**C3 / Padres Pedal the Cause 2020**

# "FEN1 Nuclease-Targeted Therapy for Ewing Sarcoma"

Jean Wang, PhD (MCC)
Richard Kolonder, PhD (Ludwig/MCC)
Sun Choo, MD (Rady/MCC)

## SCIENTIFIC ABSTRACT

Ewing sarcoma (ES) is driven by the oncogenic EWS-FLI1 fusion protein resulting from *t* (11; 22) chromosomal translocation. EWS-FLI1 sequesters BRCA1 in transcription complexes to prevent it from carrying out homologous recombination (HR). Consistent with this HR-defect, the Cancer Cell Line Drug Sensitivity project found ES cells to be sensitive to PARP inhibitors. HR is one of many pathways that suppress DNA replication errors to maintain genome integrity. Yeast genetics have elucidated synthetic lethal relationships among pathways, including HR, that resolve stalled replication forks, and those relationships can be targeted for cancer therapy. In yeast, mutations of RAD27 (human FEN1) endonuclease that functions in lagging strand DNA synthesis are synthetic lethal with mutations in HR genes. We have shown that this synthetic lethal relationship is conserved because human BRCA-mutant cancer cells are hypersensitive to FEN1 inhibition. Interestingly, we found that a PARP-inhibitor-resistant ES cell line (SK-ES-1) is as sensitive to our proprietary FEN1 inhibitor as BRCA-mutant cancer cells. From mining large-scale CRIPSR screening databases, we found FEN1 to be uniquely essential to all five ES cell lines tested. To evaluate FEN1 as a potential target for ES therapy, this collaborative pilot project will pursue three lines of investigation: 1) Extend the FEN1 inhibitor results to a panel of ES cell lines in comparative studies with PARP inhibitor and clinically relevant chemotherapeutic drugs; 2) Validate the FEN1 inhibitor studies using siRNAs to knockdown FEN1; and 3) Determine how the EWS-FLI1 fusion protein contributes to the FEN1-dependency of ES cells.

## LAY ABSTRACT

Ewing sarcoma (ES) is a bone cancer that affects children and young adults. Despite aggressive treatment survival remains poor because of resistance to chemotherapy. Our study will investigate a new drug target, FEN1, to overcome resistance to current therapy.

FEN1 is an enzyme that helps with DNA replication. Others have shown that ES cells behave like BRCA- deficient cells, which we know are hypersensitive to FEN1 inhibition. ES cells are also sensitive to removal of FEN1 by a process that uses CRISPR to knockout genes. Further, we have found that ES cells from at least one patient are hypersensitive to our FEN1 inhibitor,

SMD2485.

To explore the possibility of treating ES with FEN1 inhibitors, we will measure the effects of SMD2485 in a panel of ES cells. We will compare the effects of SMD2485 to that of Olaparib (PARP inhibitor) and traditional chemotherapy. Patients that are refractory to chemotherapy are often also resistant to PARP inhibitors. By comparing the responses to SMD2485, Olaparib, and chemo drugs, we will determine if FEN1 inhibition is efficacious in treating chemo resistant ES cells. We will silence, by using RNA interference, the abnormal EWS-FLI1 that drives ES. One of EWS-FLI1's many functions is to cause BRCA-deficiency, which then causes ES cells to be sensitive to FEN1 inhibitors. We will determine how silencing EWS-FLI1 will affect the responses of ES cells to SMD2485, Olaparib, and chemo drugs.

This project will provide the necessary pre-clinical data for evaluating FEN1 as a drug target to treat Ewing sarcoma.