

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,
optionally wherein the HERV-K pre-mRNA is a HERV-K Np9 or HERV-K Rec pre-mRNA,
preferably 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 4. An RTM according to any one of claims 1 to 3 wherein the binding region comprises

- (i) the nucleotide sequence of SEQ ID NO: 4 or a variant thereof,
- (ii) 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,
- (iii) 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, or
- (iv) the nucleotide sequence of SEQ ID NO: 5 or a variant thereof.

Claim 5. An RTM according to any one of claims 1 to 4 wherein the suicide protein is Herpes simplex virus (HSV) thymidine kinase, optionally wherein the HSV thymidine kinase comprises;

- (i) the amino acid sequence of SEQ ID NO: 6 or a variant thereof,
- (ii) 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) 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) the amino acid sequence of SEQ ID NO: 8 or a variant thereof.

Claim 6. An RTM according to any one of claims 1 to 5 further comprising a spacer located between the binding domain and the *trans*-splicing domain of the RTM, optionally wherein the spacer comprises the sequence of SEQ ID NO: 10 or a variant thereof.

Claim 7. An RTM according to any one of 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

(i) the *trans*-splicing domain comprises an acceptor site, optionally wherein the *trans*-splicing domain comprises the nucleotide sequence of SEQ ID NO: 12 or a variant thereof,

(ii) the RTM further comprises 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 wherein the 2A cleavage site comprises the sequence of SEQ ID NO: 11 or a variant thereof,

(iii) the coding sequence for the suicide protein lacks a start codon; and/or

(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 8. An RTM according to any one of claims 1 to 6 comprising in order in a 5' to 3' direction; the coding sequence, *trans*-splicing domain and the binding region, optionally wherein

(i) the *trans*-splicing domain comprises a splice donor site.

(ii) the coding sequence for the suicide protein comprises a start codon; and/or

(iii) the RTM further comprises 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 9. A nucleic acid encoding an RTM according to any one of claims 1 to 8.

Claim 10. 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,

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,

(ii) the HSV thymidine kinase comprises the amino acid sequence of SEQ ID NO: 8 or a variant thereof,

(iii) the nucleic acid comprising the nucleotide sequence of SEQ ID NO: 9 or a variant thereof; and/or

(iv) the nucleic acid is an RTM further comprising a binding region specific for a target pre-mRNA and a *trans*-splicing domain.

Claim 11. An expression vector comprising a nucleic acid according to claim 9, optionally wherein the vector is a viral vector.

Claim 12. A viral particle comprising a nucleic acid according to claim 9 or an expression vector according to claim 11.

Claim 13. An isolated cell comprising an RTM according to any one of claims 1 to 8, a nucleic acid according to claim 9 or an expression vector according to claim 11.

Claim 14. A pharmaceutical composition comprising an RTM according to any one of claims 1 to 8, a nucleic acid according to claim 9, an expression vector according to claim 11, a viral particle according to claim 12 or an isolated host cell according to claim 13.

Claim 15. A composition comprising an RTM according to any one of claims 1 to 8, a nucleic acid according to claim 9, an expression vector according to claim 11, a viral particle according to claim 12, an isolated host cell according to claim 13 or a pharmaceutical composition according to claim 14, for use in a method of treatment of cancer.

Claim 16. Use of an RTM according to any one of claims 1 to 8, a nucleic acid according to claim 9 or an expression vector according to claim 11, a viral particle according to claim 12, an isolated host cell according to claim 13 or a pharmaceutical composition according to claim 14 in the manufacture of a medicament for use in treating cancer.

Claim 17. A composition for use according to claim 15 or use according to claim 16, 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 18. A composition for use, or use according to claim 17, 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, optionally wherein the one or more further treatments occur at least 1 week after the first treatment.

Claim 19. A composition for use according to any one of claims 15 or 17-18 or use according to any one of claims 16 to 18, wherein;

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

Claim 20. A method of killing a cell *in vitro* comprising;

contacting a cell with an RTM according to any one of claims 1 to 8, a nucleic acid according to claim 9, an expression vector according to claim 11, or a viral particle according to claim 12, 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.

Claim 21. 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 8, a nucleic acid according to claim 9, an expression vector according to claim 11, a viral particle according to claim 12 and/or a pharmaceutical composition according to claim 14, 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 22. A method according to claim 21 wherein

- (i) the HERV gene expressing cells are cancer cells
- (ii) the population of cells is a sample of cells obtained from an individual or the descendants thereof, and/or
- (iii) the cells are haematopoietic cells.