CLAIMS

1. A method for identifying an agent suitable for treating a cancer, wherein the method comprises:

contacting a tumouroid co-culture with one or more candidate agents, wherein the tumouroid co-culture comprises immune cells and at least one tumouroid,

detecting the presence or absence of one or more change in the tumouroid co-culture that is indicative of candidate agent suitability for treating the cancer, and

identifying a candidate agent as suitable for treating the cancer if the presence or absence of one or more of said changes in the tumouroid co-culture is detected

wherein the immune cells are antigenically compatible with the patient from whom the at least one tumouroid is derived,

wherein the method is preceded by:

preparing the at least one tumouroid by culturing tumour epithelial cells in a tumouroid culture medium;

preparing the immune cells by obtaining immune cells from an impure immune sample, and culturing the immune cells in an immune cell expansion medium wherein the tumouroid culture medium comprises IL-2 and/or the immune cell expansion medium comprises IL-2; and

preparing the tumouroid co-culture by mixing the at least one tumouroid with the immune cells in an *in vitro* culture.

2. The method of claim 1, wherein:

(a) suitability for treating the cancer comprises efficacy for treating the cancer and/or safety for treating the cancer;

(b) the one or more change is a change in one or more cancer biomarkers;

(c) the one or more change is selected from a reduction in cell viability, a reduction in cell proliferation, an increase in cell death, a change in cell or organoid size, a change in cell motility, change in production of cytokines and cytotoxic molecules by co-cultured immune cells, dissociation or disruption of the intact/compact epithelial cell layer, and a change in the expression of one or more genes;

(d) the detecting comprises a cellular proliferation assay, a viability assay, flow cytometric analysis, ELISA for IFN-γ, analysis of gene expression and/or cellular imaging;

(e) the one or more change is a reduction in cell viability, intracellular flow cytometric staining for active Caspase 3 (BD) or positive stain for death cells;

(f) the one or more change is an increase in cell death;

(g) the co-culture comprises at least 5%, at least 6%, at least 7%, at least 8%, at least 9% or at least 10% collagen; and/or

(h) the method is preceded by preparing the tumouroid co-culture by removing the tumouroid culture medium from the at least one tumouroid.

3. The method of any one of the preceding claims, wherein the method comprises comparing the presence or absence of the one or more change of the tumouroid co-culture with a reference organoid or reference tumouroid, and wherein the method further comprises:

contacting a reference organoid co-culture or reference tumouroid co-culture with the one or more candidate agents, wherein the reference organoid co-culture or reference tumouroid co-culture comprises immune cells and at least one organoid or tumouroid, and

detecting the presence or absence of the one or more change in the reference organoid co-culture or reference tumouroid co-culture that is indicative of candidate agent suitability for treating the cancer.

4. The method of claim 3, wherein:

(a) a candidate agent is identified as a suitable agent if the presence or absence of a change is detected in the tumouroid co-culture but not in the reference organoid co-culture or reference tumouroid co-culture;

(b) the method is preceded by one or more of the following steps:

preparing the at least one organoid by culturing normal epithelial cells in an organoid culture medium; and/or

preparing the reference organoid co-culture or reference tumouroid co-culture, by removing the tumouroid culture medium organoid culture medium from the at least one tumouroid or at least one organoid, and/or subsequently mixing the at least one reference organoid or at least one reference tumouroid with the immune cells in an organoid co-culture medium or a tumouroid co-culture medium and/or wherein the impure immune sample is a tumour sample, normal colon tissue and/or peripheral blood;

(c) the normal epithelial cells are autologous with the tumour epithelial cells;

(d) the reference organoid co-culture or reference tumouroid co-culture is used as a control; and/or

(e) (I) the tumouroid co-culture medium and/or (II) the reference organoid co-culture medium or reference tumouroid co-culture medium, comprises extracellular matrix, collagen, any animal-derived or synthetic basement membrane, or rat tail collagen I; and/or

(f) both the tumouroid co-culture and the reference organoid co-culture or reference tumouroid co-culture are derived from the particular patient.

5. The method of any one of the preceding claims, wherein:

(a) the immune cells of the tumouroid co-culture and/or reference organoid co-culture and/or reference tumouroid co-culture have a motility of at least 40 μm/day, 60 μm/day, 80 μm/day, 100 μm/day, 120 μm/day, or 140 μm/day;

(b) at least 20%, at least 30%, at least 40% or at least 50% of the immune cells in the tumouroid co-culture and/or reference organoid co-culture and/or reference tumouroid co-culture are capable of moving a distance of at least 200 μm, at least 250 μm, at least 300 μm, at least 350 μm or at least 400 μm in 80 hours;

(c) the immune cells remain active for at least 4 h, 8 h, 12 h, 24 h, 48 h or 72 h;

(d) the one or more candidate agents are of known suitability for treating cancer and the method further comprises identifying the one or more candidate agents as suitable agents for treating cancer in a particular patient; and/or

(e) the method further comprises treating the patient with the candidate agent identified as suitable for treating cancer in the particular patient.

6. The method of any one of the preceding claims, wherein the one or more candidate agent is:

(a) selected from one or more of the following therapeutic classes: immunotherapeutic, tumour-specific peptides, checkpoint inhibitors, alkylating agent, antimetabolite, metabolic agonist, metabolic antagonist, plant alkaloid, mitotic inhibitor, antitumour antibiotic, topoisomerase inhibitor, radiotherapeutics, chemotherapeutics, antibodies, photosensitizing agent, stem cell transplant, vaccine, cytotoxic agent, cytostatic agent, tyrosine kinase inhibitor, proteasome inhibitor, cytokine, interferon, interleukin, intercalating agent, targeted therapy agent, small-

molecule drug, hormone, steroid, cellular therapeutic, viral vector, and nucleic acid therapeutic;

(b) selected from one or more of the following therapeutic classes: tumour-specific peptides, checkpoint inhibitors, chimeric antigen receptor (CAR)-T cell therapeutic, therapeutic TCR transgenic T cells and neoantigen;

(c) an immunotherapeutic;

(d) of unknown suitability for treating cancer and the method further comprises identifying a subset of the one or more candidate agents as suitable agents for treating cancer; and/or

(e) of known suitability for treating a first cancer and unknown suitability for treating a second cancer, and the method further comprising identifying a subset of the one or more candidate agents as suitable agents for treating the second cancer.

7. The method of any one of the preceding claims, wherein the cancer:

(a) is an epithelial cancer;

(b) is a gastrointestinal cancer;

(c) is colorectal cancer; and/or

(d) comprises cancer at or below one of Stage II, Grade II, or T2 N1 M0.

8. The method of any one of the preceding claims, wherein:

(a) the tumour epithelial cell and/or normal epithelial cell is obtained from a sample from a cancer patient;

(b) the tumour epithelial cell and normal epithelial cell are obtained from the same cancer patient, and/or from the same sample, and/or wherein the sample is a tissue biopsy, and/or wherein the tissue biopsy is taken from resected colon and/or rectum of colorectal cancer patients, from ascites of colorectal or ovarian cancer patients, and/or from urine of kidney cancer patients;

(c) the tumour epithelial cells and/or normal epithelial cells are selected from the group consisting of lung cells, liver cells, breast cells, skin cells, intestinal cells, crypt cells, rectal cells, pancreatic cells, endocrine cells, exocrine cells, ductal cells, renal cells, adrenal cells, thyroid cells, pituitary cells, parathyroid cells, prostate cells, stomach cells, oesophageal cells, ovary cells, fallopian tube cells or vaginal cells;

(d) the tumour epithelial cells and/or normal epithelial cells are intestinal cells and/or colorectal cells;

(e) the tumour epithelial cells and/or normal epithelial cells are epithelial stem cells;

(f) the immune cells comprise one or more cell types selected from the group consisting of intra-epithelial lymphocytes (IELs), tumour-infiltrating lymphocytes (TILs), peripheral blood mononuclear cells (PBMCs), peripheral blood lymphocytes (PBLs), T cells, and cytotoxic T lymphocytes (CTLs), αβ T cells, γδ T cells, B cells, NK cells, and mononuclear phagocytes;

(g) the immune cells are obtained from a sample from a cancer patient;

(h) the immune cells are obtained from a peripheral blood sample and/or a tissue biopsy;

(i) the immune cells are obtained from a peripheral blood sample, wherein peripheral blood lymphocytes (PBLs) and/or T cells are obtained from the peripheral blood sample;

(j) the immune cells are obtained from a tissue biopsy, wherein tumour-infiltrating lymphocytes (TILs) and/or intra-epithelial lymphocytes (IELs) are obtained from the tumor or normal tissue biopsy, respectively;

(k) the immune cells are obtained from the same patient as the tumour epithelial cell and/or normal epithelial cell;

(l) the immune cells are HLA-matched with the tumouroid and/or organoid; and/or

(m) the immune cells persist in the immune cell expansion medium for at least 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h, 216 h and 240 h.

9. The method of any one of the preceding claims, wherein:

(a) the at least one tumouroid and/or at least one organoid comprises or consists of autologous cells;

(b) the at least one tumouroid and/or at least one organoid are separated into populations sharing one or more genotypes, phenotypes, and/or epigenetic markers, prior to the mixing with immune cells;

(c) the at least one tumouroid and/or at least one organoid are separated into populations sharing one or more genotypes, phenotypes, and/or epigenetic markers, prior to the mixing with immune cells, wherein the genotypes, phenotypes, and/or epigenetic markers contribute to the interaction between (i) the at least one tumouroid and/or at least one organoid and (ii) the immune cells;

(d) the at least one tumouroid and/or at least one organoid are separated into populations sharing one or more genotypes, phenotypes, and/or epigenetic markers,

prior to the mixing with immune cells, wherein the populations share the presence or absence of an HLA haplotype, wherein the HLA haplotype is HLA-A2;

(e) the at least one tumouroid and/or at least one organoid are separated into populations sharing one or more genotypes, phenotypes, and/or epigenetic markers, prior to the mixing with immune cells, wherein the populations share the presence or absence of an HLA haplotype;

(f) the at least one tumouroid or at least one organoid comprises or consists of mammalian cells and/or human cells;

(g) the at least one tumouroid co-culture or at least one organoid co-culture is cultured in immune cell expansion medium or in a 50:50 (*v/v*) mixture of immune cell expansion medium and organoid culture medium or tumouroid culture medium (respectively); and/or

(h) the tumouroid co-culture persists in the tumouroid co-culture medium, or wherein the reference organoid co-culture or reference tumouroid co-culture persists in the organoid co-culture medium, for at least 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h, 216 h and 240 h.

10. The method of any one of claims 3-9, wherein the organoid culture medium comprises:

(a) one or more (or all) of a basal medium, a Wnt ligand, a Wnt agonist and/or any one of Rspondin 1-4, a BMP inhibitor, EGF, and a TGF-β inhibitor; or

(b) one or more (or all) of a basal medium, a Wnt ligand, a Wnt agonist and/or any one of Rspondin 1-4, a BMP inhibitor, EGF, and a TGF-β inhibitor, and further comprises one or more (or all) of a p38 MAPK inhibitor, gastrin, nicotinamide, prostaglandine E, N-acetylcysteine, B27 and/or an antimicrobial.

11. The method of any one of the preceding claims, wherein the tumouroid culture medium comprises:

(a) one or more (or all) of a basal medium, a Wnt agonist and/or any one of Rspondin 1-4, a BMP inhibitor, EGF, and a TGF-β inhibitor;

(b) one or more (or all) of a basal medium, a Wnt agonist and/or any one of Rspondin 1-4, a BMP inhibitor, EGF, and a TGF-β inhibitor, and further comprises one or more (or all) of a p38 MAPK inhibitor, gastrin, nicotinamide, prostaglandine E, N-acetylcysteine, B27 and/or an antimicrobial; or

(c) one or more (or all) of a basal medium, a Wnt agonist and/or any one of Rspondin 1-4, a BMP inhibitor, EGF, and a TGF-β inhibitor, and further comprises one or more (or all)

of a p38 MAPK inhibitor, gastrin, nicotinamide, prostaglandine E, N-acetylcysteine, B27 and/or an antimicrobial, wherein the tumouroid culture medium further comprises a Wnt ligand.

12. The method of any one of claims 1-11, wherein the immune cell expansion medium:
(a) comprises IL-2 at a concentration of 2000-6000 IU/mL; and/or
(b) comprises an RPMI medium.

13. The method of any one of the preceding claims, wherein the tumouroid co-culture medium and/or organoid co-culture medium comprises IL-2 at a concentration of 100-200 IU/mL.

14. A method of testing a CAR-T immunotherapy, TCR transgenic T cells, neoantigen, or checkpoint inhibitor, for efficacy and/or safety when used for treating epithelial cancer, the method comprising:

expanding tumour epithelial cells in tumouroid culture medium to form a tumouroid, and culturing the tumouroid with immune cells in a tumouroid co-culture medium comprising interleukin to form a tumouroid co-culture,

expanding normal epithelial cells in organoid culture medium to form an organoid, and culturing the organoid with immune cells in an organoid co-culture medium comprising interleukin to form a reference organoid co-culture,

contacting the tumouroid co-culture and reference organoid co-culture with the CAR-T immunotherapy, TCR transgenic T cells, neoantigen, or checkpoint inhibitor,

detecting the presence or absence of one or more change in the tumouroid co-culture and reference organoid co-culture, wherein the presence or absence of one or more change is indicative of efficacy and/or safety of the CAR-T immunotherapy, TCR transgenic T cells, neoantigen, or checkpoint inhibitor, and

comparing the tumouroid co-culture and reference organoid co-culture, and wherein the immune cells are antigenically compatible with the patient from whom the tumouroid and organoid are derived,

wherein the method is preceded by:

preparing the immune cells by obtaining immune cells from an impure immune sample, and culturing the immune cells in an immune cell expansion medium,

wherein (i) the tumouroid culture medium and/or organoid culture medium comprises IL-2, and/or (ii) the immune cell expansion medium comprises IL-2.

15. A method of testing a candidate compound for efficacy and/or safety when used for treating epithelial cancer, the method comprising:

expanding tumour epithelial cells in tumouroid culture medium to form a tumouroid, and mixing the tumouroid with immune cells in a tumouroid co-culture medium comprising interleukin to form a tumouroid co-culture,

expanding normal epithelial cells in organoid culture medium to form an organoid, and mixing the organoid with immune cells in an organoid co-culture medium comprising interleukin to form a reference organoid co-culture,

contacting the tumouroid co-culture and reference organoid co-culture with the candidate compound,

detecting the presence or absence of one or more change in the tumouroid co-culture and reference organoid co-culture, wherein the presence or absence of one or more change is indicative of efficacy and/or safety of the candidate compound, and

comparing the tumouroid co-culture and reference organoid co-culture, and wherein the immune cells are antigenically compatible with the patient from whom the tumouroid and organoid are derived,

wherein the method is preceded by:

preparing the immune cells by obtaining immune cells from an impure immune sample, and culturing the immune cells in an immune cell expansion medium,

wherein (i) the tumouroid culture medium and/or organoid culture medium comprises IL-2, and/or (ii) the immune cell expansion medium comprises IL-2.

16. A method for preparing a tumouroid-immune cell co-culture, wherein the method comprises:

culturing tumour epithelial cells in an extracellular matrix and tumouroid culture medium, and removing said extracellular matrix and tumouroid culture medium from said tumouroid;

resuspending said tumouroid in immune cell expansion medium;

preparing an immune cell suspension comprising immune cells, immune cell expansion, and collagen at least 5-10% concentration in the suspension; and

mixing the immune cell suspension comprising immune cells with the resuspended tumouroid, and wherein the immune cells are derived from a patient, wherein the immune cells are antigenically compatible with the patient from whom the tumouroid is derived,

wherein the tumouroid culture medium comprises IL-2 and/or the immune cell expansion medium comprises IL-2.

17. The method of claim 16, wherein:

(a) the collagen is rat tail collagen;

(b) the immune cell medium is an RPMI medium;

(c) the immune cells are T cells;

(d) the epithelial cells and immune cells are obtained from the same subject and/or from the same sample; and/or

(e) the epithelial cells and immune cells are human cells.