

Claims:

Claim 1. An RNA *trans*-splicing molecule (RTM) comprising;

- (i) a binding region specific for a HERV pre-mRNA,
- (ii) a *trans*-splicing domain and
- (iii) a coding sequence for a suicide protein.

Claim 2. An RTM according to claim 1 wherein the binding region specifically binds to HERV pre-mRNA in a cell, such that the coding sequence is *trans*-spliced with the HERV mRNA, causing the suicide protein to be expressed in the cell.

Claim 3. An RTM according to claim 1 or claim 2 wherein the HERV pre-mRNA is a HERV-K pre-mRNA-

~~4. — An RTM according to claim 3,~~
~~optionally~~ wherein the HERV-K pre-mRNA is a HERV-K Np9 or HERV-K Rec pre-mRNA,
~~preferably -~~

~~5. — An RTM according to claim 3 wherein the binding region specifically binds to the nucleotide sequence of SEQ ID NO: 2 within the HERV-K Np9 or HERV-K Rec pre-mRNA-~~

Claim 64. An RTM according to any one of ~~the preceding~~ claims 1 to 3 wherein the binding region comprises
~~the binding region comprises~~ the nucleotide sequence of SEQ ID NO: 4 or a variant thereof-

~~(i) — 7. — An RTM according to claim 6,~~
~~wherein the binding region comprises~~ a nucleotide sequence that is a variant of SEQ ID NO: 4 with modifications relative to SEQ ID NO: 4 at one or more positions corresponding to positions 4, 19, 20, 32 and 34 of SEQ ID NO: 4,-

~~(ii) — 8. — An RTM according to claim~~
~~7 wherein the binding region comprises~~ a nucleotide sequence that is a variant of SEQ ID NO: 4 with a C to U substitution at a position corresponding to position 4 of SEQ ID NO: 4; an A to U substitution at position corresponding to position 19; an A to U substitution at position corresponding to position 20; an A to G substitution at position corresponding to position 32; and an A to G substitution at position corresponding to position 34-

~~(iii) — 9. — An RTM according to any one of the preceding claims, or~~
~~(iv) — wherein the binding region comprises~~ the nucleotide sequence of SEQ ID NO: 5 or a variant thereof.

Claim 105. An RTM according to any one of ~~the preceding~~ claims 1 to 4 wherein the suicide protein is Herpes simplex virus (HSV) thymidine kinase, optionally -

~~11. — An RTM according to claim 10 wherein the HSV thymidine kinase comprises;~~
~~(i) the amino acid sequence of SEQ ID NO: 6 or a variant thereof,-~~

~~(ii) 12.—An RTM according to claim 10 or 11 wherein the HSV thymidine kinase comprises an amino acid sequence that is a variant of SEQ ID NO: 6 with modifications relative to SEQ ID NO: 6 at positions corresponding to positions 46 and 60 of SEQ ID NO: 6, optionally positions corresponding to positions 1, 46 and 60 of SEQ ID NO: 6;~~

~~(iii)~~

~~13.—An RTM according to claim 12 wherein the HSV thymidine kinase comprises an amino acid sequence that is variant of SEQ ID NO: 6 with an M to L substitution at a position corresponding to position 46; and an M to I substitution at a position corresponding to position 60 and optionally a deletion of M at a position corresponding to position 1 of SEQ ID NO: 6; or~~

~~-(iv)~~

~~14.—An RTM according to any of claims 10 to 13 wherein the HSV thymidine kinase comprises the amino acid sequence of SEQ ID NO: 8 or a variant thereof.~~

Claim 156. An RTM according to any one of ~~the preceding~~ claims 1 to 5 further comprising a spacer located between the binding domain and the *trans*-splicing domain of the RTM, ~~optionally~~;

~~16.—An RTM according to claim 15 wherein the spacer comprises the sequence of SEQ ID NO: 10 or a variant thereof.~~

Claim 177. An RTM according to any one of ~~the preceding~~ claims 1 to 6 that comprises in order in a 5' to 3' direction the binding region, the *trans*-splicing domain and the coding sequence;

~~optionally wherein -~~

the *trans*-splicing domain comprises an acceptor site, ~~optionally~~

~~19.—An RTM according to claim 18 wherein the *trans*-splicing domain comprises the nucleotide sequence of SEQ ID NO: 12 or a variant thereof;~~

~~20.—An RTM according to any one of claims 17 to 19(ii) the RTM further comprises ing a sequence encoding a 2A self-cleaving peptide, said sequence being located between the *trans*-splicing domain and the coding sequence of the RTM, optionally -~~

~~21.—An RTM according to claim 20 wherein the 2A cleavage site comprises the sequence of SEQ ID NO: 11 or a variant thereof;~~

~~22.—An RTM according to any one of claims 17 to 21(iii) wherein the coding sequence for the suicide protein lacks a start codon; and/or -~~

~~23.—An RTM according to any one of claims 17 to 22 comprising (iv) the RTM comprises the nucleic acid sequence of SEQ ID NO: 13; a variant of SEQ ID NO: 13; SEQ ID NO: 15; or a variant of SEQ ID NO: 15.~~

Claim 248. An RTM according to any one of claims 1 to ~~16~~ comprising in order in a 5' to 3' direction; the coding sequence, *trans*-splicing domain and the binding region;

~~optionally;~~

~~25. — An RTM according to claim 23 wherein~~
~~(i) the *trans*-splicing domain comprises a splice donor site.~~

~~26. — An RTM according to any one of claims 24 or 25 wherein (ii) the coding sequence for the suicide protein comprises a start codon; and/or, _____~~

~~27. — An RTM according to any one of claims 24 to (iii) the RTM-26 further comprising a ribozyme sequence located downstream (3') of the binding domain, wherein said ribozyme sequence cleaves the RTM 3' of the binding domain to remove downstream nucleotide sequence.~~

Claim 289. A nucleic acid encoding an RTM according to any one of ~~the preceding~~ claims 1 to 8.

Claim 2910. A nucleic acid encoding an HSV thymidine kinase comprising an amino acid sequence that is a variant of SEQ ID NO: 6 with modifications relative to SEQ ID NO: 6 at positions corresponding to positions 46 and 60 of SEQ ID NO: 6, optionally positions corresponding to positions 1, 46 and 60 of SEQ ID NO: 6, _____

~~30. — A nucleic acid according to claim 29 optionally~~ wherein
~~(i) the HSV thymidine kinase comprises an amino acid sequence that is variant of SEQ ID NO: 6 with an M to L substitution at a position corresponding to position 46; and an M to I substitution at a position corresponding to position 60 and optionally a deletion of M at a position corresponding to position 1 of SEQ ID NO: 6, _____~~

~~31. — A nucleic acid according to claim 29 or 30 wherein (ii) the HSV thymidine kinase comprises the amino acid sequence of SEQ ID NO: 8 or a variant thereof, _____~~

~~(iii) the nucleic acid 32. — A nucleic acid according to any one of claims 29 to 31 comprising the nucleotide sequence of SEQ ID NO: 9 or a variant thereof; and/or, _____~~

~~33. — A nucleic acid according to any one of claims 29 to 32 wherein (iv) the nucleic acid is an RTM further comprising a binding region specific for a target pre-mRNA and a *trans*-splicing domain.~~

Claim 3411. An expression vector comprising a nucleic acid according to claim 289, _____

~~35. — An expression vector according to claim 34 optionally, wherein the vector is a viral vector.~~

Claim 3612. A viral particle comprising a nucleic acid according to claim 928 or an expression vector according to claim 1134 or claim 35.

Claim 3713. An isolated cell comprising an RTM according to any one of claims 1 to 278, a nucleic acid according to claim 289 or an expression vector according to claim 34 or claim 3511.

Claim 3814. A pharmaceutical composition comprising an RTM according to any one of claims 1 to 278, a nucleic acid according to claim 9, 28 or an expression vector according to claim 3411 or claim 35, a viral particle according to claim 1236 or an isolated host cell according to claim 1337.

~~39. — A method of treatment of cancer comprising;
administering an RTM according to any one of claims 1 to 27, a nucleic acid according to claim 28 or an expression vector according to claim 34 or claim 35, a viral particle according to claim 36, an isolated cell according to claim 37 or a pharmaceutical composition according to claim 38, to an individual in need thereof.~~

Claim 4015. A composition comprising an RTM according to any one of claims 1 to 278, a nucleic acid according to claim 28, 9, or an expression vector according to claim 3411 or claim 35, a viral particle according to claim 3612, an isolated host cell according to claim 3713 or a pharmaceutical composition according to claim 1438, for use in a method of treatment of cancer.

Claim 4416. Use of an RTM according to any one of claims 1 to 827, a nucleic acid according to claim 28, 9 or an expression vector according to claim 1134 or claim 35, a viral particle according to claim 1236, an isolated host cell according to claim 1337 or a pharmaceutical composition according to claim 1438 in the manufacture of a medicament for use in treating cancer.

Claim 1742. ~~A method according to claim 39 and~~ a composition for use according to claim 15 40 or use according to claim 1644, wherein following the administration of the RTM, nucleic acid, expression vector, viral particle, isolated cell or pharmaceutical composition to the individual, the method further comprises,
administering to the individual a first treatment with an inactive pro-form of a cytotoxic compound that is activated by the suicide protein, such that the cytotoxic compound is activated in cancer cells in the individual.

Claim 4318. A ~~method,~~ composition for use, or use according to claim 1742, further comprising administering to the individual one or more further treatments with the inactive pro-form, such that the cytotoxic compound is activated in cells that have become or are becoming cancer cells in the individual following said first treatment.

~~44. — A method, composition for use, or use according to claim 43, optionally~~ wherein the one or more further treatments occur at least 1 week after the first treatment.

~~45. — A method of preventing cancer occurrence or recurrence in an individual undergoing cell therapy, the method comprising administering a population of cells according to claim 37 to an individual in need thereof.~~

~~46. — A method according to claim 45 comprising administering to the individual an inactive pro-form of a cytotoxic compound that is activated by the suicide protein, such that the cytotoxic compound is activated in cells in the population that have become or are becoming cancerous in the individual.~~

~~47. — A method according to claim 45 or claim 46 wherein the cells are autologous cells from the individual.~~

~~48. — A method according to any one of claims 45 to 47 wherein the cells are haematopoietic cells.~~

Claim 149. A ~~method according to any one of claims 39 or 42 to 48;~~ composition for use according to any one of claims 15 or 17-18 ~~40 or 42 to 44~~ or use according to any one of claims 41~~16~~ to 44~~18~~, wherein:

(i) the cancer is characterised by expression of an HERV gene and/or -

~~50. — A method according to any one of claims 39 or 42 to 49; composition for use according to any one of claims 40, 42 to 44 or 49 or use according to any one of claims 41 to (ii) 44 or 49, wherein the cancer is pancreatic or hepatic cancer.~~

Claim 5120. A method of killing a cell *in vitro* comprising;
 contacting a cell with an RTM according to any one of claims 1 to 278, a nucleic acid according to claim 928, an expression vector according to claim 1134 or claim 35, or a viral particle according to claim 3612, such that the cell expresses the suicide protein; and,
 contacting the cell with the inactive pro-form of a cytotoxic compound that is activated by the suicide protein, such that the cytotoxic compound is activated by the suicide protein, thereby killing the cell.

~~52. — A method according to claim 51, wherein the cell expresses an HERV gene.~~

Claim 5321. A method of depleting HERV gene expressing cells in a population comprising;
 contacting the population of cells with an RTM according to any one of claims 1 to 278, a nucleic acid according to claim 289, an expression vector according to claim 3411 or claim 35, a viral particle according to claim 1236 and/or a pharmaceutical composition according to claim 1438, such that HERV gene expressing cells in the population of cells express the suicide protein; and,
 contacting the population with the inactive pro-form of a cytotoxic compound that is activated by the suicide protein, such that the suicide protein activates the cytotoxic compound in HERV gene expressing cells in the population, thereby depleting HERV gene expressing cells in the population.

Claim 5422. A method according to claim 53-21 wherein

(i) the HERV gene expressing cells are cancer cells.

(ii) ~~55. — A method according to claim 53 or 54 wherein~~ the population of cells is a sample of cells obtained from an individual or the descendants thereof, and/or -

(iii)

~~56. — A method according to any one of claims 53 to 55 wherein~~ the cells are haematopoietic cells.