

Claims

1. A trans-splicing RNA (tsRNA) molecule comprising:
 - a) at least one binding domain specific for at least a part of a gene that associates with or is a biomarker for a cell or a disease to be treated; and
 - b) nucleic acids encoding at least one or more expressible
suicide protein or a protein that is a component of a suicide system; or
fluorescent protein, luciferase or other reporter protein; or
therapeutic protein; and
 - c) at least one splice signal; and
 - d) at least one safety domain specific for the splice signal or splice site within the trans-splicing RNA.
2. The trans-splicing RNA (tsRNA) molecule according to claim 1 wherein one or more of the following apply:
 - 1) said binding domain comprises a binding site comprising at least 25, 35, 45, 55 or more consecutive unstructured nucleotides (nt) having no internal binding and/or self-complementary sequences and; said binding domain, when of a length of 44 nt or longer, has at least one, or a plurality of, mismatch nucleotide(s) with respect to said gene;
 - 2) wherein said safety domain is an antisense binding domain specific for a splice site in the splice signal whereby the safety domain prevents off-target trans-splicing;
 - 3) wherein said safety domain is either a linear sequence of nucleic acids, termed a continuous safety domain, or a folded sequence of nucleic acids, termed a segmented safety domain, comprising one or more folds;
 - 4) wherein said tsRNA further comprises at least one binding domain specific for at least a part of a gene that is ubiquitously expressed in any cell;
 - 5) wherein said biomarker is any single biomarker or combination of biomarkers selected from the following list: a cancer marker, HCC biomarkers alpha-feta protein (AFP), Vascular endothelium growth factor (VEGF), γ -glutamyl transferase (GGT), Hepatocellular

carcinoma associated protein 2 (HCCA2), Transforming growth factor beta 1 (TGF- β 1), cluster of differentiation 24 (CD24), Cyclin D1 (CCND1), Glypican 3 (GPC3), Telomerase reverse transcriptase (TERT), α -L-fucosidase (AFU), CD19, CD34, CD44, CD49E, CD51, CD105, Collagen type XV alpha 1 (COL15A1), C-X-C motif chemokine receptor 4 (CXCR4), Denticleless E3 ubiquitin protein ligase homolog (DTL), Epithelial cell adhesion molecule (EPCAM), Golgi protein 73 (GP73), G protein signaling modulator 2 (GPSM2), Hepatocyte growth factor (HGF), Heat shock protein 70 (HSP70), Insulin like growth factor 2 (IGF2), Immunoglobulin superfamily member 3 precursor (IGSF3), Integrin Subunit Alpha 6 (ITGA6), Kell blood group glycoprotein (KEL), KIT Proto-Oncogene, Receptor Tyrosine Kinase (KIT), Minichromosome Maintenance Complex Component 3 (MCM3), Minichromosome Maintenance Complex Component 7 (MCM7), PDZ Binding Kinase (PBK), DNA Polymerase Delta 1, Catalytic Subunit (POLD1), Protein Regulator Of Cytokinesis 1 (PRC1), SRY-Box Transcription Factor 17 (SOX17), Spermatogenesis-associated serine-rich protein 2 (SPATS2), Translocon-associated protein subunit beta (SSR2), Stathmin 1 (STMN1), Thrombomodulin (THBD), ZW10 Interacting Kinetochore Protein (ZWINT), HBV- derived RNA including HBV pgRNA, Epstein-Barr virus derived RNA and pre-mRNAs including BamHI Z Epstein-Barr virus replication activator (BZLF1), Epstein-Barr virus nuclear antigen 3B (EBNA-3B), Latent membrane protein 1 (LMP1), and Latent membrane protein 2A (LMP2A), epidermal cell markers including Keratin 1 (KRT1), Keratin 2 (KRT2), Keratin 10 (KRT10), Keratin 14 (KRT14), Caspase-14 precursor (CASP14), Neuroblast differentiation-associated protein 2 (AHNAK2), basal cell markers including Keratin 15 (KRT15), Collagen 17A1 (COL17A1), Tumour protein 73 (TP73), hair follicle cell markers including Homeobox C13 (HOXC13) and Fibroblast growth factor 7 (FGF-7), senescent cell markers including Forkhead Box 04 (FOXO4) and cyclin-dependent kinase inhibitor 2A (p16), the stratum corneum markers Kallikrein related peptidase 5 (KLK5), Small proline-rich protein 4 (SPRR4), and Arachidonate 12-lipoxygenase (ALOX12B), the Stratum spinosum (Upper epidermal layers) markers HOP

homeobox (HOPX) and Kallikrein 9 (KLK9), the Stratum granulosum markers Filaggrin (FLG) and Premature ovarian failure 1B protein (POF1B), the Melanocyte markers Melan-A (MLANA) and Tyrosinase (TYR), the Langerhans cell markers CD1A and CD207, the fibroblast markers Periostin (POSTN) and Phospholipase C-eta-2 protein (PLCH2), the basal cell carcinoma markers Glioma 1 (GII1), Glioma 2 (GII2), Forkhead box protein (FOXM1), Forkhead box protein (FOXO3A), Desmoglein 2 (DSG2) and C3b, the basal cell carcinoma recurrence markers Cyclooxygenase (COX-2), Ezrin (EZR), CD25, Maspin, Glioma 3 (GII3), GalNAc3 and Gremlin1; and

- 6) wherein said at least one or more suicide protein or at least one or more protein that is a component of a suicide system is selected from the group comprising or consisting of: HSVtk, CYLD Lysine 63 Deubiquitinase (CYLD), tumor necrosis factor-like weak inducer of apoptosis (TWEAK), Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Tumor necrosis factor alpha (TNF- α).

3. The trans-splicing RNA molecule according to any one of the preceding claims wherein said disease is cancer or a viral infection or a bacterial infection or a genetic disease, preferably:

said cancer is selected from the group comprising or consisting of: hepatocellular carcinoma (HCC), cervical cancer, vaginal cancer, vulvar cancer, penile cancer, skin cancers, melanoma including malignant melanoma, squamous-cell carcinoma, basal-cell carcinoma, Merkel cell carcinoma, lung cancer, cell bladder cancer, breast cancer, colon or rectal cancer, anal cancer, endometrial cancer, kidney cancer, leukemia, acute myelogenous or myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphatic leukemia (CML), chronic myelogenous or myeloid leukemia (CML), hairy cell leukemia (HCL), T- cell prolymphocytic leukemia (P-TLL), large granular lymphocytic leukemia, adult T-cell leukemia, lymphoma, myeloma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, thyroid cancer, nasopharyngeal cancer, mouth or throat cancer, oropharyngeal cancer, nasopharyngeal cancer, stomach cancer, brain tumours, bone cancer, and stem cell cancer; or

wherein said viral infection is selected from the group comprising or consisting of: an infection with a retrovirus including the human T-cell lymphotropic virus (HTLV) lentiviruses, human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), human papillomavirus including types 16 and 18 (HPV-16 and HPV-18), a hepadnavirus including HAV, HBV, HCV, HDV, and HEV, a herpesvirus including herpes simplex (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), an adenovirus, an adeno-associated virus, an influenza virus or an integrating virus and a nasopharyngeal virus; or

wherein said bacterial infection is selected from the group comprising or consisting of: an infection caused by *Bartonella henselae*, *Francisella tularensis*, *Listeria monocytogenes*, *salmonella* species, *Salmonella typhi*, *Brucella* species, *Legionella* species, *Mycobacteria* species, *Mycobacterium tuberculosis*, *Nocardia* species, *Rhodococcus* species, *Yersinia* species, a nasopharyngeal bacteria and *Neisseria meningitidis*; or

wherein said genetic disease is selected from the group comprising or consisting of: Neurofibromatosis 1 and 2, McCune Albright, Duchenne muscular dystrophy (DMD), Epidermolysis bullosa, Fanconi A and C, Philadelphia chromosome, Hemophilia A and B, cystic fibrosis, Muckle Wells syndrome, lipoprotein lipase deficiency, B-thalassemia, Gaucher Disease types I to III- GBA gene, Ornithine transcarbamylase (OTC) deficiency-OTC, Phenylketonuria (PKU)-PAH gene, Aspartylglucosaminuria-AGA gene, Alpha-1 antitrypsin deficiency (AATD)-SERPINA 1, and pyruvate dehydrogenase complex deficiency.

4. The trans-splicing RNA molecule according to any one of the preceding claims wherein one or more of the following apply:

- 1) said tsRNA is targeted against nasopharyngeal cells or epidermal cells, basal cells, senescent cells or hair follicles;
- 2) wherein targeting residues are non-covalently linked, via antisense oligonucleotides (DNA or RNA) complementarity to at least a part of the tsRNA molecule;
- 3) wherein targeting residues are covalently linked to at least a part of the tsRNA molecule; and

- 4) wherein at least one tri-antennary GalNAc residues (GalNAc₃) or at least one homo-dimer of CD137-binding aptamer (aptCD137-2) residue is attached to said tsRNA; optionally wherein at least two GalNAc₃ residues are attached to said tsRNA.

5. The trans-splicing RNA molecule according to any one of the preceding claims wherein at least a part thereof comprises unstructured RNA, having as little as possible internal secondary structure formation, comprising a 21 nt antisense oligonucleotide binding domain with at least one spacer on at least one side; optionally wherein an 18 nt spacers is provided either side of said binding domain.

6. The trans-splicing RNA molecule according to any one of the preceding claims wherein one or more of the following apply:

- 1) said tsRNA is either 5' or 3' tsRNA;
- 2) wherein said trans-splicing RNA is dumbbell shaped; and
- 3) wherein said trans-splicing RNA comprises at least one residue or helper function for targeted delivery covalently or non-covalently attached to said ts-RNA. at least one of the loops; optionally wherein said residue or helper function is selected from the group comprising: a carbohydrate, a (GalNAc)₃ residue, a nucleic acid, a RNA or DNA or peptide aptamer, a CD137 or a PSMA binding RNA aptamer, a protein, a peptide, a cell penetrating peptide, an antibody, or a CD137-binding antibody.

7. A cell containing said tsRNA according to any one of claims 1 - 6.

8. A vector containing said tsRNA according to any one of claims 1 - 6; preferably said vector is a naked nucleic acid based vector, a non-viral vector, or a viral vector; optionally

wherein said naked nucleic acid based vector is selected from the group comprising or consisting of: a RNA molecule, a plasmid, a DNA minicircle and a dumbbell-shaped DNA vector; or

wherein said non-viral vector is selected from the group comprising or consisting of: a liposomal vesicle, a nanoparticle, a polymer conjugate, an

antibody conjugate, a cell penetrating peptide and a polymer capsule; or

wherein said viral vector is selected from the group comprising or consisting of: a retroviral vector, a lentiviral vector, an adenoviral vector, and adeno-associated viral vector, a Herpes simplex viral vector, a vaccinia viral vector, chimeric viral vectors, a sindbis-viral vector, or an alphaviral vector, semliki forest viral vector, and a Venezuelan equine encephalitis viral vector.

9. The vector according to claim 8 for use in a method of targeting a diseased cell, wherein the vector is to be administered by an administration route selected from the group comprising or consisting of: topical application; intranasal application; alveolar application; systemic application; oral application; intravenous application; intramuscular application; subcutaneous application; cutaneous application; intraperitoneal application; or injection into a tumor and, optionally, exposing said cell to at least one other component(s) of a suicide system effective to kill said cell.

10. A method of killing a cell comprising transfecting, lipofecting, transducing, electroporating, nucleofecting or transforming said cell with the tsRNA according to any one of claims 1- 6, or a vector containing the tsRNA according to any one of claims 1- 6, *ex vivo* and, optionally, exposing said cell to at least one other component(s) of a suicide system effective to kill said cell.

11. The method according to claim 9 or 10 wherein said component(s) of said suicide system is selected from the group comprising or consisting of: ganciclovir, cytosine deaminase-5-fluorocytosine, cytochrome P450-ifosfamide, cytochrome P450- cyclophosphamide, and nitroreductase-5-[aziridin-1-yl]-2,4-dinitrobenzamide.

12. The trans-splicing RNA molecule according to any one of claims 1 -6 or the cell according to claim 7, the vector according to claim 8, the use according to claim 9, or the method according to claims 10-11 wherein said cell is mammalian, preferably human.

13. A pharmaceutical composition comprising said tsRNA according to any one of claims 1- 6 or the vector according to claim 8 and, optionally, at least one further component of a suicide system effective to trigger death of a cell expressing said trans-spliced RNA; and a carrier suitable for human or veterinary use; optionally wherein said one further component of said suicide system is selected from the group comprising or consisting of: ganciclovir, cytosine deaminase-5-fluorocytosine, cytochrome P450-ifosfamide, cytochrome P450-cyclophosphamide, and nitroreductase-5-[aziridin-1-yl]-2,4- dinitrobenzamide.

14. The pharmaceutical composition of claim 13, for use in a method of treating a disease or condition or cosmetic condition wherein the method comprises transfecting, lipofecting, transducing, electroporating, nucleofecting or transforming a diseased cell with said pharmaceutical composition, *ex vivo* and, optionally, exposing said cell to at least one other component(s) of a suicide system effective to kill said cell.

15. A dumbbell-shaped DNA expression vector comprising:

- a) one or more linear or hairpin-shaped transcription cassettes each comprising a nucleotide sequence encoding a nucleic acid molecule to be expressed;
- b) two single-stranded DNA loops;
- c) operably linked to said transcription cassette a minimal transcription promoter nucleotide sequence and a transcriptional terminator;
- d) a nucleotide sequence comprising a DNA sequence that functions as nuclear targeting sequence (NTS);
- e) a nucleotide sequence comprising a spliceable intron; and
- f) at least one residue or helper function for targeted delivery covalently or non-covalently linked to at least one of the loops;

optionally one or more of the following apply:

- 1) wherein said residue or helper function is selected from the group comprising: a carbohydrate, a (GalNAc)₃ residue, a nucleic acid, a RNA or DNA or peptide aptamer, a CD137 or a PSMA binding RNA

aptamer, a protein, a peptide, a cell penetrating peptide, an antibody, or a CD137-binding antibody;

- 2) wherein said helper function is non-covalently attached to one or both of the dumbbells loops via complementary antisense oligonucleotides (RNA or DNA) to which the helper function is covalently attached at the 5' or 3' end;
- 3) wherein said helper function is covalently attached to one or both of the dumbbells loops; and
- 4) wherein said NTS comprises a binding site for a transcription factor; preferably wherein said NTS is the SV40 enhancer sequence, the minimal SV40 enhancer sequence, the smooth muscle cell γ -actin (SMGA) promoter or the oriP of the Epstein- Barr virus (EBV).

16. A pharmaceutical composition comprising said dumbbell-shaped DNA expression vector according to claim 15 and, optionally, at least one further component of a suicide system effective to trigger death of a cell expressing said dumbbell-shaped DNA vector; and a carrier suitable for human or veterinary use.

17. A dumbbell-shaped DNA expression vector comprising or encoding the trans-splicing RNA molecule according to any one of claims 1 - 6.