TP53 knockout induces sensitivity to palbociclib and 5-fluorouracil combination in breast cancer.

*John M Warrington[1], Jay R Patel[1], Changlong Liu[2], Carolyn E Banister[3], Phillip J Buckhaults[4]

[1] Undergraduate Researcher, University of South Carolina, [2] Post-doctoral fellow, University of South Carolina, [3]Research assistant professor, University of South Carolina, [4]Associate professor, University of South Carolina

Presentation Keywords: TP53, Fluorouracil, Palbociclib, synergy

Introduction: Breast cancer is the most commonly diagnosed and the deadliest malignancy among women in the United States. Of these breast cancers, 20-40% acquire a loss of function mutation in TP53, the tumor suppressor gene necessary for apoptosis to occur within cells [1]. Despite the frequent occurrence of TP53 mutations, there are currently no targeted therapies geared towards this aberration in breast cancers [2]. While mutations in this gene often lead to more aggressive tumors and worse patient outcomes, they may also create a targetable Achilles Heel within the cancer's somatic mutation landscape [3]. To identify therapies that exploit this weakness, we engineered TP53 knockouts using CRISPR-Cas9 gene editing in MCF7 breast cancer cells. These knockout and wild-type cells were then screened against 133 FDA approved anticancer agents from the NCI's approved oncology drug set (AOD-IV). Those drugs that showed TP53-dependent toxicity were identified and characterized.

Methodology: Three sgRNAs were designed to target 192bp and 5,794bp apart within exon 4 and from exon 4 to exon 10 of TP53 (crispr.mit.edu). These sgRNAs were synthesized as individual gBlocks and were co-transfected into TP53 WT MCF7 breast cancer cells along with pCas9-GFP. Transfected cells were grown in the presence of 10MNutlin-3a for 2 months. Two independent Nutlin-resistant pools were selected, one targeting exon 4, the other targeting exons 4-10. Isogenic, single cell clones were established via limiting dilution from the three pools and TP53 presence or disruption was verified by PCR, agarose gel electrophoresis, Sanger sequencing, and Western blotting. The two independent knockout derivative pools and one wild type derivative pool were screened against the 133 FDA approved anticancer agents in the AOD-IV using both resazurin endpoint assays. Drugs that showed greater inhibition of TP53 knockout cell pools were selected and further characterized on single cell clones. Fluorouracil and Palbociclib were selected for synergy experiments as they are commonly used in breast cancer therapy. Synergy experiments were performed on the single cell clones and analyzed using resazurin end point assays and the R-package 'synergyfinder'.

Results: Most TP53 mutations in breast cancers occur in exons 4-9. By targeting this region, we modeled the majority of naturally occurring null mutations. We verified the creation of TP53 knockouts using genomic analysis of the CRISPR-Cas9 targeted region and phenotypic resistance to the MDM2 inhibitor Nutlin-3a. Upon screening the MCF7 cells against the AOD-IV, several drugs routinely used in breast cancer therapy showed far less toxicity toward TP53 knockout cells, consistent with p53's essential role in apoptosis. Notably, the knockouts were more resistant to methoxsalen, mitotane, olaparib, and oxaliplatin. We also observed increased sensitivity of TP53 knockout cells when treated individually with 5-Fluorouracil (5FU) and Palbociclib, a CDK 4/6

inhibitor that is used to treat ER-positive, PR-positive, HER-2 negative breast cancers. Palbociclib also strongly synergized with 5FU against the TP53 knockout derivatives, suggesting that a novel combination therapy stratified by TP53 mutation status would be clinically useful.

Conclusions: These TP53 knockout derivatives of MCF7 cells are useful tools to screen therapeutic regimens for p53 dependent responses. Patients with TP53-mutant tumors may benefit from a combination therapy of 5FU and Palbociclib. Future studies will validate the relative susceptibilities of primary breast cancer organoids with and without TP53 mutations to this combination therapy thus illuminating the contribution of the somatic mutation landscapes with regards to drug sensitivity. Predictable sensitivity to 5FU/Palbociclib combination therapy would justify clinical trials stratified by TP53 mutation status.

References:

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. Ca Cancer J Clin 2018; 68:394–424

[2] Bertheau P, Lehmann-Che J, Varna M, Dumay A, Poirot B, Porcher R, et al. p53 in breast cancer subtypes and new insights into response to chemotherapy. *Breast* 2013; 22 Suppl 2:S27-9.

[3] Pharoah PDP, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br J Cancer*; 1999; 80(12): 1968-73.