**Synergy of Various Inhibitors of the ATR-CHK1-WEE1 axis in Merkel Cell Carcinoma**

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**Introduction**

Merkel Cell Carcinoma (MCC) is a rare neuroendocrine skin cancer. It is asymptomatic and presents as a hard red nodule on the skin. There are several risk factors including old age, fair skin, and immunosuppression. Loco-regional metastasis is already present in ~30% of patients at primary diagnosis due to frequent misdiagnosis as benign1. This has led to a 33%-46% mortality rate2.

Treatments for MCC are extremely limited and mostly consist of surgery, or chemotherapy, so new therapeutic targets need to be found and new drugs for these targets need to be tested. MCC is addicted to the ATR-CHK1-WEE1 pathway (manuscript in progress). Using synthetic lethality to test inhibitors of this pathway against known chemotherapeutic agent Gemcitabine has proven highly effective in determining optimal dosage for combination treatments.

**Methods**

MCC cells (MKL1 and Waga) were grown at 37℃ with 5% CO2 in RPMI media with 10% FBS, 1% Penstrep, and 1% Glutamax. Media changes were done evert three to four days for optimal cell growth and health. For experiments, cells were washed with PBS and treated with Versene to break up cell aggregates. Cells were counted to 10,000 cells/mL and plated in 96 well plates, 200µL per well.

Depending on which inhibitor was used, Gemcitabine, Ceralasertib, Prexasertib, or Adavosertib, different concentrations of inhibitor were used. One would be added to the plate top down and the other would be added to the plate right to left, leaving 4 wells empty in the bottom left corner as control wells. Three different treatment lengths were used, four days, eight days, and twelve days.

After the designated incubation period, 30µL of CellTiterGlo was added to each of the wells of the plate. This was left to incubate for 15-30 minutes and then read on the M2-flourescent plate reader. Originally the results were analyzed using SynergyFinder, which does statistical analysis to determine the synergistic or antagonistic effects of your drug combinations3. Recently, data has be reanalyzed using R to determine synergistic or antagonistic effects.

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