Synergy with decitabine expands the utility of talazoparib: A novel therapeutic strategy for BRCA-proficient pancreatic cancer

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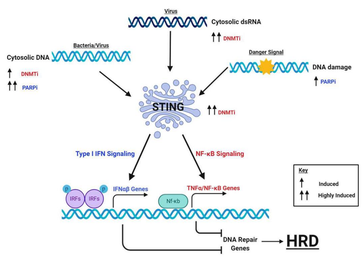
Introduction

- Pancreatic cancer (PCa) is a highly aggressive and lethal malignancy with a poor five-year survival (13%).
- Current systemic therapies for PCa include platinum or gemcitabine-based chemotherapies, which often fail to achieve long-term survival, particularly in metastatic disease.
- Thus, there is a need for improved treatment approaches.
- PARPis are used for patients with breast cancer and homologous recombination deficiency (HRD) – a state induced by BRCA1/2 and PALB2 mutations.
- Approximately 5-7% of patients with PCa have these mutations and benefit from maintenance PARPi use (POLO Trial, *NEJM*, 2019).
- DNA-methyltransferase inhibitors (DNMTis) combined with PARPis have shown promise in other cancers, including breast and AML, regardless of BRCA status.
- Epigenetic changes induced by DNMTis cause the reactivation of endogenous retroviruses that activate STING-dependent inflammatory pathways and induce HRD.



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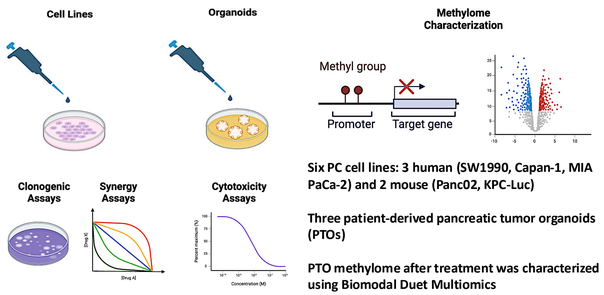
Hypothesis: The combination of DNMTi Decitabine (DAC) and PARPi Talazoparib (TAL) works synergistically to inhibit pancreatic cancer in pre-clinical models, regardless of BRCA mutation status

Methods and Materials



Six PC cell lines: 3 human (SW1990, Capan-1, MIA PaCa-2) and 2 mouse (Panc02, KPC-Luc)

Three patient-derived pancreatic tumor organoids (PTOs)

PTO methylome after treatment was characterized using Biomodal Duet Multiomics

Synergy and Cytotoxicity of DAC + TAL

Cell Lines:

Cell Line	BRCA Status	DAC+TAL Response
Panc02	WT	++
Capan-1	BRCA2 5174delT	++
SW1990	WT	+
MIA PaCa-2	WT	++
KPC-Luc	WT	+++

Table 1. PCa cell lines with corresponding BRCA status and response to DAC + TAL

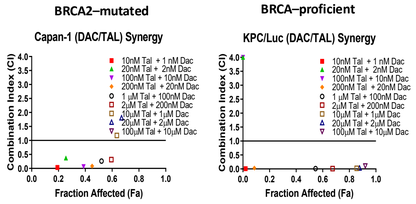


Figure 1. Two representative plots depicting synergy (Combination Index < 1) of DAC+TAL at various doses in Capan-1 (BRCA-2 mutated human PC) and KPC/Luc (BRCA-proficient mouse PC).

Patient-derived Tumor Organoids:

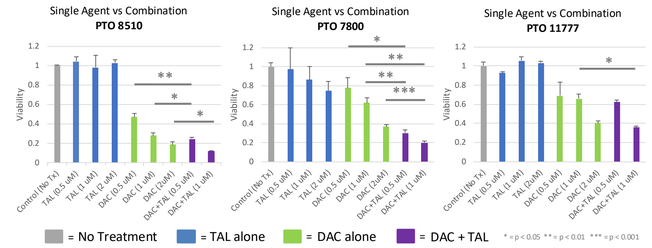


Figure 2. Viability of organoids after treatment with DAC, TAL, and DAC+TAL as determined by MTS assay.

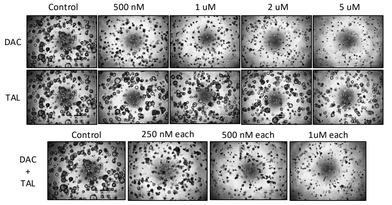


Figure 3. Brightfield microscopy of PTO 8510 after DAC, TAL, and DAC+TAL.

Organoid	Standard Chemo Response	Radiation Response	BRCA Status	DAC+TAL Response
PTO 8510	+	+++	BRCA2 del	++
PTO 7800	+++	+++	WT	+++
PTO 11777	-	-	WT	+

Table 2. Response of PTOs to standard of care chemotherapies and radiation, as previously reported, with BRCA status and graded response to DAC+TAL.

PTO Methylome Analysis

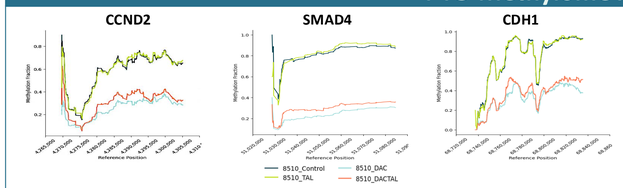


Figure 4. Methylation fraction of three genes known to be hypermethylated in PCa (*CCND2*, *SMAD4*, and *CDH1*) in PTO 8510 after treatment with DAC, TAL, and DAC+TAL.

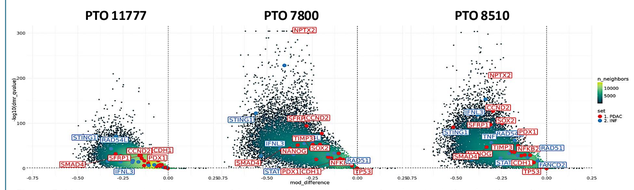


Figure 5. Volcano plots depict genome-wide changes to promoter regions' methylation fraction, with select genes labeled, after DAC+TAL. Significantly demethylated regions are above the dashed line on the x-axis, indicating -log10(DMR_qvalue) > 1.3. In red are select genes relevant to PCa, and in blue are inflammation-associated genes.

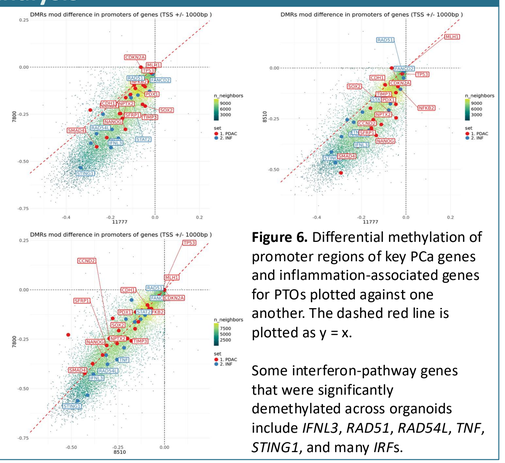


Figure 6. Differential methylation of promoter regions of key PCa genes and inflammation-associated genes for PTOs plotted against one another. The dashed red line is plotted as y = x.

Some interferon-pathway genes that were significantly demethylated across organoids include *IFNL3*, *RAD51*, *RAD54L*, *TNF*, *STING1*, and many *IRFs*.

Discussion and Future Directions

- The combination of DAC + TAL synergistically inhibits mouse (Panc02, KPC-Luc) and human PC cell lines (SW1990, Capan-1, MIA PaCa-2).
- Patient-derived PTOs were more inhibited by DAC+TAL than by low doses of single agents.
- Methylome analysis showed that DAC is driving epigenetic changes in these PTOs, which are largely conserved when combining DAC with TAL.
- PTO 11777 is less responsive to either agent alone or in combination and shows a more limited impact on the methylome compared to other PTOs.
- Genes hypermethylated in PCa were demethylated following DAC treatment in these PTOs.

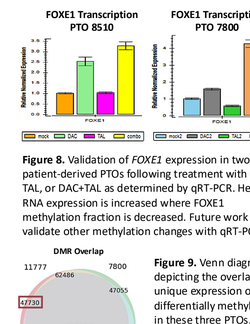


Figure 7. Heatmap depicting the effect of DAC, TAL, and DAC+TAL on the methylation fraction +/- 1000 base pairs upstream or downstream from key PCa driver genes in treated PTOs.

Figure 8. Validation of *FOXE1* expression in two patient-derived PTOs following treatment with DAC, TAL, or DAC+TAL as determined by qRT-PCR. Here, RNA expression is increased where FOXE1 methylation fraction is decreased. Future work will validate other methylation changes with qRT-PCR.

Figure 9. Venn diagram depicting the overlap and unique expression of differentially methylated regions in these three PTOs. Highlighted in the red box are DMRs unique to PTO 11777 after DAC+TAL treatment. Highlighted in the blue box are DMRs shared by PTO 7800 and PTO 8510.

Future Directions

- Validate changes in expression of key PCa driver genes and STING-dependent interferon genes with qRT-PCR (to be performed with 7 PTOs).
- Identify novel methylation regions associated with resistance by comparing PTO 11777 to PTO 7800 and PTO 8510.
- Complete *in vivo* mouse studies with DAC+TAL to justify a clinical trial.

Conclusions

- The combination of PARPi (Talazoparib – TAL) and DNMTi (Decitabine – DAC) functions synergistically to inhibit PCa cell lines and patient-derived PTOs.
- Treatment with DAC and TAL mediate epigenetic changes of known hypermethylated genes in PCa.
- Promoter regions associated with interferon pathway factors are significantly demethylated after DAC+TAL treatment.
- The resistance of PTO 11777 to DAC and TAL correlates with a lesser impact of DAC or DAC+TAL treatment on the genome-wide methylation fraction.
- A clinical trial investigating DAC + TAL for patients with PCa is warranted.

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References

1. Muvarak, Nidal E., Khadiza Chowdhury, Limin Xia, Carine Robert, Eun Yong Choi, Yi Cai, Marina Bellani, et al. "Enhancing the Cytotoxic Effects of PARP Inhibitors with DNA Demethylating Agents - A Potential Therapy for Cancer." *Cancer Cell* 30, no. 4 (October 10, 2016): 637–50. https://doi.org/10.1016/j.ccell.2016.09.002
2. Golan, Talia, Pascal Hammel, Michele Reni, Eric Van Cutsem, Teresa Macarulla, Michael J. Hall, Joon-Oh Park, et al. "Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer." *New England Journal of Medicine* 381, no. 4 (July 25, 2019): 317–27. https://doi.org/10.1056/NEJMoa1903387.
3. Abbotts, Rachel, Michael J. Topper, Christopher Biondi, Daniel Fontaine, Reena Goswami, Lora Stojanovic, Eun Yong Choi, et al. "DNA Methyltransferase Inhibitors Induce a BRCAness Phenotype That Sensitizes NSCLC to PARP Inhibitor and Ionizing Radiation." *Proceedings of the National Academy of Sciences* 116, no. 45 (November 5, 2019): 22609–18. https://doi.org/10.1073/pnas.1903765116.
4. Mclaughlin, Lena J., Lora Stojanovic, Aksinija A. Kogan, Julia L. Rutherford, Eun Yong Choi, Ray-Whay Chiu Yen, Limin Xia, et al. "Pharmacologic Induction of Innate Immune Signaling Directly Drives Homologous Recombination Deficiency." *Proceedings of the National Academy of Sciences of the United States of America* 117, no. 30 (July 28, 2020): 17785–95. https://doi.org/10.1073/pnas.2003499117.
5. Kogan, Aksinija A., Michael J. Topper, Anna J. Dellomo, Lora Stojanovic, Lena J. McLaughlin, T. Michael Creed, Christian L. Eberly, et al. "Activating STING1-Dependent Immune Signaling in TP53 Mutant and Wild-Type Acute Myeloid Leukemia." *Proceedings of the National Academy of Sciences of the United States of America* 119, no. 27 (July 5, 2022): e2123227119. https://doi.org/10.1073/pnas.2123227119.
6. Roy, Sanjit, Tijana Dukic, Zachery Keepers, Binny Bhandary, Narottam Lamichhane, Jason Molitoris, Young H. Ko, Aditi Banerjee, and Hem D. Shukla. "SOX2 and OCT4 Mediate Radiation and Drug Resistance in Pancreatic Tumor Organoids." *Cell Death Discovery* 10, no. 1 (March 1, 2024): 1–8. https://doi.org/10.1038/s41420-024-01871-1.
7. Shukla, Hem D., Tijana Dukic, Sanjit Roy, Binny Bhandary, Andrew Gerry, Yannick Poirier, Narottam Lamichhane, et al. "Pancreatic Cancer Derived 3D Organoids as a Clinical Tool to Evaluate the Treatment Response." *Frontiers in Oncology* 12 (January 12, 2023). https://doi.org/10.3389/fonc.2022.1072774.