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Article

Cellular Biophysical Markers for Rapid Evaluation of Immune Checkpoint Inhibitors in Radioimmunotherapy Against Glioblastomas

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Supplementary Figures

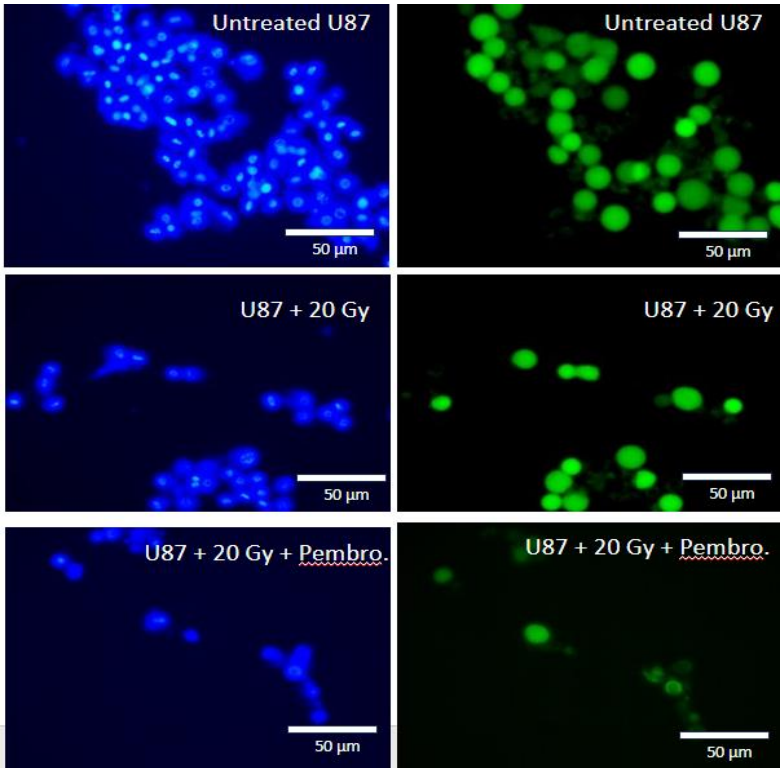

Figure S1. Green fluorescing Calcein and blue-fluorescing Hoechst-stained U87 cells. Following radiation exposure, there was limited impact on the nuclear or cellular morphology of the cells. The scale bar represents 50 µm.

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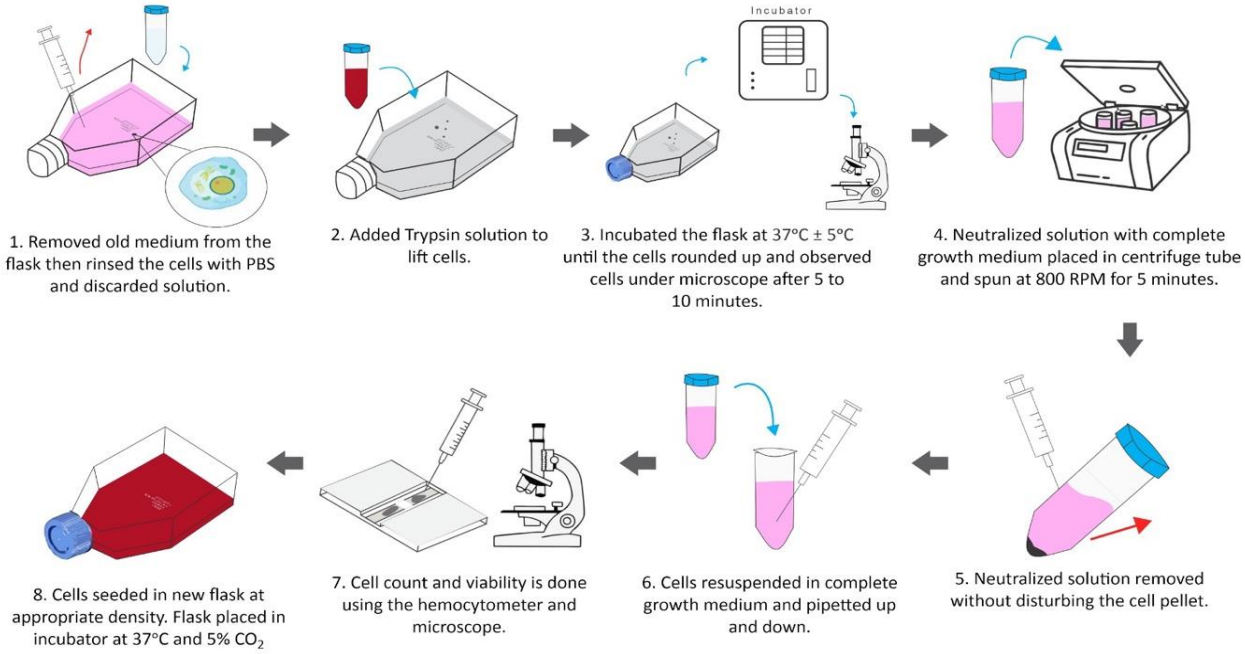

Figure S2. Cell culture protocol. Trypsin was used to detach the cells after the previous medium was discarded and rinsed with phosphate-buffered saline (PBS). After lifting, trypsin was neutralized with a culture medium, and the resulting mixture was put into a centrifuge and spun at 800 RPM for five minutes. Using a hemacytometer, cells were counted. The cell pellet was afterwards resuspended in complete growth medium, and aliquoted into a fresh culture flask at the proper cell density after the neutralized trypsin solution had been removed without disturbing it.

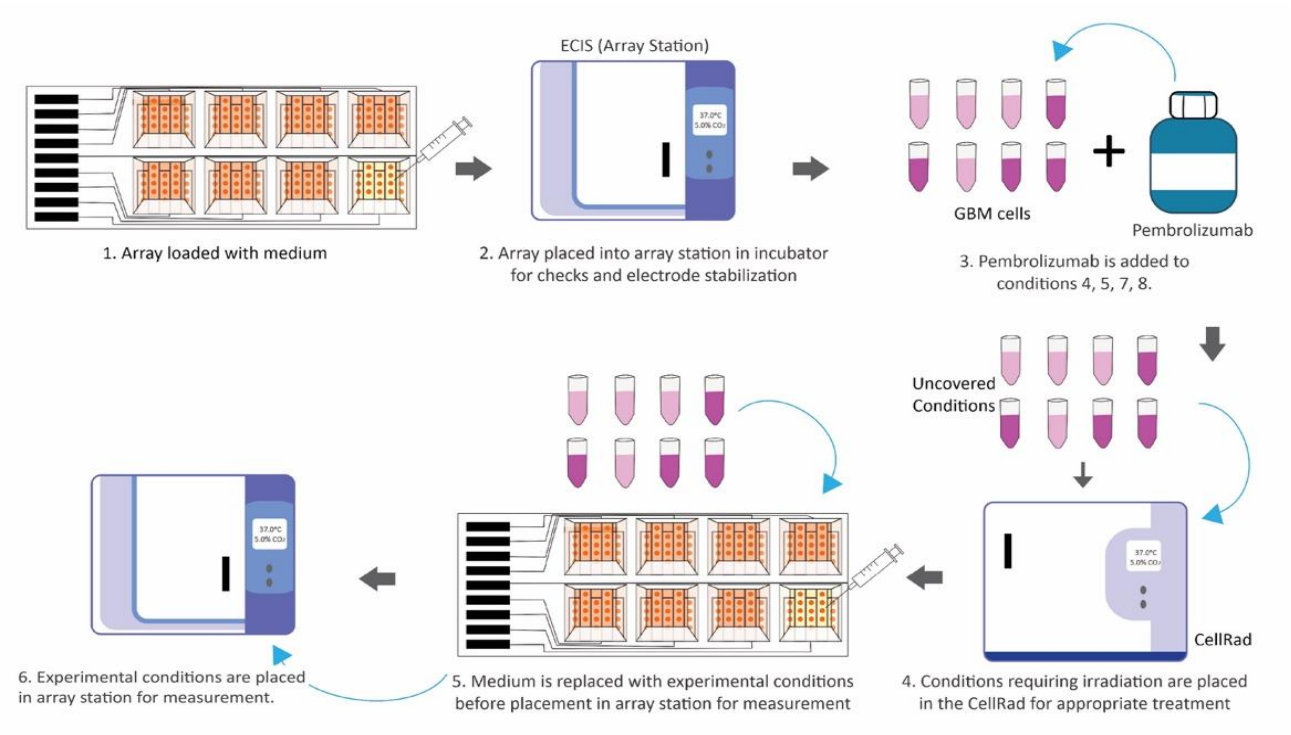

Figure S3. Loading and preparation of the ECIS. 1. 8W10E+ array is loaded with medium as a substrate for built-in "check" and "stabilize" functions prior to the experiment. 2. The array is loaded into an incubator-housed array station for calibration. 3,4. Experimental conditions are prepared separately as needed. 5. Array is removed from the incubator station and medium is replaced by experimental conditions. 6. The array is then placed back into the station and allowed to stabilize for 15 minutes before a final "check" and measurement starts.

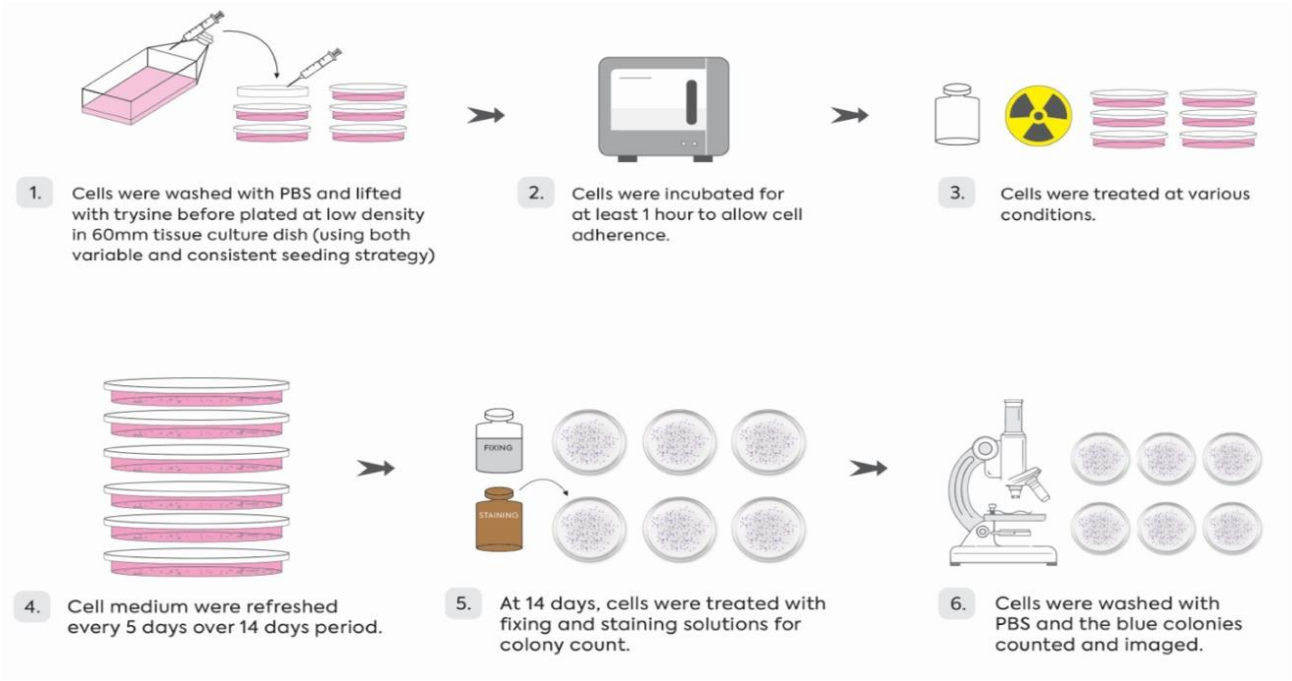

Figure S4. Preparation of clonogenic assays. Step 1: Cells were washed with PBS and lifted with trysine before plated at low density in 60mm tissue culture dish (using both variable and consistent seeding strategy) Step 2: Cells were incubated for at least 1 hour to allow cell adherence Step 3: Cells were treated at various conditions Step 4: Cell medium were refreshed every 5 days over 14 days period Step 5: A At 14 days, cells were treated with fixing and staining solutions for colony count. 6. Cells were washed with PBS and the blue colonies counted and imaged.

Supplementary Tables

Table S1: Relative alpha/beta ratio for T98G treated with radiation (T98G + IR [0 to 20 Gy]) and T98G treated with radiation and pembrolizumab (T98G +IR [0 to 20 Gy] + Pembro).

Conditions	α	β	α/β
N1_T98G_IR	0.6912	0.0154	44.8831
N1_T98G_IR_Pembro	0.7277	0.0189	38.5026
N2_T98G_IR	0.6774	0.018	37.6333
N2_T98G_IR_Pembro	0.1741	0.0025	69.64
N3_T98G_IR	0.6861	0.018	38.1167
N3_T98G_IR_Pembro	0.5624	0.0128	43.9375

Table S2: Relative alpha/beta ratio for T98G treated with radiation (T98G + IR [0 to 50 Gy]) and T98G treated with radiation and pembrolizumab (T98G +IR [0 to 50 Gy] + Pembro).

Conditions	α	β	α/β
N1_T98G_IR	0.5252	0.0069	76.1159
N1_T98G_IR_Pembro	0.5605	0.0081	69.1975
N2_T98G_IR	0.4447	0.0062	71.7258
N2_T98G_IR_Pembro	0.295	0.0037	79.7297
N3_T98G_IR	0.472	0.0071	66.4789
N3_T98G_IR_Pembro	0.4375	0.0064	68.3594