

## Claims

1. A method of regulating expression of a target gene in a cell in a context-specific manner, the method comprising:

- providing a cell with a nucleic acid construct adapted to express an inhibitory RNA suitable to inhibit expression of the target gene;
- wherein the inhibitory RNA is active in the cell in a context-specific manner such that inhibition of the expression of the target gene occurs specifically in a first context, in which the inhibitory RNA is active in said cell,

optionally wherein inhibition does not occur, or occurs in reduced amount, in at least a second context, in which the inhibitory RNA is inactive or less active, optionally

wherein the nucleic acid construct is a modified form of an endogenous sequence encoding an inhibitory RNA, preferably an miRNA, which has been modified to target a transcript from the target gene, and/or

wherein the method comprises the step of genetically modifying a sequence encoding an endogenous inhibitory RNA to alter its targeting specificity so that it targets the transcript of a target gene, and/or

wherein the cell is a context-specific hypoimmunogenic cell, preferably a context-specific hypoimmunogenic therapeutic cell, and/or

wherein the cell is an immune cell, or a progenitor thereof, such as wherein the cell is a macrophage or a T cell, or a progenitor thereof, and/or

wherein the inhibitory RNA targets a target gene associated with the MHC-I complex, optionally wherein the target gene is B2M, HLA-A, HLA-B and/or HLA-C gene such that expression of the MHC-I complex on the cell is reduced when the inhibitory RNA is active, preferably when the target gene is B2M, and/or

wherein the inhibitory RNA is specifically active in a cell which is in a context selected from the group consisting of:

- a cell at a particular state of differentiation, for example a fully-differentiated cell, a cell at an intermediate stage of differentiation or an undifferentiated cell;
- a totipotent cell, a pluripotent cell or a multipotent cell;
- a cell present in a particular tissue;

- a cancerous or pre-cancerous cell;
- a tumour associated cell, e.g. a tumour associated immune cell or an immune cell in the tumour microenvironment;
- a polarised cell, e.g. a macrophage;
- 5 - an immune cell which is activated or polarised;
- a cell which is hypoxic; or
- a cell subject to the unfolded protein response, and/or

wherein the cell is an allogeneic therapeutic cell selected from the group consisting of:

- 10 - a therapeutic immune cell or a progenitor thereof;
- a therapeutic endocrine cell or a progenitor thereof, preferably a pancreatic beta cell or a progenitor thereof;
- a therapeutic CNS cell or a progenitor thereof, suitably a neuron or a glial cell (such as an astrocyte, an oligodendrocytes, an ependymal cell, or a microglial cell) or a
- 15 progenitor thereof;
- a therapeutic epithelial cell or a progenitor thereof;
- a therapeutic myocyte or a progenitor thereof, for example a cardiac myocyte, skeletal myocyte, and smooth myocyte;
- a therapeutic pluripotent stem cell.

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2. A cell or cell population obtained by the method of claim 1.
3. A genetically modified cell which comprises a nucleic acid construct adapted to express an inhibitory RNA suitable to inhibit expression of a target gene;
- 25 wherein the inhibitory RNA is active in the cell in a context-specific manner, such that inhibition of the expression of the target gene occurs specifically in a first context, in which the inhibitory RNA is active in said cell,
- optionally wherein inhibition does not occur or is reduced in at least a second context, in which the inhibitory RNA is inactive or less active, optionally

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wherein the nucleic acid construct is a modified endogenous sequence encoding an inhibitory RNA, preferably an miRNA, which has been modified to target a transcript from the target gene.

- 35 4. The method of claim 1 or the cell of any claim 3 wherein the cell is adapted to express at least one further inhibitory RNA which is/are active in the cell in a context-specific manner,

optionally wherein inhibition of the expression of a second or further target gene occurs specifically in a first context, in which the inhibitory RNAs are active in said cell or

wherein inhibition of the expression of a second or further target gene occurs specifically in a second or further context, in which the at least one further inhibitory RNAs are active in said cell.

5. The cell of claim 3 or 4 wherein the cell comprises a genetically modified a sequence encoding an endogenous inhibitory RNA with altered targeting specificity so that it targets the transcript of a target gene, and/or

wherein the cell comprises a nucleic acid construct adapted to express an inhibitory RNA suitable to inhibit expression of the target gene, and wherein the inhibitory RNA becomes active in the cell following a change in the context of the cell, and/or

wherein the cell is a context-specific hypoimmunogenic cell, preferably a context-specific hypoimmunogenic therapeutic cell.

6. The method or cell of any preceding claim wherein the inhibitory RNAs target more than one target gene, optionally wherein one of the target genes is B2M and another one of the target genes is selected from: PPARG, IRF4, KDM6B, FOXP3, PDCD-1 and STAT6, optionally wherein one of the two or more inhibitory RNAs is selected from SEQ ID NO: 1 or 18 (Solution 8), SEQ ID NO: 5 or 22 (Solution 20), SEQ ID NO: 9 or 26 (Solution 29), SEQ ID NO: 10 or 27 (Solution 30), SEQ ID NO: 348 to SEQ ID NO:411 or SEQ ID NO: 412 to SEQ ID NO: 475 (targeting B2M), and another one of the two or more inhibitory RNAs is selected from: SEQ ID NO: 66 to SEQ ID NO: 87 or SEQ ID NO: 88 to SEQ ID NO: 109 (targeting PPARG); SEQ ID NO: 110 to SEQ ID NO: 133 or SEQ ID NO: 134 to SEQ ID NO: 157 (targeting IRF4); SEQ ID NO: 158 to SEQ ID NO: 181 or SEQ ID NO: 182 to SEQ ID NO: 205 (targeting KDM6B); SEQ ID NO: 476 to SEQ ID NO: 525 or SEQ ID NO: 526 to SEQ ID NO: 575 (targeting FOXP3); SEQ ID NO: 576 to SEQ ID NO: 624 or SEQ ID NO: 625 to SEQ ID NO: 673 (targeting PDCD-1) or SEQ ID NO: 206 to SEQ ID NO: 229 or SEQ ID NO: 230 to SEQ ID NO: 253 (targeting STAT6); or wherein one of the target genes is PPARG and another one of the target genes is selected from: IRF4, KDM6B and STAT6, or wherein one of the target genes target genes is IRF4 and another one of the target genes is selected from: PPARG, KDM6B and STAT6, or wherein one of the target genes is KDM6B and another one of the target

genes is selected from: PPARG, IRF4, and STAT6, or wherein one of the target genes is FOXP3 and another one of the target genes is PDCD-1, and/or

wherein the cell is a progenitor cell which has been modified to comprise a nucleic acid construct adapted to express an inhibitory RNA suitable to inhibit expression of the target gene, and wherein the inhibitory RNA is active in the cell following differentiation, preferably wherein the inhibitory RNA is active in the cell when the cell is fully differentiated, and/or

wherein the cell is a differentiated cell selected from the group consisting of: a leukocyte, a dendritic cell, a lymphocyte, a monocyte, a macrophage, a red blood cell, platelet, a hematopoietic cell, a pancreatic islet cell (e.g. a beta cell), a liver cell, a muscle cell, a keratinocyte, a cardiomyocyte, a neuronal cell, a skeletal muscle cell, an ocular cell, a mesenchymal cell, a fibroblast, a lung cell, a GI tract cell, a vascular cell, an endocrine cell and an adipocyte

or wherein the cell is a stem cell, suitably a stem cell which is suitable to differentiate into a differentiated cell selected from the group consisting of: a leukocyte, a dendritic cell, a lymphocyte, a red blood cell, a platelet, a hematopoietic cell, a pancreatic islet cell (e.g. a beta cell), a liver cell, a muscle cell, a keratinocyte, a cardiomyocyte, a neuronal cell, a skeletal muscle cell, an ocular cell, a mesenchymal cell, a fibroblast, a lung cell, a GI tract cell, a vascular cell, an endocrine cell or an adipocyte, and/or

wherein the inhibitory RNA is a modified form of an endogenous miRNA selected from the group consisting of: hsa-miR-375, hsa-miR-143, and hsa-miR-21, hsa-miR-30d, hsa-miR-192, hsa-miR-30a, hsa-miR-182, hsa-miR-148a, hsa-miR-127, and hsa-miR-200c and/or wherein the inhibitory RNA comprises one of the following sequences or a functional variant thereof:

- SEQ ID NO: 1 or 18 (Solution 8), SEQ ID NO: 5 or 22 (Solution 20), SEQ ID NO: 9 or 26 (Solution 29), or SEQ ID NO: 10 or 27 (Solution 30) (targeting B2M);
- SEQ ID NO: 66 to SEQ ID NO: 87 or SEQ ID NO: 88 to SEQ ID NO: 109 (targeting PPARG);
- SEQ ID NO: 110 to SEQ ID NO: 133 or SEQ ID NO: 134 to SEQ ID NO: 157 (targeting IRF4);
- SEQ ID NO: 158 to SEQ ID NO: 181 or SEQ ID NO: 182 to SEQ ID NO: 205 (targeting KDM6B);

- SEQ ID NO: 348 to SEQ ID NO:411 or SEQ ID NO: 412 to SEQ ID NO: 475 (targeting B2M);
- SEQ ID NO: 476 to SEQ ID NO: 525 or SEQ ID NO: 526 to SEQ ID NO: 575 (targeting FOXP3);
- 5     - SEQ ID NO: 576 to SEQ ID NO: 624 or SEQ ID NO: 625 to SEQ ID NO: 673 (targeting PDCD-1); or
- SEQ ID NO: 206 to SEQ ID NO: 229 or SEQ ID NO: 230 to SEQ ID NO: 253 (targeting STAT6), and/or

10     wherein the cell is a macrophage, or a progenitor thereof, and wherein the inhibitory RNA is active in a macrophage in one of the following contexts:

- the macrophage is an unpolarised macrophage;
- the macrophage is classically activated (M1);
- the macrophage is alternatively activated (M2);
- 15     - the macrophage is a regulatory macrophages (Mreg); or
- the macrophage is in the TME;

or wherein the miRNA is selected from the group consisting of: MIR146A (e.g. hsa-miR-146a-5p), MIR20A (e.g. hsa-miR-20a-5p), LET7C (hsa-miR-let-7c-5p), and LET7i (hsa-let-7i-5p), and/or

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wherein the cell is a T cell, or progenitor thereof, and wherein the inhibitory RNA is specifically active in the T cell in one of the following contexts:

- the T cell is a CD8<sup>+</sup> T cell;
- the T cell is a CD4<sup>+</sup> T cell;
- 25     - the T cell is a CD4<sup>+</sup> T helper cell, optionally a Th1, Th2, Th17, Th9, Tfh or Th22 cell;
- the T cell is a memory T cell;
- the T cell is a CD4<sup>+</sup> T<sub>reg</sub> cell, optionally a FOXP3<sup>+</sup> T<sub>reg</sub> cells or a FOXP3<sup>-</sup> T<sub>reg</sub> cell;
- the T cell is exhausted (e.g. a cell); or
- the T cell is in the TME.

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7. A nucleic acid construct for the context-specific inhibition of expression of a target gene in a cell, the nucleic acid construct encoding an inhibitory RNA suitable to inhibit expression of the target gene, wherein the inhibitory RNA is active in the cell in a context-specific manner such that inhibition of the expression of the target gene occurs in a first context, in which the inhibitory RNA is active in said cell wherein the construct comprises
- 35     a sequence selected from the following list, or a functional variant thereof:

- SEQ ID NO: 1 or 18 (Solution 8), SEQ ID NO: 5 or 22 (Solution 20), SEQ ID NO: 9 or 26 (Solution 29), or SEQ ID NO: 10 or 27 (Solution 30) (targeting B2M);
  - SEQ ID NO: 66 to SEQ ID NO: 87 or SEQ ID NO: 88 to SEQ ID NO: 109 (targeting PPARG);
  - 5 - SEQ ID NO: 110 to SEQ ID NO: 133 or SEQ ID NO: 134 to SEQ ID NO: 157 (targeting IRF4);
  - SEQ ID NO: 158 to SEQ ID NO: 181 or SEQ ID NO: 182 to SEQ ID NO: 205 (targeting KDM6B);
  - SEQ ID NO: 348 to SEQ ID NO: 411 or SEQ ID NO: 412 to SEQ ID NO: 475 (targeting B2M);
  - 10 - SEQ ID NO: 476 to SEQ ID NO: 525 or SEQ ID NO: 526 to SEQ ID NO: 575 (targeting FOXP3);
  - SEQ ID NO: 576 to SEQ ID NO: 624 or SEQ ID NO: 625 to SEQ ID NO: 673 (targeting PDCD-1); or
  - 15 - SEQ ID NO: 206 to SEQ ID NO: 229 or SEQ ID NO: 230 to SEQ ID NO: 253 (targeting STAT6).
8. An inhibitory RNA which comprises or consists of a sequence selected from the following list, or a functional variant thereof:
- 20 - SEQ ID NO: 1 or 18 (Solution 8), SEQ ID NO: 5 or 22 (Solution 20), SEQ ID NO: 9 or 26 (Solution 29), or SEQ ID NO: 10 or 27 (Solution 30) (targeting B2M);
  - SEQ ID NO: 66 to SEQ ID NO: 87 or SEQ ID NO: 88 to SEQ ID NO: 109 (targeting PPARG);
  - SEQ ID NO: 110 to SEQ ID NO: 133 or SEQ ID NO: 134 to SEQ ID NO: 157 (targeting IRF4);
  - 25 - SEQ ID NO: 158 to SEQ ID NO: 181 or SEQ ID NO: 182 to SEQ ID NO: 205 (targeting KDM6B);
  - SEQ ID NO: 348 to SEQ ID NO: 411 or SEQ ID NO: 412 to SEQ ID NO: 475 (targeting B2M);
  - 30 - SEQ ID NO: 476 to SEQ ID NO: 525 or SEQ ID NO: 526 to SEQ ID NO: 575 (targeting FOXP3);
  - SEQ ID NO: 576 to SEQ ID NO: 624 or SEQ ID NO: 625 to SEQ ID NO: 673 (targeting PDCD-1); or
  - SEQ ID NO: 206 to SEQ ID NO: 229 or SEQ ID NO: 230 to SEQ ID NO: 253 (targeting STAT6).
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9. A composition comprising a cell according to any one of claims 2-6.

10. A cell according to any one of claims 2-6 or a composition according to claim 9 for use in therapy.

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11. A reporter nucleic acid comprising or consisting of a constitutive promoter operably linked to a gene encoding a fluorescent protein and a target gene (or a portion of a target gene), wherein the fluorescent protein and the target gene are transcribed as a polycistronic mRNA, optionally

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wherein the target gene is inserted into the 3' untranslated region of the fluorescent protein, and/or

wherein the target gene is a target gene for an inhibitory RNA and/or wherein the target gene is selected from: PDCD-1, B2M, PPARG, IRF4, KDM6B, FOXP3 or STAT6, and/or

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wherein the constitutive promoter is EF1a and/or wherein the fluorescent protein is GFP, optionally CopGFP, and/or

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wherein the reporter nucleic acid further comprises:

- WPRE;
- Flanking Long Terminal Repeat (LTR) sequences; and/or
- a gene for a cell surface protein operably linked to a constitutive promoter, optionally wherein the constitutive promoter is PGK, optionally wherein the cell surface protein is tNGFR.

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12. The reporter nucleic acid according to claim 11 for use in detecting the inhibition or silencing of the target gene by one or more inhibitory RNAs.

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13. Use of the reporter nucleic acid according to claims 11 in detecting the inhibition or silencing of the target gene by one or more inhibitory RNAs.

14. An expression system comprising:

- the reporter nucleic acid according to claim 11 or 12; and
- an expression nucleic acid comprising a constitutive promoter operably linked to a nucleic acid sequence encoding an inhibitory RNA, optionally

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wherein:

- the expression nucleic acid is flanked by Long Terminal Repeat (LTR) sequences;
- the constitutive promoter in the expression nucleic acid is EF1a;
- the inhibitory RNA is an endogenous non-coding RNA encoding sequence, e.g. an endogenous inhibitory RNA encoding sequence, which has been modified to retarget the endogenous inhibitory RNA to silence or partially silence a target gene via GEiGS approach, or wherein the inhibitory RNA is a GEiGS extended Solution and/or
- the expression nucleic acid further comprises a further fluorescent protein operably linked to the constitutive promoter, optionally wherein the further fluorescent protein is dsRed, and/or

wherein the reporter nucleic acid and the expression nucleic acid are provided in two separate lentiviral vectors, and/or

wherein the expression system comprises two or more expression nucleic acids, optionally

wherein the two or more expression nucleic acids are provided in two or more separate lentiviral vectors.

15. A method of detecting the inhibitory or silencing activity of one or more inhibitory RNAs on a target gene comprising:

- transfecting a cell or cells with the expression system according to claim 14
- binding of the inhibitory RNA to the target gene within the polycistronic mRNA;
- degradation of the polycistronic mRNA; and
- reduction in the fluorescent signal from the fluorescent protein in the cell or cells, optionally
- incubating the cell or cells under conditions suitable for expression of the reporter nucleic acid and the expression nucleic acid; and/or
- detecting the reduction in the fluorescent signal, optionally via flow cytometry, and/or

wherein the cell is a macrophage, an iPSC or a T cell, optionally a primary T cell or a T cell line.

16. A method of selecting inhibitory RNAs which show silencing or inhibition of a target gene from a pool of test inhibitory RNAs, comprising:

- transfecting a cell or cells with the expression system according to claims 14; and

- selecting inhibitory RNAs which were transfected into the cell or cells which show reduction in the fluorescent signal from the fluorescent protein, optionally
  - incubating the cell or cells under conditions suitable for expression of the reporter nucleic acid and the expression nucleic acid; and/or
  - detecting the reduction in the fluorescent signal, optionally via flow cytometry, and/or
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wherein the cell is a macrophage, an iPSC or a T cell, optionally a primary T cell or a T cell line.