Claims

1. A method for evaluating primary patient-derived tumor cell spheroids in a three-dimensional microfluidic device, the method comprising:

obtaining primary patient-derived tumor cell spheroids from an enzyme treated tumor sample,

suspending a first aliquot of the primary patient-derived tumor cell spheroids in biocompatible gel;

suspending a second aliquot of the primary patient-derived tumor cell spheroids in biocompatible gel;

placing the first aliquot of the primary patient-derived tumor cell spheroids in biocompatible gel in a first three-dimensional microfluidic device,

contacting the first aliquot with a first fluorophore dye selective for dead cells, the first fluorophore dye emitting fluorescence at a first wavelength when bound to a dead cell,

contacting the first aliquot with a second fluorophore dye selective for live cells, the second fluorophore dye emitting fluorescence at a second wavelength different from the first wavelength when bound to a live cell,

measuring total fluorescence emitted by each of the first and second fluorophore dyes in the primary patient-derived tumor cell spheroids in the first aliquot within the first three-dimensional microfluidic device,

placing the second aliquot of the primary patient-derived cell spheroids in biocompatible gel in a second three-dimensional microfluidic device,

contacting the second aliquot with the first fluorophore dye,

contacting the second aliquot with the second fluorophore dye, wherein the contacting of the second aliquot with the first fluorophore dye and second fluorophore dye is carried out at least 24 hours after the contacting of the first aliquot with the first fluorophore dye and second fluorophore dye,

measuring total fluorescence emitted by each of the first and second fluorophore dyes in the primary patient-derived tumor cell spheroids in the second aliquot within the second three-dimensional microfluidic device, and

wherein an increase or decrease in the ratio of live cells to dead cells in each of the aliquots may be assessed.

2. The method of claim 1, wherein the total fluorescence emitted by each of the first and second fluorophore dyes is measured using a camera, preferably at a resolution of at least 2X, at least 3X, at least 4X or more, from directly above or below each three-dimensional microfluidic device, and preferably wherein the three-dimensional microfluidic devices are placed on a moveable stage permitting the camera to capture the total fluorescence in each aliquot.

3. The method of claim 1 or 2, wherein the second aliquot is contacted with at least one test compound after it is placed in the second three-dimensional microfluidic device and wherein the second aliquot is cultured in the presence of the test compound for at least 24 hours, at least two days, at least three days, at least four days, at least five days, or at least 6 days.

4. The method of claim 1 or 2, wherein the second aliquot is contacted with at least two test compounds after it is placed in the second three-dimensional microfluidic device and wherein the second aliquot is cultured in the presence of the test compounds for at least 24 hours, at least two days, at least three days, at least four days, at least five days, or at least 6 days, and preferably wherein at least one of the test compounds is an immune checkpoint inhibitor.

5. The method of any one of claims 1-4, wherein the first and the second aliquots each contains between about 15 and 30 spheroids, preferably between about 20 and 25 spheroids.

6. The method of any one of claims 1-5, wherein the first aliquot in the first three-dimensional microfluidic device is cultured for less than 6 hours, less than 3 hours, less than 2 hours, or even less than 1 hour prior to contacting the first aliquot with the first and second fluorophore dyes.

7. The method of claim 6, wherein the first aliquot in the first three-dimensional microfluidic device is not cultured prior to contacting the first aliquot with the first and second fluorophore dyes.

8. The method of any one of claim 1-7, wherein the enzyme is collagenase.

9. The method of any one of claims 1-8, wherein the first fluorophore dye is propidium iodide, DRAQ7, 7-AAD, eBioscience Fixable Viability Dye eFluor® 455UV, eBioscience Fixable Viability Dye eFluor® 450, eBioscience Fixable Viability Dye eFluor® 506, eBioscience Fixable Viability Dye eFluor® 520, eBioscience Fixable Viability Dye eFluor® 660, eBioscience Fixable Viability Dye eFluor® 780, BioLegend Zombie Aqua™, BioLegend Zombie NIR™, BioLegend Zombie Red™, BioLegend Zombie Violet™, BioLegend Zombie UV™, or BioLegend Zombie Yellow™, and/or the second fluorophore dye is acridine orange, nuclear green LCS1 (ab138904), DRAQ5 (ab108410), CyTRAK Orange, NUCLEAR-ID Red DNA stain (ENZ-52406), SiR700-DNA, calcein AM, calcein violet AM, calcein blue AM, Vybrant® DyeCycle™ Violet, Vybrant® DyeCycle™ Green, Vybrant® DyeCycle™ Orange, or Vybrant® DyeCycle™ Ruby.

10. The method of any one of claims 1-9, wherein the primary patient-derived tumor cell spheroids are obtained by

mincing a primary tumor sample in a medium supplemented with serum;

treating the minced primary tumor sample with an enzyme; and

harvesting tumor spheroids from the enzyme treated sample, and preferably wherein the minced primary tumor sample is treated with the enzyme in an amount or for a time sufficient to yield a partial digestion of the minced primary tumor sample, and preferably wherein the treatment is for between 10 minutes and 60 minutes, and more preferably between 15 minutes and 45 minutes at a temperature of 25 °C to 39 °C.

11. The method of any one of claims 1-10, wherein the biocompatible gel is collagen, a gel formed from Basement Membrane Matrix, or fibrin hydrogel.

12. The method of any one of claims 1-11, wherein the primary patient-derived tumor cell sample is derived from murine tissue of a Bladder MBT-2, Breast 4T1, EMT6, Colon, Colon26, CT-26, MC38, Fibrosarcoma WEHI-164, Kidney Renca, Leukemia C1498, L1210, Liver H22,

KLN205, LL/2, LewisLung, Lymphoma A20 S, E.G7-OVA, EL4, Mastocytoma P815, Melanoma B16-BL6, B16-F10, S91, Myeloma MPC-11, Neuroblastoma Neuro-2a, Ovarian: ID8, Pancreatic Pan02, Plasmacytoma J558, or Prostate RM-1 murine model.

13. The method of any one of claims 1-11, wherein the primary patient-derived tumor cell sample is a patient derived xenograft (PDX).

14. The method of any one of claims 1-13, wherein the first three-dimensional microfluidic device and/or the second three-dimensional microfluidic device comprises:

one or more fluid channels flanked by one or more gel cage regions, wherein the one or more gel cage regions comprises the biocompatible gel in which the tumor cell spheroids are suspended, and wherein the device recapitulates *in vivo* tumor microenvironment.

15. The method of any one of claims 1-14, wherein the first three-dimensional microfluidic device and/or the second three-dimensional microfluidic device comprises:

a substrate comprised of an optically transparent material and further comprising

i) one or more fluid channels;

ii) one or more fluid channel inlets;

iii) one or more fluid channel outlets;

iv) one or more gel cage regions; and

v) a plurality of posts;

wherein all or a portion of each gel cage region is flanked by all or a portion of one or more fluid channels, thereby creating one or more gel cage region-fluid channel interface regions; each gel cage region comprises at least one row of posts which forms the gel cage region; and the one or more gel cage region has a height of less than 500 μm.

16. The method of any one of claims 3-15, wherein the first test compound is a small molecule, a nucleic acid molecule, an RNAi compound, an aptamer, a protein or a peptide, an antibody or antigen-binding antibody fragment, a ligand or receptor-binding protein, a gene therapy vector, or a combination thereof.

17. The method of any one of claims 3-15, wherein the first test compound is a chemotherapeutic compound, an immunomodulatory compound, or radiation.

18. The method of claim 17, wherein the first test compound is an alkylating compound, an antimetabolite, an anthracycline, a proteasome inhibitor, or an mTOR inhibitor.

19. The method of any one of claims 3-15, wherein the first test compound is an immune modulator.