosan, cellulose, dextran, polyurethanes, polyacrylic acids, pseudo-poly(amino acids), polyhydroxybutyrate-related copolymers, polyanhydrides, polymethylmethacrylate, poly(ethylene oxide). Preferably, the deliver agent is a biodegradable polymer, such

as, a in vivo jetPEI (Polyethylenimine), hyperbranched Poly(β-amino ester)s (HPAEs), lipid nanoparticle (LNP), liposomes or exosome.

The term "effective amount" used herein refers to an amount of an agent or a combination of agents, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition can: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), preventing or delaying the spread (e.g., metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of the disease. The methods of the invention contemplate any one or more of these aspects of treatment.

"Percent (%) amino acid sequence identity" , "homology" or "homologous to" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the

sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

The present invention provides genetic engineering strategies implementing novel hybrid promoters with cancer cell selectivity. These hybrid promoters may be used in gene therapy to treat various types of cancers. These novel hybrid promoters are based on the combination of one or more anti-oxidative response enhancers/elements (ARE) operably linked to engineered human telomerase reverse transcriptase and other tumor specific core promoters. It is shown that the expression of a transgene is significantly increased in various cancer cells when hybrid promoter including ARE elements and enhancer element following poly (A) sequence. Cytotoxin driven by ARE-hTERT promoters efficiently kills cancer cells and presents good safety in primary normal cells.

An aspect of invention relates to a nucleic acid molecule comprising one or a plurality of anti-oxidative response enhancers linker to an hTERT or other tumor specific promoter.

For simultaneously superior transcriptional efficiency and specificity in cancer cells, we comprised an hTERT promoter, anti-oxidative response elements from NQO1 or GCLM and SV40 enhancer to generate an expression cassette or expression construct. An preferred hTERT promoter is a 243bp nucleotide fragment cloned from 5' flank region and partial transcript sequence of hTERT gene (-190…53bp referred to transcript initiating site as 0). We proposed that anti-oxidative response cis elements amplify transcriptional activity of hTERT promoter producing sufficient mRNA for anti-cancer efficacy.

The nucleotide sequence of the hTERT promoter is show in SEQ ID NO: 1:

GCACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTGGG
GACCCGGGCACCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCG
CGGACCCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCC

GGGCCCTCCCAGCCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGC GGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCTGCGCACGTGGGA (SEQ ID NO:1, hereafter referred to as "hTERT core promoter"). In another embodiment, the hTERT sequence is a homologue of SEQ ID NO: 1. In another embodiment, the hTERT sequence is a variant of SEQ ID NO: 1. In another embodiment, the hTERT sequence is a fragment of SEQ ID NO: 1. In another embodiment, the hTERT sequence is a homologue of a fragment of SEQ ID NO: 1. In another embodiment, the hTERT sequence is a variant of a fragment of SEQ ID NO: 1. Each possibility represents a separate embodiment of the present invention. In another embodiment, the hTERT sequence is at least 60% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 65% homologous to SEQ ID NO: 1.In another embodiment, the hTERT sequence is at least 70% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 72% homologous to SEQ ID NO: 1.In another embodiment, the hTERT sequence is at least 74%homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 76% homologous to SEQ ID NO: 1.In another embodiment, the hTERT sequence is at least 78% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 80% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 82% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 84% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 88% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 94% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 96% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 98% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 99% homologous to SEQ ID NO: 1. In another embodiment, the hTERT sequence is at least 60%, 65%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 88%, 94%, 96%, 98%, or 99% homologous to any one of SEQ ID NO: 2-7.

The nucleotide sequence of an ARE cis element from NQO1 (hereafter referred to as "ARE1") is show in SEQ ID NO: 8:

GATCCAGTCACAGTGACTCAGCAGAATCTG (SEQ ID NO: 8). In another embodiment, the ARE1 sequence is a homologue of SEQ ID NO: 8. In another embodiment, the ARE1 sequence is a variant of SEQ ID NO: 8. In another embodiment, the ARE1 sequence is a fragment of SEQ ID NO: 8. In another

embodiment, the ARE1 sequence is a homologue of a fragment of SEQ ID NO: 8. In another embodiment, the ARE1 sequence is a variant of a fragment of SEQ ID NO: 8. Each possibility represents a separate embodiment of the present invention. In another embodiment, the ARE1 sequence is at least 60%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 65% homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 70%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 72% homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 74%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 76% homologous to SEQ ID NO: 8.In another embodiment, the ARE1 sequence is at least 78%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 80% homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 82%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 84%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 88%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 94%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 96%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 98%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 99%homologous to SEQ ID NO: 8. In another embodiment, the ARE1 sequence is at least 60%, 65%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 88%, 94%, 96%, 98%, or 99% homologous to SEQ ID NO: 9. In another embodiment, the ARE1 sequence comprises the sequence TCCAGTCA (ARE1-a).

The nucleotide sequence of an ARE cis element from GCLM (hereafter referred to as ARE2) is show in SEQ ID NO: 10.

CCGCGGGATGAGTAACGGTTACGAAGCACTTTCTCGGCTACGATTTCTG CTTAGTCATTGTCT (SEQ ID NO:10). In another embodiment, the ARE2 sequence is a homologue of SEQ ID NO: 10. In another embodiment, the ARE2 sequence is a variant of SEQ ID NO: 10. In another embodiment, the ARE2 sequence is a fragment of SEQ ID NO: 10. In another embodiment, the ARE2 sequence is a homologue of a fragment of SEQ ID NO: 10. In another embodiment, the ARE2 sequence is a variant of a fragment of SEQ ID NO: 10. Each possibility represents a separate embodiment of the present invention. In another embodiment, the ARE2 sequence is at least 60%homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at

least 65% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 70%homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 72% homologous to SEQ ID NO: 01. In another embodiment, the ARE2 sequence is at least 74%homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 76% homologous to SEQ ID NO: 10.In another embodiment, the ARE2 sequence is at least 78%homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 80% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 82% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 84% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 88% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 94% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 96% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 98% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 99% homologous to SEQ ID NO: 10. In another embodiment, the ARE2 sequence is at least 60%, 65%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 88%, 94%, 96%, 98%, or 99% homologous to SEQ ID NO: 11 (ARE2-b). In another embodiment, the ARE1 sequence comprises the sequence TTAGTCA (ARE2-a) or TGAGTAA (ARE2-c).

The nucleotide sequence of a SV40 enhancer element following poly (A) sequence or a polyadenylation signal sequence is show in SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is a homologue of SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is a variant of SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is a fragment of SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is a homologue of a fragment of SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is a variant of a fragment of SEQ ID NO: 12. Each possibility represents a separate embodiment of the present invention. In another embodiment, the SV40 enhancer sequence is at least 60%homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 65% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 70%homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 72% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 74%homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least

76% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 78% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 80% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 82% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 84% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 88% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 94% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 96% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 98% homologous to SEQ ID NO: 12. In another embodiment, the SV40 enhancer sequence is at least 99% homologous to SEQ ID NO: 12.

The nucleotide sequence of a SCGB2A2 enhancer element cloned from 5' flank regulatory region of SCGB2A2 gene is show in SEQ ID NO: 13:

CTGTCCTGAACATTGTTAGATGTTAAGAAGGTTTCAGTTGATGTTTAATT
CCTGGCCTCTGTCACTTGACCTCAATAGGACACTCACCCCCATCCAGTTGG
GACAAGCAAAACCCACTATTGGGAAAGGTTCTTCAGGGTGGGGAAAGTG
AAAAAGTGTCCCCACTGAGACCCATTGCTCCAGCAGCAGGGATACTTTGT
ATAAGTTCCTCCATATGCCTCTCCTGAGGCCCTGGCGTAACAGAGGCTCAT
GACAGTGGGCTGACTACTGTTTTGGGATAATGTTGGCATTGAAGGGAGGG
GAAAATTACTAACTCACTGGCAAAGGTACTAGTGAAGGTATCTGATGAGC
CAAGGTAAACTTCCTCTCGCATATGCGCATTAACATTTTTAACCACACACA
ATGTGGGGACGTGGCGAGTTGTATAATTTCTGGGTTTTGGTACGGGAAAG
AATTAGCCAGGGGCTGACATTGACCTTGTCTCTGCTTTCAAATCCGGTTCC
AGGTTCCAACTCATTAGATACATCCTGGTCAGATGGTGTCACACTAACAG
TCACTTCTAGGGCAAAAGGACACTGAGGTGGCCCAGGCTCTCATTAGCAG
GGTTTCCAGCCATCTTCTTTCTTCACTACCTTCTTCCTACCTGACCCCCAGT
TTCCCTCCAGGAAAGTACAAATGGTGTTGTATTTACCATGTCCAGGAAGA
CACAGTGGTGTCTGATTTAAGTGTTACGTCTGATGTCTGTGTTTCGTAAAA
CTTTAATTTTAAATGAGTTCCTTTTCCTGAATAGATCTAGTAATAAAACAT
AAAATACAGTCAATACACTCTTTGTGTAAAATATCAAAATCTGCTCTTGAA
AATGAGTTACACAATAGTAAATTAAGGAAAATAAGTAGTTTTCATCACTA
TAATACAAACAAGAAGTGCCCAAGCCATGAGCTCAATGAGCAGGGACAG
GGAGGAAGGGTCAGGGTGAGGGTCCTGGAGATGTCAGATGGAACTGCCT

ATGGACAGATACAGGGTCCTGTGAACAGGGAGGTCCCCAGAGATGCAGG
GCCAGCAAAGAACACAAGGCATTAGCCATCTTTGATGTGGCTTTTGGAAT
GAAGTG (SEQ ID NO:13). In another embodiment, the SCGB2A2 enhancer sequence is a homologue of SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is a variant of SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is a fragment of SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is a homologue of a fragment of SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is a variant of a fragment of SEQ ID NO: 13. Each possibility represents a separate embodiment of the present invention. In another embodiment, the SCGB2A2 enhancer sequence is at least 60%homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 65% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 70%homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 72% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 74% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 76% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 78% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 80% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 82% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 84% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 88% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 94% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 96% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 98% homologous to SEQ ID NO: 13. In another embodiment, the SCGB2A2 enhancer sequence is at least 99% homologous to SEQ ID NO: 13.

The nucleotide sequence of a SCGB2A2 core promoter cloned from SCGB2A2 promoter is show in SEQ ID NO: 14:

CTAGGCCCACTGCTGGGATAAGCTGGCACAGATACACGGGACATGATG
CTTTCATTCTCACCTTAAAGAAAGAGACAGAGAAGAAAAGGAAGGGGAG
CAAGTGGAGAAGGAATACAAGAAAGAGGAAGAGAAGCAGGAAGATTCT

ACATACAGGCTGGCTGTGTTTCCCCTGGGGCATGCTCCTGTTTACTGGTCC CATGCCAGGTTGACTCATTGCCTCGTTCATGGGTGGAATTAAGATGCCTAC CTGGGGAATAAATAGAGCAAGGCTGGGTGCTCA (SEQ ID NO: 14). In another embodiment, the SCGB2A2 promoter sequence is a homologue of SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is a variant of SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is a fragment of SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is a homologue of a fragment of SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is a variant of a fragment of SEQ ID NO: 14. Each possibility represents a separate embodiment of the present invention. In another embodiment, the SCGB2A2 promoter sequence is at least 60% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 65% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 70%homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 72% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 74%homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 76% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 78% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 80% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 82% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 84% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 88% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 94% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 96% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 98% homologous to SEQ ID NO: 14. In another embodiment, the SCGB2A2 promoter sequence is at least 99% homologous to SEQ ID NO: 14.

The nucleotide sequence of a hCCRK promoter cloned from hCCRK promoter is show in SEQ ID NO: 15: ACCCAGGTACCTATGTTCAAAAGTGCCTCAATCCTAGTTAACAAGGGCAG AGACCACGAGAAACAACACTGTGTTTAGTAGCAACTTAACAACCAGCCAG

CAGTTCTGTCCACACACACACCACCGGGCATGGTTCCAAAGCTAAAAAGG
CACTAATTGCTTTTCTATAAGGAGGTAGAACACAGTCCCTCCGTGTTCTTT
AGGCCTGATGGTCTGCATTATCGGATCTGTTACCGTGTTAATTGTTCCTGT
CTCACACAGCCGGTTTGGGCTTTCTTCTGCATATGTCTGGGATGGTGACGG
GTTCCTATATAGAGGAGTACTGGGGAAGCCTCTGTGTGTGTGTGTGTGTCC
GTGCATATGTACACATGTGTGTAAAAAGCAGCCACACGCTGAGAATGGTT
AACGGGTAGCCAGGCTGTCTGTACTCAGGGCCCTAAGACTGGCCCAGGAA
AGGGCCGGGGGAGGTGGGGCGGGGTGAAGGTGGAGCGGGCTTGGCTTGT
GCTCACTGCCTTTTCCACAACAGGAGTACAAATGCTGGAGTGAGTGAGGT
GAACTCAAGTCGCCTTTAGGAATGGCTGAAAAAGCCCACACCTGGAAATC
ACTCCCTCCCTGCTCCTCCACGGCAGGTTGCATCTGCGAGACGCTTCGGTC
ATTAGAGGAATGAGCCGGGAGTGAGCAATTCACCAGCTCTCCAGCACTTG
GTGGAAAGCAGCAGGCAACC (SEQ ID NO: 15). In another embodiment, the hCCRK promoter sequence is a homologue of SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is a variant of SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is a fragment of SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is a homologue of a fragment of SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is a variant of a fragment of SEQ ID NO: 15. Each possibility represents a separate embodiment of the present invention. In another embodiment, the hCCRK promoter sequence is at least 60% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 65% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 70%homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 72% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 74%homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 76% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 78% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 80% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 82% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 84% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 88% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 94%

homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 96% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 98% homologous to SEQ ID NO: 15. In another embodiment, the hCCRK promoter sequence is at least 99% homologous to SEQ ID NO: 15.

The nucleotide sequence of a CMV mini promoter is show in SEQ ID NO: 16: GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAA CCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAA GACACCGGGACCGATCCAGCCTCCGCGGCCCCGCATTCGAGCTCGGTACC CGG (SEQ ID NO: 16). In another embodiment, the CMVmini promoter sequence is a homologue of SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is a variant of SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is a fragment of SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is a homologue of a fragment of SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is a variant of a fragment of SEQ ID NO: 16. Each possibility represents a separate embodiment of the present invention. In another embodiment, the CMVmini promoter sequence is at least 60% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 65% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 70% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 72% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 74%homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 76% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 78% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 80% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 82% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 84% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 88% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 94% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 96% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter

sequence is at least 98% homologous to SEQ ID NO: 16. In another embodiment, the CMVmini promoter sequence is at least 99% homologous to SEQ ID NO: 16.

The nucleotide sequence of one H19 814 promoter refers to patent US_9173964_B2; the nucleotide sequences of other tumor specific elements, including TYR, TRP1 and AFP refers to In vivo gene product documents.

In some embodiments, the expression cassette comprises, from 5'-3', a SV40 enhancer, an hTERT promoter, a CMVmini promoter, a CDS sequence and a SV40 poly A addition sequence (Fig. 1, CG010201). In some embodiments, SV40-hTERT-CMVmini region of the expression cassette comprises the sequence of SEQ ID NO: 18 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette comprises, from 5'-3', an hTERT promoter, a CMVmini promoter, a CDS sequence and a SV40 poly A addition sequence (Fig. 1, CG010301). In some embodiments, hTERT-CMVmini region of the expression cassette comprises the sequence of SEQ ID NO: 19 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette comprises, from 5'-3', an hTERT promoter, a E1F TATA promoter, a CDS sequence, a SV40 poly A addition sequence and a SV40 enhancer (Fig. 1, CG010401). In some embodiments, hTERT- E1F TATA region of the expression cassette comprises the sequence of SEQ ID NO: 20 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette comprises, from 5'-3', an ARE1 sequence, an hTERT promoter, a CMVmini promoter, a CDS sequence, a SV40 poly A addition sequence and a SV40 enhancer (Fig. 1, CG010501). In some embodiments, ARE1-hTERT-CMVmini region of the expression cassette comprises the sequence of SEQ ID NO: 21 or a sequence having at least 90% sequence identity thereto.

In some embodiments, the expression cassette comprises, from 5'-3', an ARE2 sequence, an hTERT promoter, a CMVmini promoter, a CDS sequence, a SV40 poly A addition sequence and a SV40 enhancer (Fig. 1, CG010601). In some embodiments, ARE2-hTERT-CMVmini region of the expression cassette comprises the sequence of SEQ ID NO: 22 or a sequence having at least 90% sequence identity thereto.

The expression cassette of the present invention may comprise one or more ARE cis elements (ARE1 or ARE2), such as 1, 2, 3 or more ARE1 sequences, which can be linked directly or spaced by short linker(s).

The CDS sequence of any one of the expression cassette of the present invention may encode any gene product which can be used as a cytotoxic (or cytostatic) agent for tumor cells. The gene product includes but not limited to: a cytotoxin, such as, DTA, tk-HSV, GZMA, GZMB, pro- and cleaved- caspase1, 3, 8 and 11, Bcl-2, FADD and its variants; a Membrane penetrator, such as, perforin, GSDMA, GSDMB, GSDMD, GSDME and relative proteins in various species and N terminal or active mutant variants, MLKL and its variants; a siRNA targeting, such as, EGFR, HER2, Kras, c-Myc, hTERT, IGFR, AKT1, AKT2, AKT3, mTOR, cAMPK, YAP, NRF2, ESCRT subunits, or NQO1; a Intracellular & membrane recombinant protein, such as, p53 and its mutant variants, MDM2/4 and their mutant variants, p21, PTEN, NOTCH1, NOTCH2, NOTCH3, NOTCH4, CD44, DR3, CD40L, CD83; an antibody targeting, such as, PD-1, PD-L1, VEGF, CTLA-4, CD40, 4-1BB, CD80, CD83, CD86, CD3, CXCR4, CXCL1, HSP, HMGB1, IL-1β, TGF-β, FAP, CLDN18.2, CD47, CD16, TIGIT, CD28; a cytokine, such as IFN-γ, IL-2, IL-12 p40, p35 and p70, IL-18, IL-1 α and β, IL4, IL13, GM-CSF. When introduced in a subject, the CDS sequence in the expression cassette of the present invention is expressed only in tumor or cancer cells or is expressed at a much higher level in tumor or cancer cells than that in normal cells.

In the expression cassette of the present invention, a site at which the CDS sequence or gene of interest is to be inserted may be present as a multiple cloning site. In this case, the gene of interest may be inserted at the multiple cloning site (insertion site) by utilizing a sequence to be recognized by a restriction enzyme. A gene expression cassette that does not contain DNA of the gene of interest itself but contains a portion at which the DNA is to be inserted as a multiple cloning site as described above is also encompassed in the present invention.

The expression cassette of the present invention may be utilized by being incorporated into a gene expression vector. The present invention also encompasses a vector containing the gene expression cassette of the present invention.

Examples of the vector into which the gene expression cassette of the present invention is inserted include: a plasmid; viral vectors, such as an adenovirus (Ad) vector, an adeno-associated virus (AAV) vector, a lentivirus vector, a retrovirus vector, a herpes virus vector, and a Sendai virus vector; and non-viral vectors, such as a biodegradable polymer. The vector into which the gene expression cassette has been introduced may be introduced into cells by a known method, such as infection or

electroporation. Further, a commercially available vector may be modified so as to contain the expression cassette of the present invention. Further, the present invention also encompasses a viral vector containing the expression cassette for the gene of interest described above. Among viral vectors, for example, an Ad vector and an AAV vector each enable specific diagnosis or treatment of a disease such as cancer, but the gene expression cassette of the present invention can allow the gene to be expressed stably and sustainably, and hence the viral vectors are desirably used as appropriate for applications of gene expression. The viral vector may be generated by inserting the expression cassette for the gene of interest described above onto a viral genome usable as a vector.

In certain embodiments, the present invention concerns formulation of one or more of the gene expression cassettes disclosed herein in pharmaceutically acceptable carrier or associated with a delivery agent for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. Thus, the present invention contemplates a composition comprising: 1) one or more of the gene expression cassettes and 2) a pharmaceutically acceptable carrier and/or a deliver agent. In particular, the present invention contemplates the formulation of one or more viral vectors, virions, or virus particles (or pluralities thereof) that comprise one or more of the disclosed gene expression cassettes. In such compositions, it will also be understood that, if desired, the gene expression cassette or vectors containing the same as disclosed herein may be administered in combination with other agents as well, such as, e.g., peptides, proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the normal cells or host tissues. The viral vector compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA, DNA, or PNA compositions. Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, topical, sublingual,

subcutaneous, transdermal, parenteral, intravenous, intranasal, and intramuscular administration, intratumoral, lymph node administration and formulation.

Further embodiments of the present invention generally pertain to methods of treating a subject with a hyperproliferative disease, that include: (1) obtaining a pharmaceutical composition of a polynucleotide that includes any of the gene expression cassettes described above; and (2) administering a pharmaceutically effective amount of the composition to the subject. As discussed above, the subject can be any subject, such as a mammal. The mammal may be a human, such as a patient with a disease. The disease can be any disease that can afflict a subject. In certain particular embodiments, the subject is a human, and the disease is a hyperproliferative disease such as tumor or cancer. The cancer may be any cancer, such breast cancer, lung cancer, prostate cancer, ovarian cancer, brain cancer, liver cancer, cervical cancer, colon cancer, renal cancer, skin cancer, head and neck cancer, bone cancer, esophageal cancer, bladder cancer, uterine cancer, lymphatic cancer, stomach cancer, pancreatic cancer, testicular cancer, lymphoma, or leukemia. In some embodiments, the subject is undergoing secondary anticancer therapy. Any secondary anti-cancer therapy known to those of ordinary skill in the art is contemplated, such as chemotherapy, surgical therapy, radiation therapy, immunotherapy, or additional gene therapy.

In another embodiment, the present invention provides a method for treating a tumor in a subject in need thereof, comprising administering to the subject a gene expression cassette, a vector or a pharmaceutical composition of the present invention, thereby treating a tumor in a subject in need thereof. In another embodiment, the present invention provides a method for inhibiting tumor progression in a subject in need thereof, comprising administering to the subject a gene expression cassette, a vector or a pharmaceutical composition of the present invention, thereby inhibiting tumor progression in a subject in need thereof. In another embodiment, the present invention provides a method for inhibiting tumor metastasis in a subject in need thereof, comprising administering to the subject a gene expression cassette, a vector or a pharmaceutical composition of the present invention, thereby inhibiting tumor metastasis in a subject in need thereof.

In certain embodiments, the tumor is solid tumors include, but are not limited to, breast cancer, endometrial cancer, ovarian cancer, cervical cancer, lung cancer, gastric cancer, esophageal cancer, colorectal cancer, anal cancer, urothelial cancer, pancreatic

cancer, salivary gland cancer, melanoma, skin cancer, head and neck cancer, sarcoma and brain cancer. In certain embodiments, the tumor is a hematological malignancy include but not limited to, multiple myeloma (MM), lymphoma, chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), or acute myelogenous leukemia (AML), diffuse large B cell lymphoma (DLBCL), non-Hodgkin lymphoma (NHL), extranodal NK/T cell lymphoma (ENKL), Hodgkin Lymphoma (HL), plasmablastic lymphoma, Burkitt's lymphoma, marginal zone lymphoma (MZL), or mantle cell lymphoma (MCL).

In certain embodiments, the cis element or the expression cassette is constructed or packaged in a vector. In certain embodiments, the vector is a viral vector, such as an adenovirus (Ad) vector, an adeno-associated virus (AAV) vector, a lentivirus vector, a retrovirus vector, a herpes virus vector, or a Sendai virus vector. In another embodiment, the vector is a non-viral vector, such as in vivo jetPEI (Polyethylenimine), hyperbranched Poly(β-amino ester)s (HPAEs), lipid nanoparticle (LNP), liposomes or exosome.

## EXEMPLES

## EXAMPLE 1. EXPRESSION EFFICIENCY DRIVEN BY ARE-hTERT PROMOTERS IN IMMORTAL HEK293FT CELL LINE

First, considering essential role of hTERT in limitless proliferation and stemness, the transcription efficiency of tumor specific promoters including hTERT core and hybrid promoters linked with luciferase was tested in vitro in immortalized cell line HEK293FT. Transcript activity was determined by measurement of luminance value following after plasmid transfection with tumor specific promoters 24 hours referring manufacturer's (Vazyme, Bio-Lite$^{TM}$ Luciferase assay system) recommendations. HEK293FT cells were seeded in each well of 96-well plate and transfection was performed when confluent is 60 to 80%. 0.1μg of pDNA loaded with jetPrime in 100ul jetPRIME buffer were used for individual well. All transient transfections were performed using jetPRIME according to the manufacturer's (Polyplus) recommendations in $2 \times 10^4$ HEK293FT. Tumor specific plasmids are constructed via engineering tumor specific promoters (CG010201, CG010301, CG010401, CG010501, CG010601, CG010801, CG010A01, CG010C01, CG011001, CG011101, CG011201, CG011301, CG010D01, CG010E01 and CG010F01, synthesized and purified by GenScript.) and Luciferase into pcDNA3.4 backbone (GenScript) via

GIBSON recombination. The structure of control group is described in FIG.1 CMV-CDS-Poly(A). CG010501 (ARE1-hTERT) and CG010601 (ARE2-hTERT) were able to drive relative high level of luciferase (FIG 2).

Thus, an hTERT hybrid promoter comprised vector efficiently drives gene expression in immortalized human cell line, showing potential for further analysis in cancer cells.

## EXAMPLE 2. HIGH EFFICIENCY OF GENE EXPRESSION DRIVEN BY ARE-hTERT PROMOTERS IN COLORECTAL CANCER CELL LINES

For further study of expression efficiency in various cancers, hTERT and its derived hybrid promoter candidates described in Example 1 were tested in vitro on mouse and human colorectal carcinoma cell lines, MC38 and HCT116. Experiment procedure is performed as described in EXAMPLE 1. As seen with the immortalized HEK293T kidney epithelial cells. CG010501 (ARE1-hTERT) and CG010601 (ARE2-hTERT) exhibited superior expressing efficiency in both colorectal carcinoma cell lines, relative to hTERT single promoter constructs (FIGS 3A-B, CG010301 and CG101401).

Thus, an ARE-hTERT hybrid promoter comprised vector efficiently drives gene expression in colorectal carcinoma cell lines and shows potential for anti-tumor gene therapy.

## EXAMPLE 3. HIGH EFFICIENCY OF GENE EXPRESSION DRIVEN BY ARE-hTERT PROMOTERS IN MELANOMA CELL LINES

For further study of expression efficiency in various cancers, hTERT and its derived hybrid promoter candidates described above were tested in vitro on human melanoma cell lines, A375 and G361. Experiment procedure is performed as described in above. As seen with the above cancer cell lines. CG010501 (ARE1-hTERT) and CG010601 (ARE2-hTERT) exhibited superior expressing efficiency in both melanoma cell lines, relative to hTERT single promoter constructs (FIGS 4A-B, CG010301 and CG101401) and melanoma specific promoters (FIG 4C, CG011001, CG011101, CG011201 and CG011301).

## EXAMPLE 4. HIGH EFFICIENCY OF GENE EXPRESSION DRIVEN BY ARE-hTERT PROMOTERS IN PANCREATIC ADENOCARCINOMA CELL LINES

For further study of expression efficiency in various cancers, hTERT and its derived hybrid promoter candidates described above were tested in vitro on mouse and human pancreatic adenocarcinoma cell lines, Pan02. Experiment procedure is performed as described in above. As seen with the above cancer cell lines, CG010601 (ARE2-hTERT) exhibited higher expressing efficiency in both pancreatic adenocarcinoma cell lines, relative to hTERT single promoter constructs and pancreatic cancer specific promoters (FIG 5, CG010A01 and CG010C01).

Thus, an ARE-hTERT hybrid promoter comprised vector efficiently drives gene expression in pancreatic adenocarcinoma cell lines and shows potential for anti-tumor gene therapy.

## EXAMPLE 5. HIGH EFFICIENCY OF GENE EXPRESSION DRIVEN BY ARE-hTERT PROMOTERS IN HEPATOCELLULAR CARCINOMA CELL LINES

For further study of expression efficiency in various cancers, hTERT and its derived hybrid candidates described in above were tested in vitro on mouse and human hepatocellular carcinoma cell lines, Bel7402 and HepG2. Experiment procedure is performed as described in above. As seen with the above cancer cell lines, CG010601 (ARE2-hTERT) exhibited higher expressing efficiency in both hepatocellular carcinoma cell lines, relative to hTERT single promoter constructs (FIGS 6A-B, CG010301 and CG101401) and liver cancer specific promoters (FIGS 6C-D, CG010D01, CG010E01 and CG010F01).

Thus, an ARE-hTERT hybrid promoter comprised vector efficiently drives gene expression in hepatocellular carcinoma cell lines and shows potential for anti-tumor gene therapy.

## EXAMPLE 6. NONE OR POOR EFFICIENCY OF GENE EXPRESSION DRIVEN BY hTERT AND ARE-hTERT PROMOTERS IN MULTIPLE PRIMARY CELLS

For studying tumor selectivity of hybrid promoters, hTERT and its derived hybrid candidates described in above were tested in primary cells. Human umbilical

vein endothelial cell and smooth muscle cell are used. Experiment procedure is performed as described in above. CG010601 (ARE2-hTERT) exhibited little transcription activity in both primary cells (FIGS 7A-B). In comparison, CG010501 (ARE1-hTERT) showed higher luciferase expression in human smooth muscle cell, but the expression level is still poor (FIG 7B). These data indicate that ARE-hTERT hybrid promoters do not alter tumor specificity of single hTERT promoter.

Thus, an ARE-hTERT hybrid promoter comprised vector shows wonderful tumor selectivity to prevent unwanted side-effect of interested genes in normal tissues, implying good safety of ARE-hTERT hybrid promoters in clinical application.

## EXAMPLE 7. SUPERIOR ANTI-COLORECTAL ADENOCARCINOMA ACTIVITY BY ARE-hTERT HYBRID PROMOTER DRIVEN NEUTROPHIL ELASTASE

The anti-cancer therapeutic potential of hybrid promoter construct ARE2-hTERT-ELANE was further test in vitro by determining their ability to induce apoptosis in both mouse and human colorectal adenocarcinoma lines, relative to CMV promoter. Anti-tumor activity was determined by cell viability with CTG assay, measuring ATP level in liver cells, MC38 and HCT116 colorectal adenocarcinoma cancer cell lines were transfected with ARE2-hTERT-ELANE, ARE2-hTERT-luci and CMV-ELANE at the concentration of 1, 2ug and 5ug/ml for 24 hours. ARE2-hTERT-ELANE exhibited comparable cell killing with CMV-ELANE in MC38 mouse colorectal cancer cell line, while ARE2-hTERT-luci showed no anti-tumor effect. Similar results were obtained when the experiment was repeated with human HCT116 colorectal cancer cell line. The result shows an ARE-hTERT hybrid promoter comprised vector, expressing neutrophile elastase, exhibited significantly superior ability to induce apoptosis in colorectal adenocarcinoma cell lines.

## EXAMPLE 8. SUPERIOR ANTI-COLORECTAL ADENOCARCINOMA ACTIVITY BY ARE-hTERT HYBRID PROMOTER DRIVEN DIPHTHERIA TOXIN

The anti-cancer therapeutic potential of hybrid promoter constructs ARE-hTERT-DTA were further test in vitro by determining their ability to lyse both mouse and human colorectal adenocarcinoma lines, MC38 and HCT116 colorectal adenocarcinoma cancer cell lines, relative to CMV promoter. Anti-tumor activity was

determined by cell lysis with LDH assay, measuring released extracellular lactate dehydrogenase activity. The transfection procedure majorly refers to above EXAMPLE 7. The concentration of transfect plasmid was altered to 0.5ug/ml. ARE2-hTERT-ELANE exhibited comparable ability of cell lysis with CMV-ELANE in both colorectal carcinoma cell lines.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing diphtheria toxin, exhibited significantly superior ability to induce cell lysis in colorectal adenocarcinoma cell lines.

## EXAMPLE 9. SUPERIOR ANTI-MELANOMA ACTIVITY BY ARE-hTERT HYBRID PROMOTER DRIVEN DIPHTHERIA TOXIN

The anti-cancer therapeutic potential of hybrid promoter constructs ARE-hTERT-DTA were further test in vitro by determining their ability to lyse 2 human colorectal adenocarcinoma lines, G361 and A375 melanoma cell lines, relative to CMV promoter. Anti-tumor activity was determined by cell lysis with LDH assay, measuring released extracellular lactate dehydrogenase activity. The transfection procedure majorly refers to EXAMPLE 8. The ARE2-hTERT-DTA exhibited comparable ability of cell lysis with CMV-DTA in both colorectal carcinoma cell lines.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing diphtheria toxin, exhibited significantly superior ability to induce cell lysis in melanoma cell lines.

## EXAMPLE 10. SUPERIOR ANTI- HEPATOCELLULAR CARCINOMA ACTIVITY BY ARE-hTERT HYBRID PROMOTER DRIVEN DIPHTHERIA TOXIN

The anti-cancer therapeutic potential of hybrid promoter constructs ARE-hTERT-DTA were further test in vitro by determining their ability to lyse 2 human colorectal adenocarcinoma lines, Bel7042 and HepG2 hepatocellular carcinoma cell lines, relative to CMV promoter. Anti-tumor activity was determined by cell lysis with LDH assay, measuring released extracellular lactate dehydrogenase activity. The transfection procedure majorly refers to EXAMPLE 8. The ARE2-hTERT-DTA exhibited comparable ability of cell lysis with CMV-DTA in both hepatocellular carcinoma cell lines.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing diphtheria toxin, exhibited significantly superior ability to induce cell lysis in hepatocellular adenocarcinoma cell lines.

## EXAMPLE 11. EFFICENT EXPRESSION OF INTERLEUKIN 2 IN VARIOUS CANCER CELLS DRIVEN BY ARE-hTERT HYBRID PROMOTER

The anti-cancer therapeutic potential of hybrid promoter constructs ARE2-hTERT-hIL-2 were further test in vitro by determining their ability to secret functional recombinant hIL-2 in different cancer cell lines, relative to CMV promoter. The expression level of IL-2 was determined by ELISA assay, measuring tagged extracellular recombinant human IL-2. The transfection procedure majorly refers to above, while the concentration of IL-2 expressing vectors is altered to 5ug/ml. The ARE2-hTERT-IL-2 exhibited comparable IL-2 level in culture medium with CMV-IL-2 in multiple cancer cell lines. For functional analysis, culture medium from transfected cancers were used for CSFE labeled isolated CD3 T cells proliferation. The procedure of T cell isolation and CSFE assay were performed according to manufacturers' protocols. After co-culture 48 hours, the cells were collected for flow cytometry analysis. Compared to the CMV-IL-2 group, ARE2-hTERT-hIL-2 expressed similar capacity of facilitating T cell proliferation.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing recombinant human IL-2, exhibited significantly superior ability to active T cell immune response.

## EXAMPLE 12. EFFICENT EXPRESSION OF ANTIBODIES IN VARIOUS CANCER CELLS DRIVEN BY ARE-hTERT HYBRID PROMOTER

The anti-cancer therapeutic potential of hybrid promoter constructs ARE2-hTERT-αPD1 were further test in vitro by determining their ability to secret functional recombinant hIL-2 in different cancer cell lines, relative to CMV promoter. The expression level of αPD1 antibody was determined by ELISA assay, measuring tagged extracellular recombinant αPD1 antibody. The transfection procedure majorly refers to EXAMPLE 11. The ARE2-hTERT- αPD1 exhibited comparable antibody level in culture medium with CMV- αPD1 in multiple cancer cell lines. For functional analysis, culture medium from transfected cancers were used for CSFE labeled isolated CD3 T cells proliferation. The procedure of T cell isolation and CSFE assay

were performed according to manufacturers' protocols. After co-culture 48 hours, the cells were collected for flow cytometry analysis. Compared to the CMV- αPD1 group, ARE2-hTERT- αPD1 expressed similar capacity of facilitating T cell proliferation.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing recombinant human αPD1, exhibited significantly superior ability to active T cell immune response.

## EXAMPLE 13. SUPERIOR ANTI-LIVER CARINOMA ACTIVITY BY ARE-hTERT HYBRID PROMOTER DRIVEN GRANZYM B

The anti-cancer therapeutic potential of hybrid promoter constructs ARE2-hTERT-GZMB were further test in vitro by determining their ability to kill different cancer cell lines, relative to CMV promoter. Anti-tumor activity was determined by cell lysis with LDH assay, measuring released extracellular lactate dehydrogenase activity. The transfection procedure majorly refers to above EXAMPLE 7. ARE2-hTERT-GZMB exhibited comparable ability of cell lysis with CMV-GZMB in both colorectal carcinoma cell lines.

Thus, an ARE-hTERT hybrid promoter comprised vector, expressing recombinant human GZMB, exhibited significantly superior ability to kill cancer cells.

Sequences mentioned or used in the present invention:

hTRET core promoter:

GCACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTGGGGACCCGGGCA
CCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCGCGGACCCCGCCCCGTCCC
GACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCC
TTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCT
GCGCACGTGGGA (SEQ ID NO: 1)

hTERT-a:

CCACGTGGCGGAGGGACTGGGGA (SEQ ID NO: 2)

hTERT-b:

CTCCCGGGTCCCCGGCCCAGCCCCCTCCGGG (SEQ ID NO: 3)

hTERT-c:

CCCGGGCACCCGTCCTGCCCCTTC (SEQ ID NO: 4)

hTERT-d:

CCCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCA
GCCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCC (SEQ ID NO: 5)
hTERT-e:
CGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCC (SEQ ID NO: 6)
hTERT-f:
CAGCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCCTTTCCGCGGCCCCGC (SEQ ID NO: 7)

ARE1 cis elements from NQO1:
GATCCAGTCACAGTGACTCAGCAGAATCTG (SEQ ID NO: 8)
ARE1-a:
TCCAGTCA
ARE1-b:
AGCAGAATCTG (SEQ ID NO: 9)
ARE2 cis elements from GCLM:
CCGCGGGATGAGTAACGGTTACGAAGCACTTTCTCGGCTACGATTTCTGCTTAGTCAT
TGTCT (SEQ ID NO: 10)
ARE2-a:
TTAGTCA
ARE2-b:
TGCTTAGTCAT (SEQ ID NO: 11)
ARE2-c:
TGAGTAA

SV40 enhancer element:
GATCTGAACCATGGAGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATG
GGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATA
CTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGAT
GCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTG
ACACACATTCCAC (SEQ ID NO: 12)
SCGB2A2 enhancer:
CTGTCCTGAACATTGTTAGATGTTAAGAAGGTTTCAGTTGATGTTTAATTCCTGGCCTC
TGTCACTTGACCTCAATAGGACACTCACCCCCATCCAGTTGGGACAAGCAAAACCCA
CTATTGGGAAAGGTTCTTCAGGGTGGGGAAAGTGAAAAAGTGTCCCCACTGAGACCC

ATTGCTCCAGCAGCAGGGATACTTTGTATAAGTTCCTCCATATGCCTCTCCTGAGGCC
CTGGCGTAACAGAGGCTCATGACAGTGGGCTGACTACTGTTTTGGGATAATGTTGGC
ATTGAAGGGAGGGGAAAATTACTAACTCACTGGCAAAGGTACTAGTGAAGGTATCTG
ATGAGCCAAGGTAAACTTCCTCTCGCATATGCGCATTAACATTTTTAACCACACACAA
TGTGGGGACGTGGCGAGTTGTATAATTTCTGGGTTTTGGTACGGGAAAGAATTAGCCA
GGGGCTGACATTGACCTTGTCTCTGCTTTCAAATCCGGTTCCAGGTTCCAACTCATTA
GATACATCCTGGTCAGATGGTGTCACACTAACAGTCACTTCTAGGGCAAAAGGACAC
TGAGGTGGCCCAGGCTCTCATTAGCAGGGTTTCCAGCCATCTTCTTTCTTCACTACCTT
CTTCCTACCTGACCCCCAGTTTCCCTCCAGGAAAGTACAAATGGTGTTGTATTTACCA
TGTCCAGGAAGACACAGTGGTGTCTGATTTAAGTGTTACGTCTGATGTCTGTGTTTCG
TAAAACTTTAATTTTAAATGAGTTCCTTTTCCTGAATAGATCTAGTAATAAAACATAA
AATACAGTCAATACACTCTTTGTGTAAAATATCAAAATCTGCTCTTGAAAATGAGTTA
CACAATAGTAAATTAAGGAAAATAAGTAGTTTTCATCACTATAATACAAACAAGAAG
TGCCCAAGCCATGAGCTCAATGAGCAGGGACAGGGAGGAAGGGTCAGGGTGAGGGT
CCTGGAGATGTCAGATGGAACTGCCTATGGACAGATACAGGGTCCTGTGAACAGGGA
GGTCCCCAGAGATGCAGGGCCAGCAAAGAACACAAGGCATTAGCCATCTTTGATGTG
GCTTTTGGAATGAAGTG (SEQ ID NO: 13)

SCGB2A2 core promoter:

CTAGGCCCACTGCTGGGATAAGCTGGCACAGATACACGGGACATGATGCTTTCATTCT
CACCTTAAAGAAAGAGACAGAGAAGAAAAGGAAGGGGAGCAAGTGGAGAAGGAAT
ACAAGAAAGAGGAAGAGAAGCAGGAAGATTCTACATACAGGCTGGCTGTGTTTCCCC
TGGGGCATGCTCCTGTTTACTGGTCCCATGCCAGGTTGACTCATTGCCTCGTTCATGG
GTGGAATTAAGATGCCTACCTGGGGAATAAATAGAGCAAGGCTGGGTGCTCA (SEQ
ID NO: 14)

hCCRK promoter:

ACCCAGGTACCTATGTTCAAAAGTGCCTCAATCCTAGTTAACAAGGGCAGAGACCAC
GAGAAACAACACTGTGTTTAGTAGCAACTTAACAACCAGCCAGCAGTTCTGTCCACA
CACACACCACCGGGCATGGTTCCAAAGCTAAAAAGGCACTAATTGCTTTTCTATAAG
GAGGTAGAACACAGTCCCTCCGTGTTCTTTAGGCCTGATGGTCTGCATTATCGGATCT
GTTACCGTGTTAATTGTTCCTGTCTCACACAGCCGGTTTGGGCTTTCTTCTGCATATGT
CTGGGATGGTGACGGGTTCCTATATAGAGGAGTACTGGGGAAGCCTCTGTGTGTGTGT
GTGTGTCCGTGCATATGTACACATGTGTGTAAAAAGCAGCCACACGCTGAGAATGGT
TAACGGGTAGCCAGGCTGTCTGTACTCAGGGCCCTAAGACTGGCCCAGGAAAGGGCC
GGGGGAGGTGGGGCGGGGTGAAGGTGGAGCGGGCTTGGCTTGTGCTCACTGCCTTTT

CCACAACAGGAGTACAAATGCTGGAGTGAGTGAGGTGAACTCAAGTCGCCTTTAGGA
ATGGCTGAAAAAGCCCACACCTGGAAATCACTCCCTCCCTGCTCCTCCACGGCAGGTT
GCATCTGCGAGACGCTTCGGTCATTAGAGGAATGAGCCGGGAGTGAGCAATTCACCA
GCTCTCCAGCACTTGGTGGAAAGCAGCAGGCAACC (SEQ ID NO: 15)

CMVmini promoter:

GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAG
ATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGA
TCCAGCCTCCGCGGCCCCGCATTCGAGCTCGGTACCCGG (SEQ ID NO: 16)

E1F TATA promoter

AATTCGTGTAGTGTATTTATACCCGGTGAGTAGATCTG (SEQ ID NO: 17)

CG010201: SV40-hTERT-CMVmini:

GATCTGAACCATGGAGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATG
GGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATA
CTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGAT
GCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTG
ACACACATTCCACGCACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTG
GGGACCCGGGCACCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCGCGGAC
CCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCAG
CCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTCAGGCAGC
GCTGCGTCCTGCTGCGCACGTGGGAGGTAGGCGTGTACGGTGGGAGGCCTATATAAG
CAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGAC
CTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCCCGCATTCGAGCTCGGT
ACCCGG (SEQ ID NO: 18)

CG010301: hTERT-CMVmini:

GCACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTGGGGACCCGGGCA
CCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCGCGGACCCCGCCCCGTCCC
GACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCC
TTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCT
GCGCACGTGGGAGGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTA
GTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGA
CACCGGGACCGATCCAGCCTCCGCGGCCCCGCATTCGAGCTCGGTACCCGG (SEQ ID
NO: 19)

CG010401: hTERT-E1F TATA:

GCACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTGGGGACCCGGGCA
CCCGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCGCGGACCCCGCCCCGTCCC
GACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCC
TTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCT
GCGCACGTGGGAAATTCGTGTAGTGTATTTATACCCGGTGAGTAGATCTG (SEQ ID NO:
20)

CG010501: ARE1-hTERT-CMVmini:

GATCCAGTCACAGTGACTCAGCAGAATCTGGATCCAGTCACAGTGACTCAGCAGAAT
CTGGATCCAGTCACAGTGACTCAGCAGAATCTGGCACAGACGCCCAGGACCGCGCTC
CCCACGTGGCGGAGGGACTGGGGACCCGGGCACCCGTCCTGCCCCTTCACCTTCCAG
CTCCGCCTCCTCCGCGCGGACCCCGCCCCGTCCCGACCCCTCCCGGGTCCCCGGCCCA
GCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCG
CGGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCTGCGCACGTGGGAGGTAGGCGTGTA
CGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCG
GCCCCGCATTCGAGCTCGGTACCCGG (SEQ ID NO: 21)

CG010601: ARE2-hTERT-CMVmini:

TGAGTAACGGTTACGAAGCACTTTCTCGGCTACGATTTCTGCTTAGTCATTGTCTTGC
ACAGACGCCCAGGACCGCGCTCCCCACGTGGCGGAGGGACTGGGGACCCGGGCACC
CGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCCTCCGCGCGGACCCCGCCCCGTCCCG
ACCCCTCCCGGGTCCCCGGCCCAGCCCCCTCCGGGCCCTCCCAGCCCCTCCCCTTCCT
TTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTCAGGCAGCGCTGCGTCCTGCTG
CGCACGTGGGAGGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAG
TGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGAC
ACCGGGACCGATCCAGCCTCCGCGGCCCCGCATTCGAGCTCGGTACCCGG (SEQ ID
NO: 22)

CG010801: SCGB2A2-SCGB2A2-CMVmini:

CTGTCCTGAACATTGTTAGATGTTAAGAAGGTTTCAGTTGATGTTTAATTCCTGGCCTC
TGTCACTTGACCTCAATAGGACACTCACCCCCATCCAGTTGGGACAAGCAAAACCCA
CTATTGGGAAAGGTTCTTCAGGGTGGGGAAAGTGAAAAAGTGTCCCCACTGAGACCC
ATTGCTCCAGCAGCAGGGATACTTTGTATAAGTTCCTCCATATGCCTCTCCTGAGGCC
CTGGCGTAACAGAGGCTCATGACAGTGGGCTGACTACTGTTTTGGGATAATGTTGGC
ATTGAAGGGAGGGGAAAATTACTAACTCACTGGCAAAGGTACTAGTGAAGGTATCTG
ATGAGCCAAGGTAAACTTCCTCTCGCATATGCGCATTAACATTTTTAACCACACACAA

TGTGGGGACGTGGCGAGTTGTATAATTTCTGGGTTTTGGTACGGGAAAGAATTAGCCA
GGGGCTGACATTGACCTTGTCTCTGCTTTCAAATCCGGTTCCAGGTTCCAACTCATTA
GATACATCCTGGTCAGATGGTGTCACACTAACAGTCACTTCTAGGGCAAAAGGACAC
TGAGGTGGCCCAGGCTCTCATTAGCAGGGTTTCCAGCCATCTTCTTTCTTCACTACCTT
CTTCCTACCTGACCCCCAGTTTCCCTCCAGGAAAGTACAAATGGTGTTGTATTTACCA
TGTCCAGGAAGACACAGTGGTGTCTGATTTAAGTGTTACGTCTGATGTCTGTGTTTCG
TAAAACTTTAATTTTAAATGAGTTCCTTTTCCTGAATAGATCTAGTAATAAAACATAA
AATACAGTCAATACACTCTTTGTGTAAAATATCAAAATCTGCTCTTGAAAATGAGTTA
CACAATAGTAAATTAAGGAAAATAAGTAGTTTTCATCACTATAATACAAACAAGAAG
TGCCCAAGCCATGAGCTCAATGAGCAGGGACAGGGAGGAAGGGTCAGGGTGAGGGT
CCTGGAGATGTCAGATGGAACTGCCTATGGACAGATACAGGGTCCTGTGAACAGGGA
GGTCCCCAGAGATGCAGGGCCAGCAAAGAACACAAGGCATTAGCCATCTTTGATGTG
GCTTTTGGAATGAAGTGCTAGGCCCACTGCTGGGATAAGCTGGCACAGATACACGGG
ACATGATGCTTTCATTCTCACCTTAAAGAAAGAGACAGAGAAGAAAAGGAAGGGGA
GCAAGTGGAGAAGGAATACAAGAAAGAGGAAGAGAAGCAGGAAGATTCTACATACA
GGCTGGCTGTGTTTCCCCTGGGGCATGCTCCTGTTTACTGGTCCCATGCCAGGTTGAC
TCATTGCCTCGTTCATGGGTGGAATTAAGATGCCTACCTGGGGAATAAATAGAGCAA
GGCTGGGTGCTCA (SEQ ID NO: 23)

CG010A01: hCCRK:

ACCCAGGTACCTATGTTCAAAAGTGCCTCAATCCTAGTTAACAAGGGCAGAGACCAC
GAGAAACAACACTGTGTTTAGTAGCAACTTAACAACCAGCCAGCAGTTCTGTCCACA
CACACACCACCGGGCATGGTTCCAAAGCTAAAAAGGCACTAATTGCTTTTCTATAAG
GAGGTAGAACACAGTCCCTCCGTGTTCTTTAGGCCTGATGGTCTGCATTATCGGATCT
GTTACCGTGTTAATTGTTCCTGTCTCACACAGCCGGTTTGGGCTTTCTTCTGCATATGT
CTGGGATGGTGACGGGTTCCTATATAGAGGAGTACTGGGGAAGCCTCTGTGTGTGTGT
GTGTGTCCGTGCATATGTACACATGTGTGTAAAAAGCAGCCACACGCTGAGAATGGT
TAACGGGTAGCCAGGCTGTCTGTACTCAGGGCCCTAAGACTGGCCCAGGAAAGGGCC
GGGGGAGGTGGGGCGGGGTGAAGGTGGAGCGGGCTTGGCTTGTGCTCACTGCCTTTT
CCACAACAGGAGTACAAATGCTGGAGTGAGTGAGGTGAACTCAAGTCGCCTTTAGGA
ATGGCTGAAAAAGCCCACACCTGGAAATCACTCCCTCCCTGCTCCTCCACGGCAGGTT
GCATCTGCGAGACGCTTCGGTCATTAGAGGAATGAGCCGGGAGTGAGCAATTCACCA
GCTCTCCAGCACTTGGTGGAAAGCAGCAGGCAACC (SEQ ID NO: 24)

CG010C01: H19:

CCAGTCTCCACTCCACTCCCAACCGTGGTGCCCCACGCGGGCCTGGGAGAGTCTGTGA
GGCCGCCCACCGCTTGTCAGTAGAGTGCGCCCGCGAGCCGTAAGCACAGCCCGGCAA
CATGCGGTCTTCAGACAGGAAAGTGGCCGCGAATGGGACCGGGGTGCCCAGCGGCTG
TGGGGACTCTGTCCTGCGGAAACCGCGGTGACCAGCACAAGCTCGGTCAACTGGATG
GGAATCGGCCTGGGGGGCTGGCACCGCGCCCACCAGGGGGTTTGCGGCACTTCCCTC
TGCCCCTCAGCACCCCACCCCTACTCTCCAGGAACGTGAGGTCTGAGCCGTGATGGTG
GCAGGAAGGGGCCCTCTGTGCCATCCGAGTCCCCAGGGACCCGCAGCTGGCCCCCAG
CCATGTGCAAAGTATGTGCAGGGCGCTGGCAGGCAGGGAGCAGCAGGCATGGTGTCC
CCTGAGGGGAGACAGTGGTCTGGGAGGGAGAAGTCCTGGACCCTGAGGGAGGTGAT
GGGGCAATGCTCAGCCCTGTCTCCGGATGCCAAAGGAGGGGTGCGGGGAGGCCGTCT
TTGGAGAATTCCAGGATGGGTGCTGGGTGAGAGAGACGTGTGCTGGAACTGTCCAGG
GCGGAGGTGGGCCCTGCGGGGGCCCTCGGGAGGGCCCTGCTCTGATTGGCCGGCAGG
GCAGGGGCGGGAATTCTGGGCGGGGCCACCCCAGTTAGAAAAAGCCCGGGCTAGGA
CCGAGGAGCAGGGTGAGGGAGGGGGTGGGATGGGTGGGGGGTAACGGGGGAAACTG
GGGAAGTGGGGAACCGA (SEQ ID NO: 25)

CG011001: TYR:

GACCTTTATTCATAAGAGATGATGTATTCTTGATACTACTTCTCATTTGCAAATTCCAA
TTATTATTAATTTCATATCAATTAGAATAATATATCTTCCTTCAATTTAGTTACCTCAC
TATGGGCTATGTACAAACTCCAAGAAAAAGTTAGTCATGTGCTTTGCAGAAGATAAA
AGCTTAGTGTAAAACAGGCTGAGAGTATTTGATGTAAGAAGGGGAGTGGTTATATAG
GTCTTAGCCAAAACATGTGATAGTCACTCCAGGGGTTGCTGGAAAAGAAGTCTGTGA
CACTCATTAACCTATTGGTGCAGAATTTGAATGATCTAAAGGAGACC (SEQ ID NO: 26)

CG011101: TYR-TYR:

CCTGCAGGTCATAGTTCCTGCCAGCTGACTTTGTCAAGACAGTGATGTCTGTGTTCCA
GCAGTTGTTCTGAGTATCCTTTTCATTATCCACTGTCCTTTCTTCTTAAATTCCACCCCC
AACATTGTAAATAGCTTCTTTCTTAAACTCTGTTCAAAGAACCAGCTTGAGTGTGTCA
GCTGCTTCCTGCTGGGGTCCTGGCAAACCAGACCTTTATTCATAAGAGATGATGTATT
CTTGATACTACTTCTCATTTGCAAATTCCAATTATTATTAATTTCATATCAATTAGAAT
AATATATCTTCCTTCAATTTAGTTACCTCACTATGGGCTATGTACAAACTCCAAGAAA
AAGTTAGTCATGTGCTTTGCAGAAGATAAAAGCTTAGTGTAAAACAGGCTGAGAGTA
TTTGATGTAAGAAGGGGAGTGGTTATATAGGTCTTAGCCAAAACATGTGATAGTCACT
CCAGGGGTTGCTGGAAAAGAAGTCTGTGACACTCATTAACCTATTGGTGCAGAATTT
GAATGATCTAAAGGAGACC (SEQ ID NO: 27)

CG011201: SV40-TYR:

CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGC
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CCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGA
AAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCA
GCAACCATAGTCCCACTGCGACCTTTATTCATAAGAGATGATGTATTCTTGATACTAC
TTCTCATTTGCAAATTCCAATTATTATTAATTTCATATCAATTAGAATAATATATCTTC
CTTCAATTTAGTTACCTCACTATGGGCTATGTACAAACTCCAAGAAAAAGTTAGTCAT
GTGCTTTGCAGAAGATAAAAGCTTAGTGTAAAACAGGCTGAGAGTATTTGATGTAAG
AAGGGGAGTGGTTATATAGGTCTTAGCCAAAACATGTGATAGTCACTCCAGGGGTTG
CTGGAAAAGAAGTCTGTGACACTCATTAACCTATTGGTGCAGAATTTGAATGATCTAA
AGGAGACC (SEQ ID NO: 28)

CG011301: TRP1:

CAGGCTTACATTTGAATGTTGGCTACATATGTATGAGTTTTCAACTTCCAGGAGAAAA
CGTCTCTTTAAAAGAGAACAACCAAAAGCTAACAGAAAATACAAGTGTGACATTGGC
CTTAGTTCGACCAAGAAAGCAATTCATCTTGTTTCTTCCTTTGTGGTATACAGATAAA
GAAAAATAAAATCACTACAACGAAAGCAAAATCTCTTCAGCGTCTCTAATACATCTT
CCAAATCAGTGTGTCTGACCTTTTCTTAAGACTTTAACCATCACAAGGAAACCAGTGG
GGAGGGAGTCATGTGCTGCCTAGTAGTTAAAGGGCAGGAGAATTCACTGGTGTGAGA
AGGGATTAGTGAGAGCTGGAAGAGAGGACCAGCCCCTCCCAGTGTGAGGAATCTGGC
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TGAAAGATTCCATTTCCGGAGTGGGTTGAAAAAGTATGCAAAAGAACTTTTGCAACT
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GCAAAGAGCAGCATAGGAGACCTGTGTTCTGAACTCTTGCTTCGAGAAC (SEQ ID NO:
29)

CG010D01: AFP-:

AGTTTGAGGAGAATATTTGTTATATTTGCAAAATAAAATAAGTTTGCAAGTTTTTTTTT
TCTGCCCCAAAGAGCTCTGTGTCCTTGAACATAAAATACAAATAACCGCTATGCTGTT
AATTATTGGCAAATGTCCCATTTTCAACCTAAGGAAATACCATAAAGTAACAGATAT
ACCAACAAAAGGTTACTAGTTAACAGGCATTGCCTGAAAAGAGTATAAAAGAATTTC
AGCATGATTTTCCATATTGTGCTTCCACCACTGCCAATAACAC (SEQ ID NO: 30)

CG010E01: AFP-AFP:

CCTGCAGGCTTAGAAATATGGGGGTAGGGGTGGTGGTGGTAATTCTGTTTTCTCCCCA
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ATTAAGGGACAGACTATGGGCTGGAGGACTTTGAGGATGTCTGTCTCATAACACTTG
GGTTGTATCTGTTCTATGGGGCTTGTTTTAAGCTTGGCAACTTGCAACAGGGTTCACT
GACTTTCTCCCCAGGCCCAAGGTACTGTCCTCTTTTCATATCTGTTTTGGGGCCTCTGG
GGCTTGAATATCTGAGAAAATATAAACATTTCAATAATGTTCTGTGGTGAGATGAGTA
TGAGAGATGTGTCATTCATTTGTATCAATGAATGAATGAGGACAATTAGTGTATAAAT
CCTTAGTACAACAATCTGAGGGTAGGGGTGGTACTATTCAATTTCTATTTATAAAGAT
ACTTATTTCTATTTATTTATGCTTGTGACAAATGTTTTGTTCGGGACCACAGGAATCAC
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TAATAAGTTGTCAAAGGTGAGAGGGCTCTTCTCTAGCTAGAGACTAATCAGAAATAC
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CTAACATCATGCTAATAATGATAATAATTACTGTCATTTTTTAATGTCTATAAGTACCA
GGCATTTAGAAGATATTATTCCATTTATATATCAAAATAAACTTGAGGGGATAGATCA
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AAACGGAATCAGAATTACATCAATGCAAAAGCATTGCTGTGAACTCTGTACTTAGGA
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GCTTATGATTCCCAAATgTCTATCTCTAGCCTCAATCTTGTTCCAGAAGATAAAAAGT
AGTATTCAAATGCACATCAACGTCTCCACTTGGAGGGCTTAAAGACGTTTCAACATAC

AAACCGGGGAGTTTTGCCTGGAATGTTTCCTAAAATGTGTCCTGTAGCACATAGGGTC
CTCTTGTTCCTTAAAATCTAATTACTTTTAGCCCAGTGCTCATCCCACCTATGGGGAGA
TGAGAGTGAAAAGGGAGCCTGATTAATAATTACACTAAGTCAATAGGCATAGAGCCA
GGACTGTTTGGGTAAACTGGTCACTTTATCTTAAACTAAATATATCCAAAACTGAACA
TGTACTTAGTTACTAAGTCTTTGACTTTATCTCATTCATACCACTCAGCTTTATCCAGG
CCACTagAGTTTGAGGAGAATATTTGTTATATTTGCAAAATAAAATAAGTTTGCAAGTT
TTTTTTTTCTGCCCCAAAGAGCTCTGTGTCCTTGAACATAAAATACAAATAACCGCTA
TGCTGTTAATTATTGGCAAATGTCCCATTTTCAACCTAAGGAAATACCATAAAGTAAC
AGATATACCAACAAAAGGTTACTAGTTAACAGGCATTGCCTGAAAAGAGTATAAAAG
AATTTCAGCATGATTTTCCATATTGTGCTTCCACCACTGCCAATAACAC (SEQ ID NO:
31)

CG010F01: SV40-AFP:

CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGC
TGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCC
CCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGA
AAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCA
GCAACCATAGTCCCACTGCAGTTTGAGGAGAATATTTGTTATATTTGCAAAATAAAAT
AAGTTTGCAAGTTTTTTTTTTCTGCCCCAAAGAGCTCTGTGTCCTTGAACATAAAATAC
AAATAACCGCTATGCTGTTAATTATTGGCAAATGTCCCATTTTCAACCTAAGGAAATA
CCATAAAGTAACAGATATACCAACAAAAGGTTACTAGTTAACAGGCATTGCCTGAAA
AGAGTATAAAAGAATTTCAGCATGATTTTCCATATTGTGCTTCCACCACTGCCAATAA
CAC (SEQ ID NO: 32)

# CLAIMS

1. An expression cassette, comprising:

1) a chimeric promoter, comprising an hTERT promoter and a minimal promoter;

2) a coding sequence (CDS); and

3) a poly(A) sequence or a polyadenylation signal sequence.

2. The expression cassette of claim 1, wherein the hTERT promoter is an hTERT core promoter, preferably comprising at least one of the sequences of SEQ ID NOs: 1-7 or a sequence having at least 90% sequence identity thereto.

3. The expression cassette of claim 1 or 2, wherein the minimal promoter is a minimal viral promoter or a minimal TATA box promoter.

4. The expression cassette of any one of claims 1-3, wherein the minimal promoter is a minimal CMV promoter, preferably comprising the sequence of SEQ ID NO: 16 or a sequence having at least 90% sequence identity thereto.

5. The expression cassette of any one of claims 1-4, wherein the minimal TATA box promoter is an E1F TATA box promoter, preferably comprising the sequence of SEQ ID NO: 17 or a sequence having at least 90% sequence identity thereto.

6. The expression cassette of any one of claims 1-5, further comprising one or more anti-oxidative response elements.

7. The expression cassette of any one of claims 1-6, wherein the anti-oxidative response element is from NQO1 or GCLM.

8. The expression cassette of any one of claims 1-7, wherein the anti-oxidative response element comprises the sequence of SEQ ID NO: 8 or a sequence having at least 90% sequence identity thereto, or the anti-oxidative response element comprises the sequence of SEQ ID NO: 10 or a sequence having at least 90% sequence identity thereto.

9. The expression cassette of any one of claims 1-8, further comprising one or more enhancers.

10. The expression cassette of any one of claims 1-9, wherein the enhancer is a SV40 enhancer, preferably comprising the sequence of SEQ ID NO: 12 or a sequence having at least 90% sequence identity thereto.

11. The expression cassette of any one of claims 1-10, wherein the coding sequence encodes a protein that is capable of inducing death of a cell once the protein is expressed by the cell.

12. The expression cassette of any one of claims 1-11, wherein the protein is selected from the group consisting of a cytotoxin, a tumor-associated antigen, a tumor specific antigen, a cytokine, a membrane penetrator, a checkpoint protein and an antibody.

13. The expression cassette of any one of claims 1-12, wherein the protein is neutrophile elastase, diphtheria toxin, IL-2 or granzyme B.

14. The expression cassette of any one of claims 1-13, wherein, when introduced into a non-tumor cell, the expression cassette is not expressed in the non-tumor cell, or is expressed at a level lower than that in a tumor cell.

15. A nucleic acid molecule comprising the expression cassette of any one of claims 1-14.

16. A vector comprising the expression cassette of any one of claims 1-14 or the nucleic acid molecule of claim 15.

17. The vector of claim 16, wherein the vector is a viral vector, preferably, a lentivirus vector, a retrovirus vector, an adeno-associated virus (AAV) vector, or an adenovirus vector.

18. A composition comprising

1) the nucleic acid molecule of claim 15 or the vector of any one of claims 16-18; and

2) a pharmaceutically acceptable carrier and/or a deliver agent.

19. The composition of claim 18, wherein the deliver agent is a biodegradable polymer, preferably, a in vivo jetPEI (Polyethylenimine), hyperbranched Poly(β-amino ester)s (HPAEs), lipid nanoparticle (LNP), liposomes or exosome.

20. The composition of claim 18 or 19 further comprising one or more anti-cancer agents.

21. Use of the expression cassette of any one of claims 1-14, the nucleic acid molecule of claim 15 or the vector of claim 16 or 17 in the manufacture of a medicament for the treatment of a cancer.

22. A method of treating cancer, comprising administrating to a subject in need thereof a therapeutically effective amount of the nucleic acid molecule of claim 15, the vector of claim 16 or 17, or the composition of any one of claims 18-20.

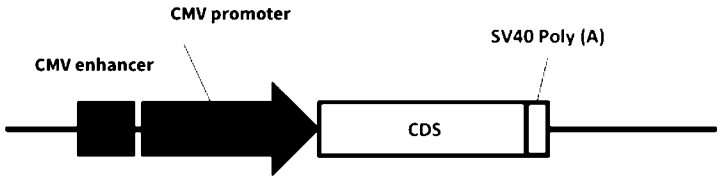
23. The method of claim 22, wherein the cancer is a solid tumor, preferably, a breast cancer, endometrial cancer, ovarian cancer, cervical cancer, lung cancer, gastric cancer, esophageal cancer, colorectal cancer, anal cancer, urothelial cancer, pancreatic

cancer, salivary gland cancer, melanoma, skin cancer, head and neck cancer, sarcoma or brain cancer.

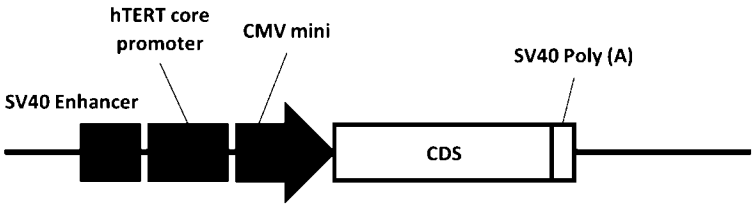
24. The method of claim 22, wherein the cancer is a hematological malignancy, preferably, a multiple myeloma (MM), lymphoma, chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), or acute myelogenous leukemia (AML), diffuse large B cell lymphoma (DLBCL), non-Hodgkin lymphoma (NHL), extranodal NK/T cell lymphoma (ENKL), Hodgkin Lymphoma (HL), plasmablastic lymphoma, Burkitt's lymphoma, marginal zone lymphoma (MZL), or mantle cell lymphoma (MCL).

**FIGURE 1.**

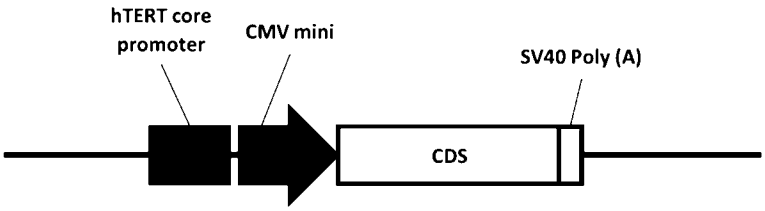
CMV-CDS-Poly(A):



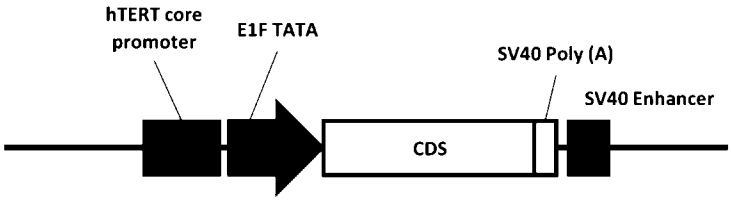

CG010201: SV40-hTERT-CMVmini-CDS-Poly(A):




CG010301: hTERT-CMVmini-CDS-Poly(A):




CG010401: hTERT-E1F TATA-CDS-Poly(A)-SV40:




CG010501: ARE1-hTERT-CMVmini-CDS-Poly(A)-SV40:

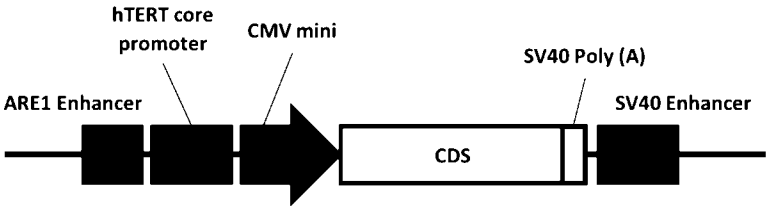
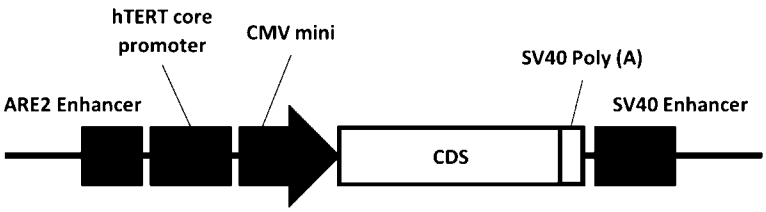
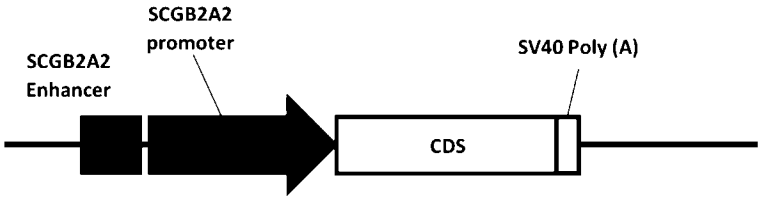


**FIGURE 1 (cont.)**

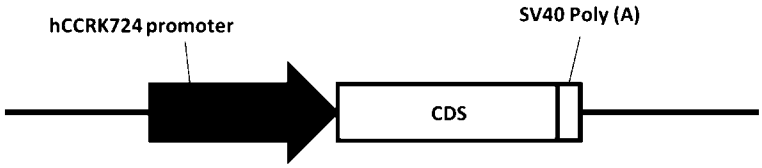
CG010601: ARE2-hTERT-CMVmini-CDS-Poly(A)-SV40:



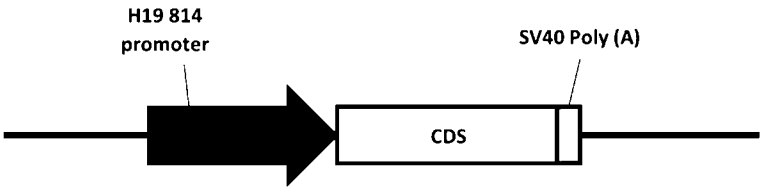

CG010801: SCGB2A2-SCGB2A2-CMVmini-CDS-Poly(A):




CG010A01: hCCRK-CDS-Poly(A):




CG010C01: H19-CDS-Poly(A):




CG011001: TYR-CDS-Poly(A):

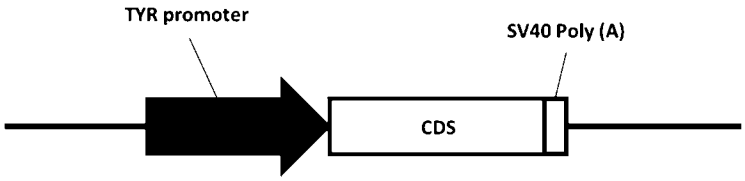
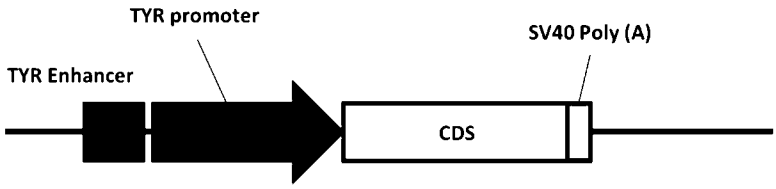
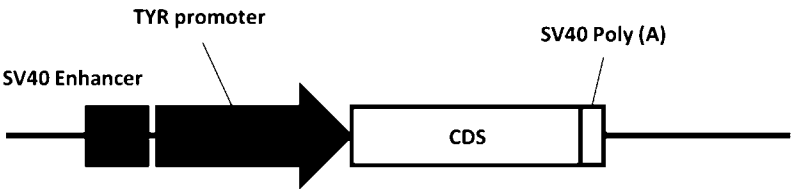


**FIGURE 1 (cont.)**

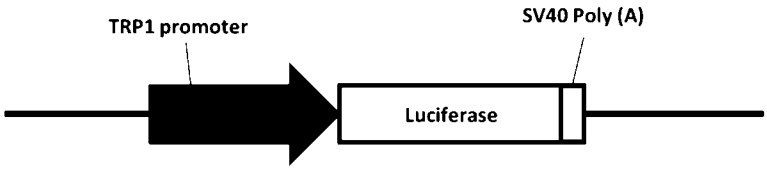
CG011101: TYR-TYR-CDS-Poly(A):



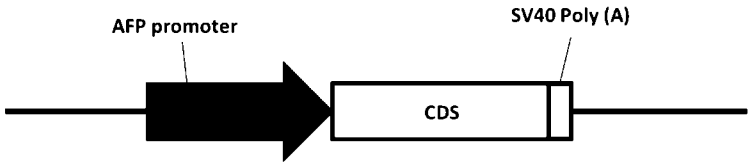

CG011201: SV40-TYR-CDS-Poly(A):




CG011301: TRP1-CDS-Poly(A):




CG010D01: AFP-CDS-Poly(A):




CG010E01: AFP-AFP-CDS-Poly(A):

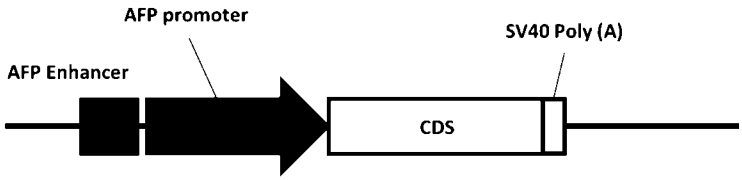


**FIGURE 1 (cont.)**

CG010F01: SV40-AFP-CDS-Poly(A):

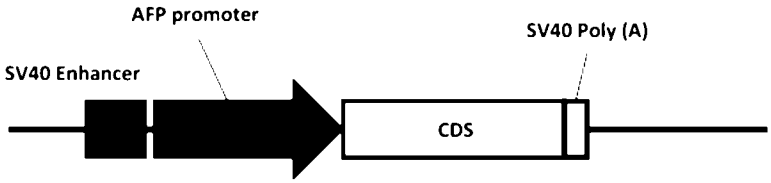



**FIGURE 2.**

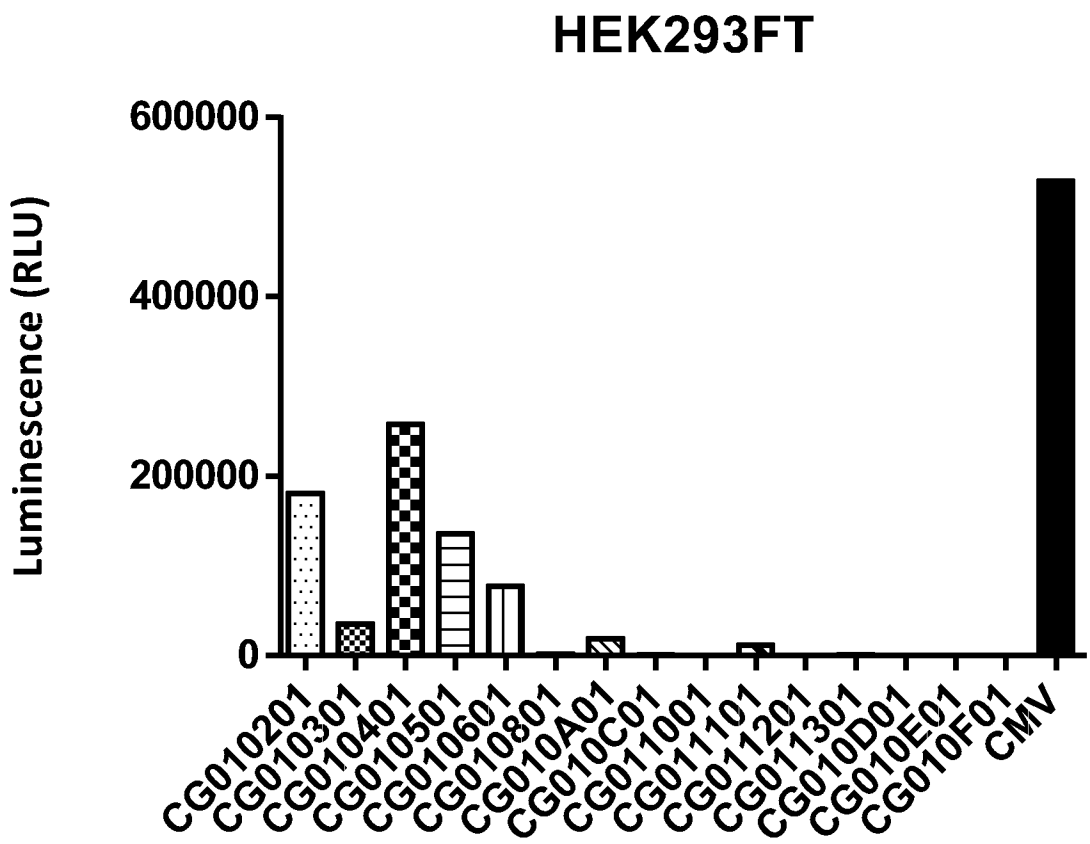
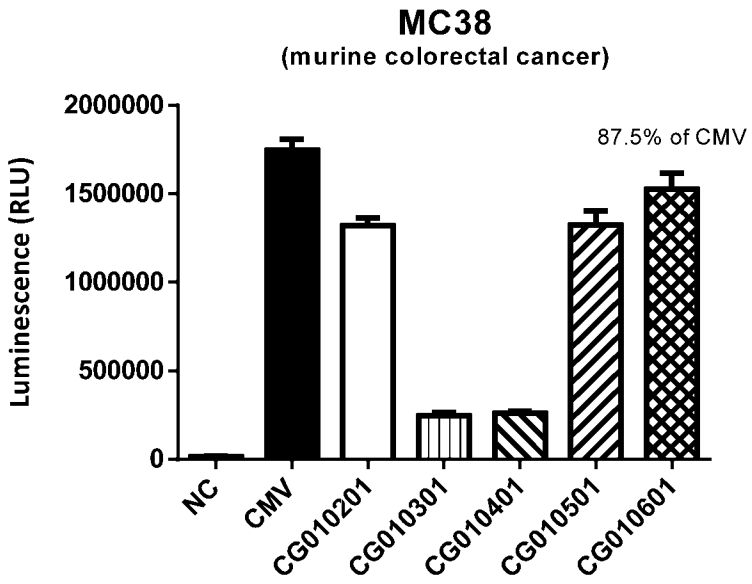


FIGURE 3.

A)


MC38
(murine colorectal cancer)
Luminescence (RLU)
2000000
1500000
1000000
500000
0
87.5% of CMV
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

B)

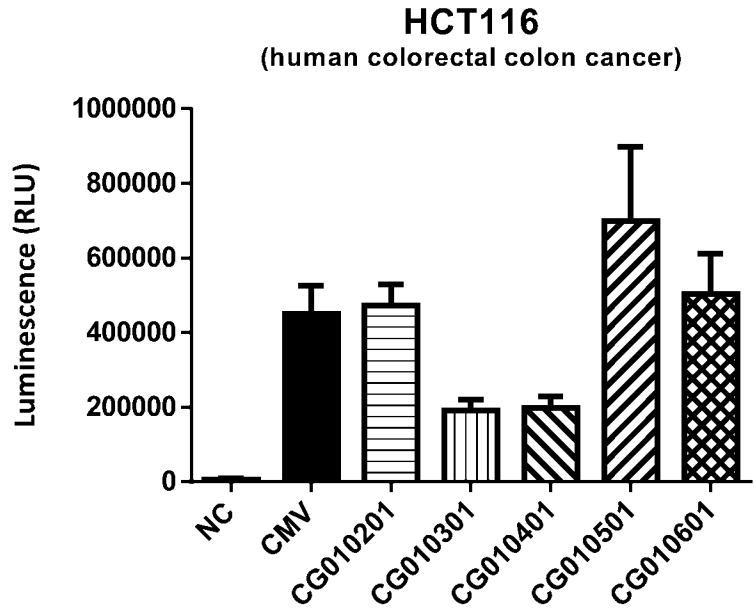
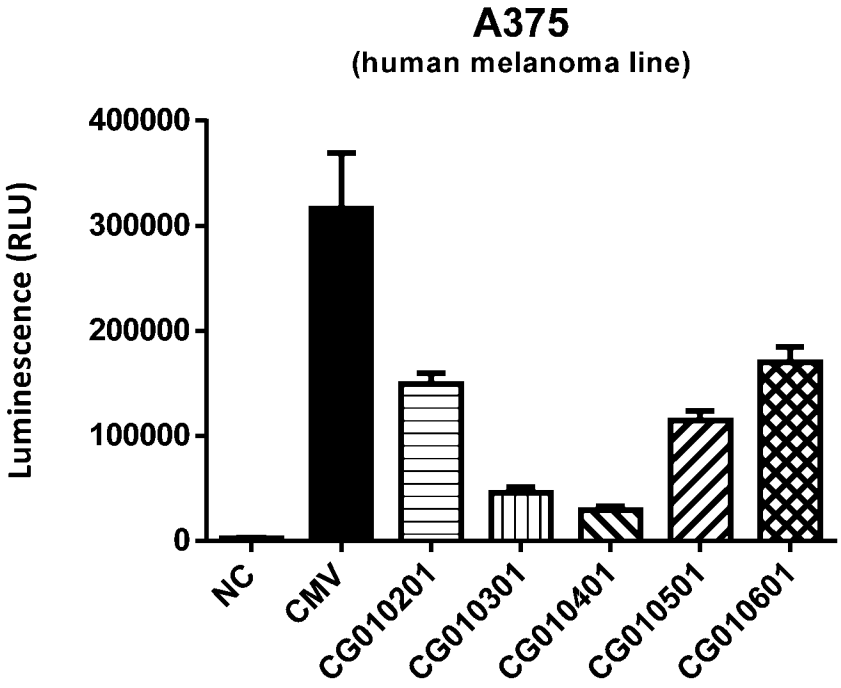

HCT116
(human colorectal colon cancer)
Luminescence (RLU)
1000000
800000
600000
400000
200000
0
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

FIGURE 4.

A)


A375
(human melanoma line)
Luminescence (RLU)
400000
300000
200000
100000
0
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

B)

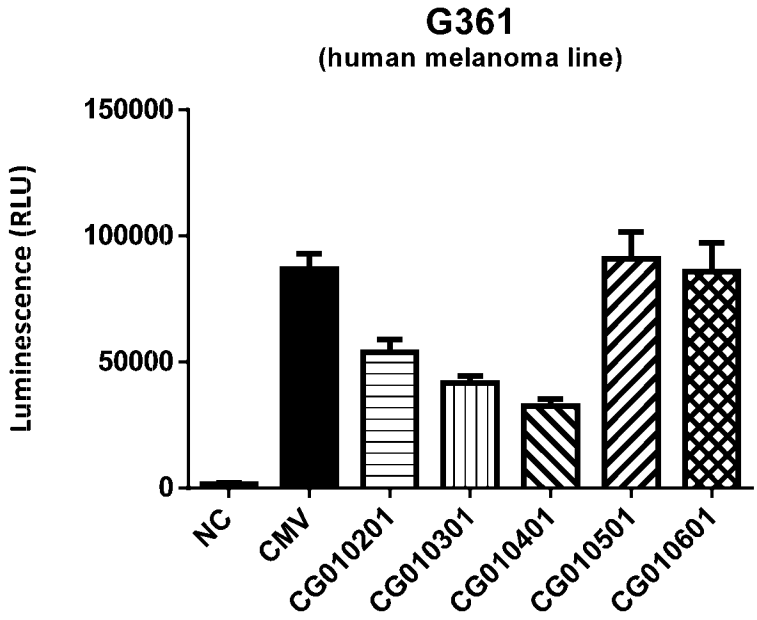

G361
(human melanoma line)
Luminescence (RLU)
150000
100000
50000
0
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

FIGURE 4. (cont.)

C)

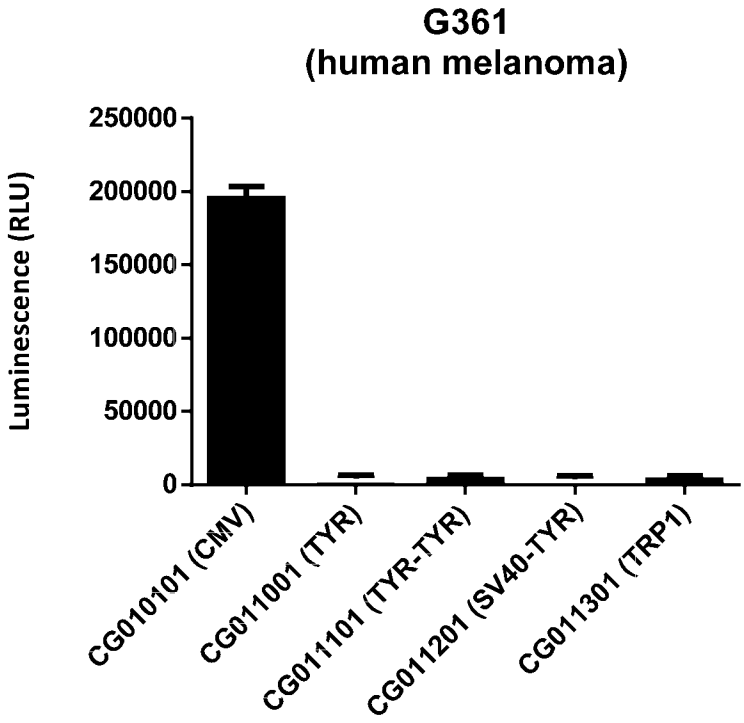


FIGURE 5.

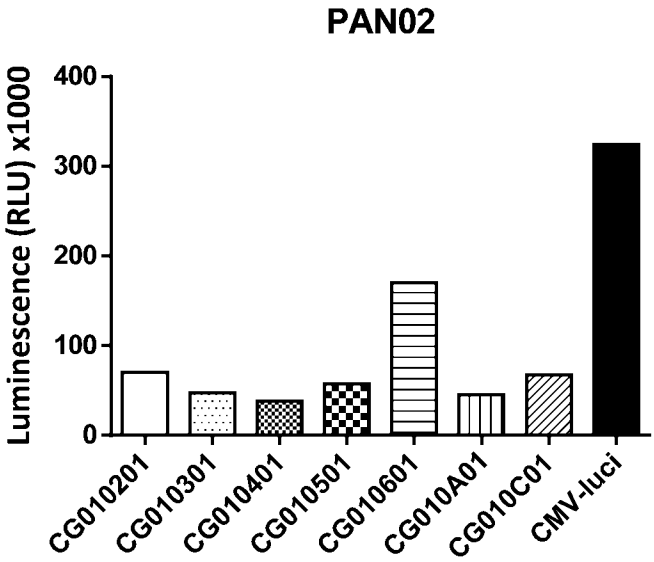
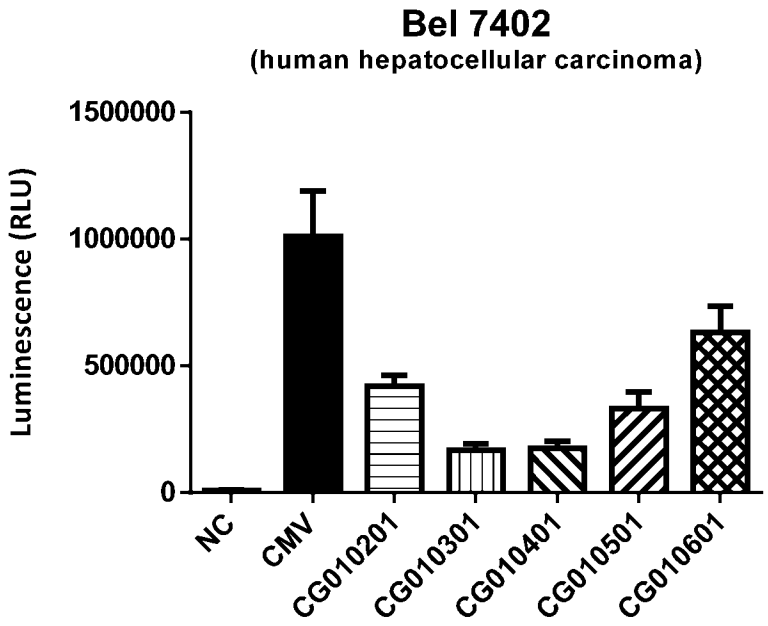

PAN02
Luminescence (RLU) x1000
400
300
200
100
0
CG010201
CG010301
CG010401
CG010501
CG010601
CG010A01
CG010C01
CMV-luci

FIGURE 6.

A)


Bel 7402
(human hepatocellular carcinoma)
Luminescence (RLU)
1500000
1000000
500000
0
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

B)

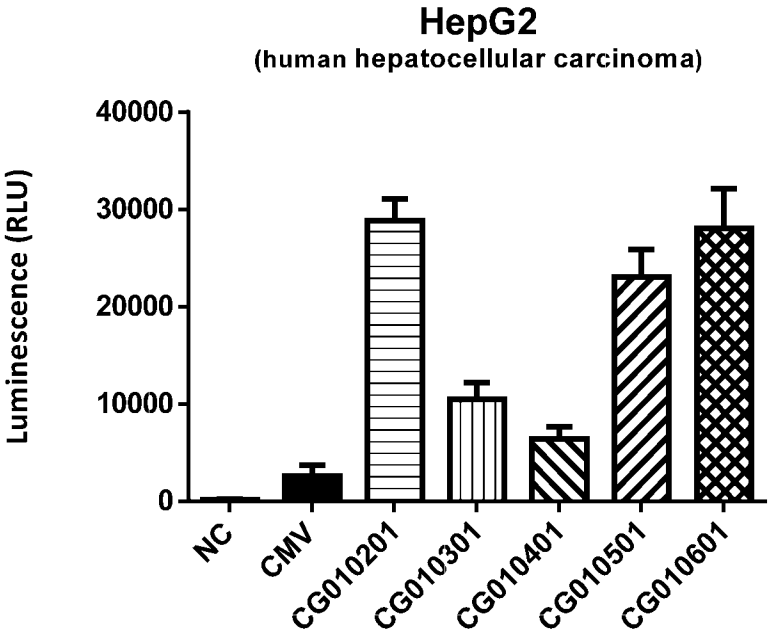
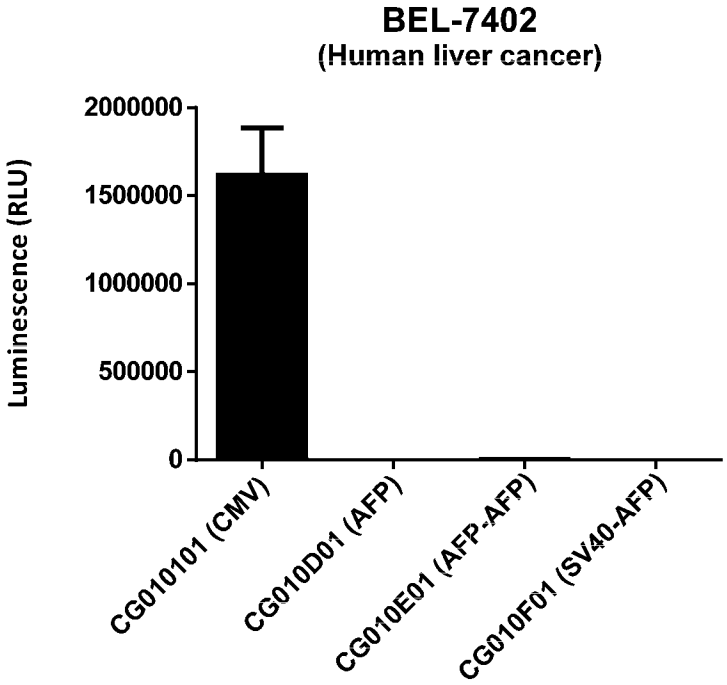

HepG2
(human hepatocellular carcinoma)
Luminescence (RLU)
40000
30000
20000
10000
0
NC
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

FIGURE 6. (cont.)

C)




D)

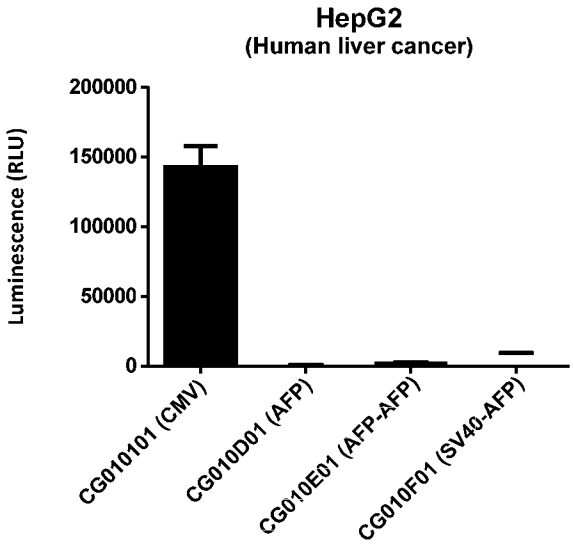
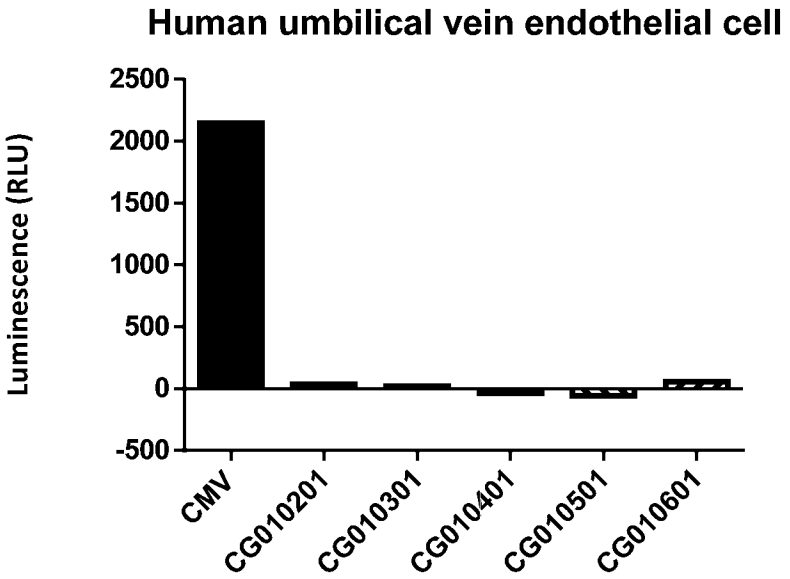
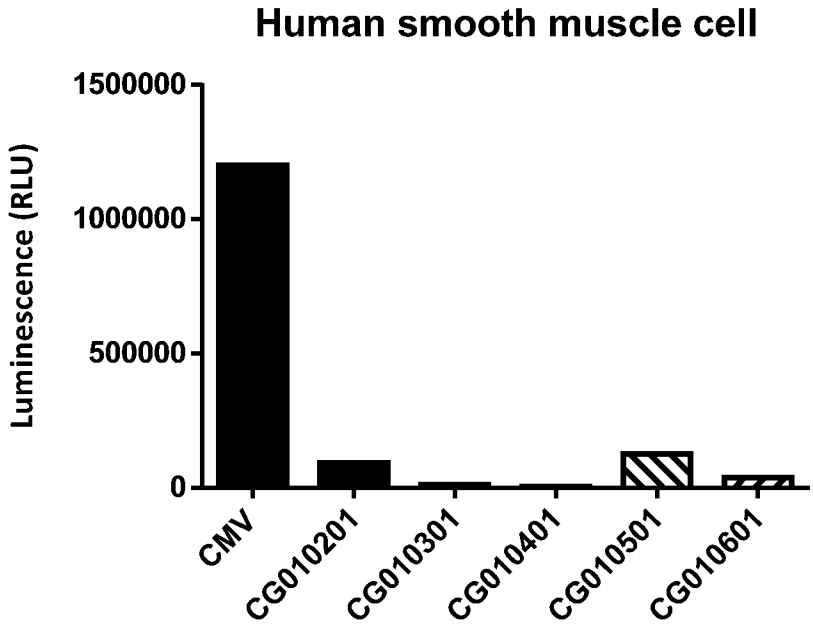


FIGURE 7.

A)


Human umbilical vein endothelial cell
Luminescence (RLU)
2500
2000
1500
1000
500
0
-500
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

B)


Human smooth muscle cell
Luminescence (RLU)
1500000
1000000
500000
0
CMV
CG010201
CG010301
CG010401
CG010501
CG010601

# INTERNATIONAL SEARCH REPORT

International application No.
**PCT/CN2024/099233**

## A. CLASSIFICATION OF SUBJECT MATTER

C12N15/11(2006.01)i; C12N15/861(2006.01)i; C12N15/867(2006.01)i; A61K31/711(2006.01)i; A61K48/00(2006.01)i; A61P35/00(2006.01)i; A61P35/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:C12N,A61K,A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNTXT, ENTXT, VCN, DWPI, VEN, duxiu, chinese patent biological sequence search system, CNKI, wanfang, ISI web of knowledge, Elsevier Science Direct, Springerlink, pubmed, genbank, ENA, EMBL, Uniprot, STN, baidu, patentics, incopat: NANJING CUREGENE TECHNOLOGY CO, XIAO Qi, SHAO Cuiying, hTERT, promoter, polyadenylation, poly(A), polyA, minimal viral promoter , TATA box, minimal CMV promoter, anti-oxidative response elements, antioxidative response elements, AREs NQO1, GCLM, SV40, enhancer, surfactant, cancer, tumor, SEQ ID NOs:1-12

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2007092968 A1 (JI,L.X. et al.) 26 April 2007 (2007-04-26)<br>abstract, claims 1-117, description paragraphs 19,26,66-131,183,232 | 1-4,9,11-24 |
| Y | US 2007092968 A1 (JI,L.X. et al.) 26 April 2007 (2007-04-26)<br>abstract, claims 1-117, description paragraphs 19,26,66-131,183,232 | 4-24 |
| Y | KALINICHENKO,S.V. et al. "A Novel Hybrid Promoter ARE-hTERT for Cancer Gene Therapy"<br>*Acta Naturae.*, Vol. 9, No. 4, 31 December 2017 (2017-12-31),<br>page 66 abstract, page 67 left column paragraph 2 to right column paragraph 1 | 6-24 |
| Y | SHEPELEV,M.V. et al. "hTERT and BIRC5 gene promoters for cancer gene therapy: A comparative study"<br>*Oncol Lett.*, Vol. 12, No. 2, 15 June 2016 (2016-06-15),<br>page 1204 abstract, page 1205 left column paragraph 2 and figure.1C | 4-24 |

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| **20 September 2024** | **26 September 2024** |
| Name and mailing address of the ISA/CN<br>**CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION**<br>**6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China** | Authorized officer<br>**LI,Nan**<br>Telephone No. **(+86) 010-53962096** |

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | CN 103596929 A (REATA PHARM CO.) 19 February 2014 (2014-02-19)<br>description paragraph 274 | 7-24 |
| Y | CN 101993892 A (CHANGSHA YINGRUN BIOTECHNOLOGY CO., LTD.) 30 March 2011 (2011-03-30)<br>abstract, claims 1-9 | 10-24 |
| A | SONG,J.S. "Activity of the human telomerase catalytic subunit (hTERT) gene promoter could be increased by the SV40 enhancer"<br>*Biosci Biotechnol Biochem.,* Vol. 68, No. 8, 31 August 2004 (2004-08-31),<br>page 1634 abstract | 1-24 |

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/CN2024/099233**

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
**PCT/CN2024/099233**

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **22-24**
because they relate to subject matter not required to be searched by this Authority, namely:

The inventions set forth in claims 22-24 pertain to methods for treating cancer and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of PCT Rule 39.1(iv), to search. However, the search is carried out on the basis of the nucleic acid molecule, the vector, or the composition in the manufacture of a medicament for treating cancer.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**INTERNATIONAL SEARCH REPORT**
**Information on patent family members**

International application No.
**PCT/CN2024/099233**

| Patent document cited in search report | Publication date (day/month/year) | Patent family member(s) | Publication date (day/month/year) |
|---|---|---|---|
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| | | JP 2008532521 A | 21 August 2008 |
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| | | CY 1120032 T1 | 12 December 2018 |
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| | | HRP 20171859 T1 | 26 January 2018 |
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| | | SI 2651902 T1 | 28 February 2018 |
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| | | KR 102000301 B1 | 15 July 2019 |
| | | JP 2014501250 A | 20 January 2014 |
| | | JP 5857068 B2 | 10 February 2016 |
| | | EP 2651902 A2 | 23 October 2013 |
| | | EP 2651902 B1 | 08 November 2017 |

**INTERNATIONAL SEARCH REPORT**
**Information on patent family members**

International application No.
**PCT/CN2024/099233**

| Patent document cited in search report | Publication date (day/month/year) | Patent family member(s) | Publication date (day/month/year) |
|---|---|---|---|
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| | | AU 2011343466 B2 | 05 January 2017 |
| | | TW 201249785 A | 16 December 2012 |
| | | TWI 527788 B | 01 April 2016 |
| | | MX 2013006902 A | 17 October 2013 |
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| | | CO 6910185 A2 | 31 March 2014 |
| CN 101993892 A | 30 March 2011 | None | |