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TITLE: TP53 Synthetic Lethal Screen in Organoid Avatars to Discover Novel Therapeutic Targets for Colon Cancer

PRINCIPAL INVESTIGATOR: Phillip Buckhaults

CONTRACTING ORGANIZATION: University of South Carolina

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Phillip J. Buckhaults

E-Mail:buckhaul@cop.sc.edu

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University of South Carolina
1600 Hampton St. #414
Columbia, SC 29208-3403

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14. ABSTRACT:

This application addresses the FY18 PRCRP topic area of Colorectal Cancer. Military relevant risk factors such as ionizing radiation often produce cancers with mutations in TP53. This proposal seeks to address a gap in colorectal cancer treatment that has profound impact on the health and well-being of military service Veterans, their beneficiaries, and extends to the American public as a whole. TP53 mutations are common in colon cancers (50%), and most often lead to resistance to chemotherapy. A systematic screen for synthetic lethal interactions between gene knockout, TP53 mutation, and chemotherapy such as Cisplatin will produce novel targets useful for treating a large fraction of colon cancers.

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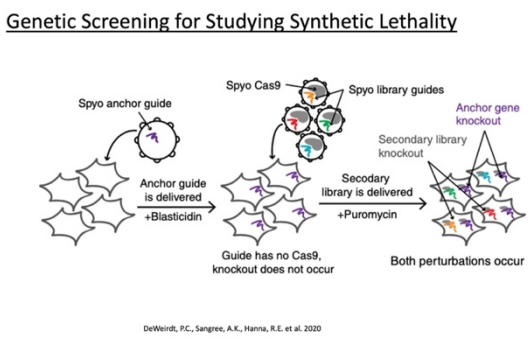
Introduction.

This progress report describes our work during the reporting period 09012020 to 09012021. The overall progress has continued to be hindered by disruptions caused by the global SARS-CoV2 pandemic. This event caused a shutdown of the lab operations, loss of key personnel, and significant delays due to COVID illness of the PI. Nonetheless, in spite of these challenges, some progress has been achieved and work is continuing with remaining resources and personnel.

Keywords: Colon Cancer. TP53. Drug Resistance. CRISPR. Gene Knockout. Synthetic Lethal Interaction.

Accomplishments.

1. We are using the strategy described by DeWeirdt, et al to screen a Brunello library in organoids. We made improvements on the strategy by designing multi-cistronic guide RNAs processed by an intervening tRNA sequence (adapted from Zhao, et al, 2019). The system is being used on two TP53 WT organoids that have been modified with empty anchor vector or TP53-knockout generating anchor vector. We have optimized large scale screening strategy and the Brunello library in Lenti-CRISPRV2 is being screened in the Anchor Vector system. The library screen will be done using long term culture conditions in absence and presence of Ibrutinib, Cisplatin, Olaparib, or Irinotecan with deep amplicon sequencing to detect slight changes in evolutionary fitness. Positives will be confirmed by our novel scar-sequencing methodology developed in previous year of this proposal. Brunello library screens using the modified strategy of DeWirdt et al will be completed over the next several months.



2. Darwinian Fitness during 2 months of chemotherapy selection. Identify somatic mutation evolution under pressure.

12 organoids have been bar coded with individual isolated CLONTRACER library barcode clones and these bar codes are being used to track Darwinian fitness of mixtures of organoids treated with chemotherapies. These bar codes form a simple proxy for private somatic mutations located at different positions in the genome, and standardize the ability to track evolution in mixtures. This is a much improved strategy for assessing evolutionary advantages among mixtures of organoids.

IN the upcoming year of a no-cost extension, we will validate candidate genes identified in CRISPR knockout screen in the presence of chemotherapy by constructing specific clonal knockouts in colon tumor organoids that are TP53 WT, KO and somatically mutated TP53 (in diverse and relevant somatic mutation backgrounds) and determining IC50s for select chemotherapies as a function of gene disruption. We will also use our SCAR-sequencing technology to validate knockouts in the initial screen.

Impact.

Technical advances in library screening in organoids (clonagenic survival, large scale culturing, and bar coding for mixture tracking) have overcome barriers to library screening in organoids. Screens are ongoing now.

Changes/Problems

The pandemic of 2020 caused by SARS-CoV-2 emerging from Wuhan China has had substantial negative impact on this project. Key personnel were lost during the shutdown and operations were harmed in the following manners.

1. Postdoctoral Fellow Candace Poole. She was unable to return to lab due to hazards of being exposed to SARS-CoV-2.
2. Organoid Biobanking / Honest Broker Gabriella Freeze. She was unable to return to the lab due to the hazards of being exposed to SARS-CoV-2.
3. CO-investigator Dr Carolyn Banister has devoted a large percentage of her time to establishing and running a COVID19 CLIA-certified testing laboratory, and her time and effort will be minimal going forward.
4. Surgeries for colon cancers slowed and the influx of new organoids stopped. We are making use of existing bio-banked specimens (about 100 patients) but are behind on our goals for recruiting new samples.
5. Lab was shut down and university closed from March to August, with limited / distanced occupancy during August to December. Research infrastructure is running on skeleton crew, with most people working from home.
6. Out of necessity to keep lab open, the Lead PI developed a novel saliva-based covid test during university lockdown, and shepherded statewide adoption of testing pipeline to allow reopening. These activities, combined with getting COVID TWICE, were more than a little disruptive.
7. PI was infected a second time with Delta in August 2020, and suffered serious long covid effects over subsequent six months.

Changes going forward.

A one year extension is requested

Products.

NA

Participants and other collaborating organizations
NA

Special Reporting Requirements
NA