

Claims

1. A trans-splicing RNA (tsRNA) molecule comprising:
 - at least one binding domain specific for at least a part of a gene that associates with or is a biomarker for a disease to be treated;
 - nucleic acid encoding at least one expressible suicide protein or a protein that is a component of a suicide system; and
 - at least one splice signal; wherein
- said binding domain comprises a binding site comprising at least 25 consecutive unstructured nucleotides (nt) having no internal binding and/or self-complementary sequences and within or outside said binding site 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. The trans-splicing RNA molecule according to claim 1 wherein said binding site comprises a sequence of nucleotides selected from the list comprising or consisting of: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300 or more nucleotides.
3. The trans-splicing RNA molecule according to claim 1 or 2 wherein said binding domain comprises a sequence of nucleotides that is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%

complementary to said part of a gene that associates with or is a biomarker for a disease to be treated.

4. The trans-splicing RNA molecule according to any one of claims 1 - 3 wherein the mismatches in said binding domain are positioned to avoid any stretches of 45 nt or longer that are perfectly complementary to the target, including or excluding said binding site.
5. The trans-splicing RNA molecule according to claim 4 wherein said mismatches are at least 5 nucleotides from the 5' or 3' end.
6. The trans-splicing RNA molecule according to any preceding claim wherein said at least one expressible suicide protein is the herpes simplex virus thymidine kinase (HSVtk).
7. The trans-splicing RNA molecule according to any preceding claim wherein said nucleic acid encodes a plurality of expressible suicide proteins or a plurality of proteins that are components of a suicide system.
8. The trans-splicing RNA molecule according to claim 7 wherein said tsRNA triggers 3'ER.
9. The trans-splicing RNA molecule according to any preceding claim wherein said tsRNA also includes a spacer sequence adjacent said binding domain.
10. The trans-splicing RNA molecule according to any one of claim 1 - 9 wherein said trans-splicing RNA molecule comprises a plurality of said binding domains which are complementary to the same or different parts of a gene that associates with or is a biomarker for a disease to be treated.
11. The trans-splicing RNA molecule according to any one of claims 1 - 9 wherein said binding domains are complementary to different genes that associate with or are biomarkers for a disease to be treated.
12. The trans-splicing RNA molecule according to any preceding claim wherein said tsRNA, outside the binding domain, comprises at least one cis-binding or self-binding domain.
13. The trans-splicing RNA molecule according to any preceding claim wherein said tsRNA comprises, outside said binding domain and 3' of said molecule, a highly structured sequence of RNA that is folded, or pairs with itself, due to the presence of self-complementary sequences.
14. The trans-splicing RNA molecule according to claim 13 wherein the highly structured RNA is adjacent a spacer located between it and a polyA site.
15. The trans-splicing RNA molecule according to claim 14 wherein the highly structured RNA is an active or inactive ribozyme.

16. The trans-splicing RNA molecule according to claim 14 or 15 wherein said tsRNA triggers 5'ER.
17. The trans-splicing RNA molecule according to claims 1 - 7 or 9 - 15 wherein said tsRNA triggers 5' or 3' ER.
- 5 18. The trans-splicing RNA molecule according to any preceding claim wherein said disease is cancer or a viral infection or a bacterial infection or an acquired genetic disease caused by mutations triggered by transposable elements, radiation, chemicals, or unknown triggers.
- 10 19. The trans-splicing RNA molecule according to claim 18 wherein said cancer is 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 lymphocytic leukemia (CLL), 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,
- 15 20. The trans-splicing RNA molecule according to claim 18 wherein said viral infection is an infection with a retrovirus including the human T-cell lymphotropic virus (HTLV) lentiviruses including the 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 any other integrating virus.
- 20 21. The trans-splicing RNA molecule according to claim 18 wherein said bacterial infection is an infection with bacteria such as Bartonella henselae, Francisella tularensis, Listeria monocytogenes, salmonella species, Salmonella typhi, Brucella species, Legionella species, Mycobacteria species, Mycobacterium tuberculosis, Nocardia species, Rhodococcus species, Yersinia species, Neisseria meningitidis
- 30 and others.
- 35

22. The trans-splicing RNA molecule according to claim 18 wherein said acquired genetic disease is Neurofibromatosis 1 and 2, Mc Cune 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, pyruvate dehydrogenase complex deficiency, or others.
23. A cell containing said tsRNA according to any one of claims 1 – 22.
24. A vector containing said tsRNA according to any one of claims 1 – 22.
25. The vector according to claim 24 wherein said vector is a naked nucleic acid based vector, a non-viral vectors, or a viral vector.
26. The vector according to claim 25 wherein said naked nucleic acid based vector comprises a RNA molecule, a plasmid, a DNA minicircle, or a dumbbell-shaped DNA minimal vector.
27. The vector according to claim 25 wherein said non-viral vector comprises a liposomal vesicle, a nanoparticle, a polymer conjugate, an antibody conjugate, a cell penetrating peptide, or a polymer capsule.
28. The vector according to claim 25 wherein said viral vector is 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.
29. A method of targeting a diseased cell comprising: 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 with tsRNA, or a vector containing the tsRNA, according to any of the claims 1 – 22 in vivo and, and, optionally, exposing said cell to other component(s) of said suicide system effective to kill said cell.
30. A method of killing a cell comprising transfecting, lipofecting, transducing, electroporating, nucleofecting or transforming said cell with tsRNA, or a vector containing the tsRNA, according to any one of claims 1 – 22 ex vivo or in vivo and, optionally, exposing said cell to other component(s) of said suicide system effective to kill said cell.
31. A method of treating a disease comprising transfecting, lipofecting, transducing, electroporating, nucleofecting or transforming a diseased cell with tsRNA, or a vector containing the tsRNA, according any one of claims 1 – 22 ex vivo or in vivo and,

optionally, exposing said cell to other component(s) of said suicide system effective to kill said cell.

32. The method according to any one of claims 29-31 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.
33. The trans-splicing RNA molecule according to any one of claims 19 – 22 or the cell according to claim 23, or the method according to claims 29 - 32 wherein said cell is mammalian.
34. The trans-splicing RNA molecule according to any one of claims 19 – 22 or the cell according to claim 23, or the method according to claims 29 - 32 wherein said cell is human.
35. A medicament comprising said tsRNA according to any one of claims 1 – 22 or the vector according to claims 24-28 and, optionally, at least one further component of said suicide system effective to trigger death of a cell expressing said trans-spliced RNA.
36. A pharmaceutical composition comprising said tsRNA according to any one of claims 1 – 22 or the vector according to claims 24-28; optionally, at least one further component of said suicide system effective to trigger death of a cell expressing said trans-spliced RNA; and a carrier suitable for human or veterinary use.
37. The pharmaceutical composition according to claim 36 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.