Material and Methods

Cell Line and Cell Culture: The TNBC cell line CAL139 was transduced with several clones of retroviral constructs targeting p53 and grown in DMEM medium and supplemented with 10% FBS. The cells were seeded at 10^4 cells per well in 96-well plates and treated with puromycin (100 µg/mL) to allow for selection of cells that have successfully been transduced.

Cell Harvest for Experiments: The cells were harvested at least 24 hours post transduction and washed with cold PBS buffer to remove any unbound plasmid DNA and debris. Enzymatic digestion of the cells was performed using 0.25% Trypsin-EDTA (PAA Laboratories) for 2-3 minutes at room temperature. The cell suspension was immediately washed with DMEM and counted using a hemocytometer to determine cell density.

Agent Exposure: DU145 cells were seeded at 10^4 cells per well in 96-well plates and treated with the indicated concentrations of ENMD-2076. Cells were transfected with either a non-targeting control or a p53 shRNA construct targeting p53 using lipofectamine 2000. The cells were harvested 24 hours post transduction and the proliferation assay was performed.

Flow Cytometry Analysis: The cells were harvested and fixed with 70% ethanol, and then stained with a propidium iodide (PI) solution (100 µg/mL) for 30 minutes at room temperature. The DNA content of the cells was then analyzed by flow cytometry.

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