Dual Inhibition of PARP and Akt Induces Metabolic Collapse and Apoptosis in Breast Cancer Cells

Thesis for the Doctor of Philosophy (Ph.D.) Degree

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1. INTRODUCTION

1.1 Breast Cancer Background and Clinical Challenges

Breast cancer is the most commonly diagnosed cancer among women worldwide. The incidence of the disease is around 2.3 million, whereas the mortality rate is 670,000 deaths, according to the World Health Organization (WHO) in 2022. Although age and female sex are major risk factors, about half of all cases occur in women without identifiable risk factors. Early detection and access to comprehensive multidisciplinary treatment are crucial for reducing global disparities in outcomes (WHO). Breast cancer is considered a heterogeneous chronic disease regulated by multiple connections including the genetic, cellular metabolism, and signaling pathways. A lack of treatment targets, and its malignant biological characteristic features render it a substantial concern. In clinical management of BC genetic backgrounds and metabolic phenotypes, which are based on the presence of molecular markers. These include the human epidermal growth factor receptor 2 (HER2) progesterone receptor (PR), and estrogen receptor (ER) (Tan et al., 2021). Triple-negative breast cancer (TNBC), which is characterized by the absence of progesterone receptors, estrogen receptors, and HER2 expression, is considered one of the highly aggressive forms of breast cancer with a poor prognosis, characterized by high proliferative capacity, genomic instability, and limited responsiveness to targeted therapy and recurrent neoplastic tumors, posing remarkable challenges (Jeffrey et al.2000).

1.2 Akt pathway and Akt Inhibitors in Breast Cancer Therapy

The PI3K pathway is a highly conserved signal transduction network in eukaryotic cells that coordinates different cellular metabolic processes, such as growth, proliferation, biosynthesis of macromolecules (Glaviano et al.,2023). inhibiting Akt could activate alternative pathways, including increased dependency on glycolysis and nutrient consumption changes. Capivasertib (AZD5363), an ATP-competitive pan-Akt inhibitor, has been shown to have medical effectiveness in breast neoplasms with PI3K/ AKT modulation. However promising, Akt inhibition as monotherapy usually initiates alternative responses, such as enhancing aerobic mitochondrial respiration or triggering of different metabolic pathways, which decrease its treatment outcome (Gris-Oliver et al., 2020).

1.3 PARP Enzymes: Biological Function and Therapeutic Targeting

Poly (ADP-ribose) polymerases particularly PARP1 and PARP2, are nuclear proteins mediators of deoxyribonucleic acid (DNA) damage repair processes. PARP1 is the most common and abundant and will characterize members of the PARP enzyme family. Mainly activated by DNA single-strand breaks (SSBs), PARP1 mediates the transfer of ADP-ribose molecules from nicotinamide adenine dinucleotide (NAD^+) onto itself and target proteins producing poly (ADP-ribose) chains that attract DNA repair mechanisms (Sukhanova et al.,2016). These mechanisms act as essential processes for genomic stability but could also modify chromatin dynamics, and cellular stress responses. The therapeutic effect of inhibiting PARP is commonly hypothesized to attenuate DNA repair. In contrast, the inhibition of PARP regulates redox signaling pathways and mitochondrial homeostasis, leading to increased ROS generation and sensitization to metabolic stress (Zong et al.,2022).

1.4 Combination therapy

The combination treatment in medical oncology started in 1965, represented by multi-therapeutic agent programs like the prednisone, oncovin(vincristine), methotrexate, and 6-mercaptopurine POMP protocol for pre-adulthood acute lymphoblastic leukemia, which revealed that targeting some signaling pathways can reduce therapeutic resistance and enhance recovery rates (Bayat Mokhtari et al., 2017). The combination of antineoplastic therapy could overcome drug resistance and develop new treatment choices. The favorable effect of combination treatment convers more than disseminated tumors (Jaaks et al., 2022). Ibrahim et al., demonstrated that inhibition of PI3K sensitizes *BRCA*-Proficient triple negative breast cancer cells, including the aggressive type MDA-MB-231 cells, to PARP inhibition by downregulating *BRCA* expression and promoting DNA damage.

2. Research Objective

General Objective

Breast cancer is the most diagnosed cancer in women worldwide, demonstrates aggressive clinical behavior especially TNBC and has poor response to existing targeted therapy rendering it a substantial clinical concern. Resistance to PARP inhibitors often arises from the restoration of homologous recombination or metabolic adaptation, whereas resistance to Akt inhibitors typically involves the activation of parallel signaling pathways. This study investigated whether the combination of PARP and AKT inhibitors enhances cytotoxic and anti-clonogenic effects by altering cellular energy production, mitochondrial metabolic function, and reactive oxygen species generation to overcome the resistance raised in breast cancer cell lines with monotherapy.

Specific Objectives

- To compare the effects of PARP inhibition, Akt inhibition, and their combination on cell viability in MDA-MB-231 and MCF7 breast cancer cell lines.
- To investigate long-term treatment effects on reducing proliferative capacity using the colony formation assay.
- To quantify the generation of reactive oxygen species (ROS) following the treatment protocol.
- To evaluate cellular bioenergetic using the Seahorse Mito Stress Test, the core mitochondrial parameters, the extracellular acidification rate, and oxygen consumption rate were measured.
- To determine apoptotic induction, we used Annexin V/ propidium iodide flow cytometry.

3. RESULTS

3.1 The effects of Olaparib (Op) and Capivasertib (Cap), and the Combination Treatment, on Breast Cancer Cell Viability

To assess antineoplastic effects, MCF7 and MDA-MB-231 human cell types were treated with 1 μM capivasertib, 5 μM olaparib, and the combination for 72 hours. The viability of breast cancer cell lines was evaluated using the sulforhodamine B (SRB) colorimetric method. In MCF7 cell lines, viability decreased significantly with olaparib and capivasterib. Furthermore, the combinatorial treatment significantly reduced the viability in both cellular models more than monotherapy.

3.2 The Effect of Olaparib, Capivasertib, and The Combination on Colony Formation

These assays were used to evaluate the capacity of the single cells to form colonies, and to evaluate the cytostatic effects of olaparib and capivasertib and the combination on human breast cancer cell lines during long term-treatment. The combination treatment in two cell types has the most significant decrease in the number of colonies.

3.3 Effects of Olaparib, Capivasertib, and The Combination Therapy on Reactive Oxygen Species Generation in Breast Cancer Cells

The reactive oxygen species were measured with dihydrorhodamine 123. In MCF7 cell lines, capivasertib increased reactive oxygen species generation significantly, in contrast, olaparib had no significant effect on ROS production in both cell types. Whereas the combination therapy significantly increased reactive oxygen species production in both cell types more than other treatments.

3.4 The Effect of Olaparib, Capivasertib, and The Combination on Inducing Cell Death Types

Flowcytometric analysis was used to demonstrate the type of cell death induced by the treatments. MCF7 and MDA-MB-231 breast cell lines were treated with 5 μM Olaparib, 1 μM Capivasertib, and the combination for 72 hours. In the MCF7 human breast cells, olaparib had different effects on cell distribution, with a slightly significant decrease in the live cell population. Whereas in the MDA-MB-231 cells, during the late apoptotic cell death stage, olaparib had a significant effect. In contrast, the capivasertib had no significant effect on the distribution of MDA-MB-231 cells,

moreover, capivasertib significantly decreased the viable cell number among MCF7 cell lines. Interestingly, the combination therapy had a significant effect on the cell distribution in the two cell lines, with an increased rate of apoptotic cell death.

3.5 Effects of Olaparib, Capivasertib, and The Combination Therapy on Energy Metabolism in Breast Cancer Cells

The Seahorse technology was used to measure an essential mitochondrial bioenergetic parameter. MCF7 and MDA-MB-231 cell lines were exposed to treatment with 5 μM Olaparib, 1 μM Capivasertib, and the combination for 72 hours. Olaparib and capivasertib demonstrated a significant reduction in basal respiration in the MCF7 cell line. Whereas the combination treatment decreased the basal respiration significantly in both cell types. Interestingly, in MCF7 cells, all treatments had a significant decrease in ATP production, and the combinatorial therapy had the most significant reduction level. In contrast, the combination therapy alone significantly decreased the ATP generation in MDA-MB-231 cell lines. The carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP), a mitochondrial membrane potential disruptor, was used to quantify maximal respiration. In MCF7 cells, all therapeutic agents reduced maximal respiration, and the combination therapy had the most significant reduction rate. On the contrary, in MDA-MB-231 human cancer cells, the combination treatment is the only therapy that significantly decreases maximal respiration. Furthermore, Rotenone and antimycin A were used to measure non-mitochondrial respiration to inhibit complexes I and III. Capivasertib decreased non-mitochondrial respiration significantly in MCF7 cells, while among the two cell types, the combination therapy showed the most significant reduction in non-mitochondrial respiration. In MCF7, the combination treatment had a marked significant decrease in proton leak compared with other treatments. Furthermore, the combination treatment significantly decreased proton leak in MDA-MB-231 cell lines. The spare respiratory capacity was calculated from the difference between maximal and basal respiration. In the MCF7 cell line, capivasertib, was significantly reduced the spare respiratory capacity. Importantly, in both cell lines, the combination treatment significantly reduced spare respiratory capacity. This study revealed that the combination of Poly (ADP-ribose) polymerase and AKT inhibitors disrupts mitochondrial energy production in cellular breast cancer. The combination therapy reduced the essential mitochondrial bioenergetic function, such as basal respiration, maximal respiration, adenosine triphosphate generation, proton leak, and spare respiratory capacity. Interestingly, these findings demonstrated that dual inhibition of poly(ADP-

ribose) polymerase and AKT impaired the important mitochondrial energetic parameters in breast cancer cell types.

3.6 The Combination Therapy of Olaparib and Capivasertib Selectively Impaired Oxidative Phosphorylation in Human Breast Cancer

The Seahorse Mito stress test was used to demonstrate the metabolic effects of the olaparib and capivasertib combination. The MCF7 and MDA-MB-231 cell lines were exposed to treatment with 5 μM Olaparib, 1 μM Capivasertib, and the combination therapy for 72 hours. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured during 75 min. The results indicated that combination therapy had a most decrease in OCR and ECAR ratios compared to monotherapy in both cell types. The adenosine triphosphate production rate from oxidative phosphorylation had been significantly altered with the combination treatment. Interestingly, adenosine triphosphate production rate from glycolysis had no significant difference between the groups. The results were further supported by calculating the oxygen consumption rate to extracellular acidification rate ratio. The two cell lines demonstrated a decrease in energy generation with combination therapy, derived from the oxidative phosphorylation, with no compensatory rise in glycolysis.

4. SUMMARY

The current study revealed that the dual inhibition of poly (ADP-ribose) polymerase and the Akt signaling pathway using olaparib and capivasertib respectively, exhibits highly potent anti-neoplastic cell activity in MDA-MB-231 and MCF7 human cell lines. Combination therapy significantly decreased cellular viability and reduced single-cell ability to form colonies more than each treatment alone. Moreover, this dual inhibition approach triggered marked disruption of mitochondrial metabolic function, altering oxidative phosphorylation, decreasing adenosine triphosphate generation, and increasing ROS production.

Importantly, the breast cancer cell lines did not compensate by remodeling metabolism toward glycolysis, leading to cellular metabolic collapse and triggering the redox-dependent apoptotic cell death. Seahorse technology was used to assess the essential mitochondrial bioenergetic parameter and the combination treatment decreased significantly basal respiration, maximal respiration, non-mitochondrial oxygen consumption, spare respiratory capacity and proton leak more than monotherapy. Furthermore, in vivo experiments are needed to assess the anticancer efficacy and investigate ATP generation and fatty acid oxidation in tissues with high metabolic activity. These investigations are important for evaluating the therapeutic potential of combination therapy.

5. PUBLICATIONS

List of publications

- Adam, Nasreldeen Mohamed Karshom, Eszter Vámos, Hamid Ahmadi, Geofrey Ouma Maloba, Arshi Arshi, and Ferenc Gallyas Junior. 2025. "Dual Inhibition of PARP and Akt Induces Metabolic Collapse and Apoptosis in Breast Cancer Cells" *Cancers* 17, no. 23: 3828. https://doi.org/10.3390/cancers17233828 (IF:4.5).

- Maloba, Geofrey Ouma, Tom Were, Erick Barasa, Nasreldeen Mohamed, Arshi Arshi, and Ferenc Gallyas. 2025. "Synergistic Effects of 2-Deoxyglucose and Diclofenac Sodium on Breast Cancer Cells: A Comparative Evaluation of MDA-231 and MCF7 Cells" *Inter*national Journal of Molecular Sciences 26, no. 10: 4894. https://doi.org/10.3390/ijms26104894 (IF: 4.9).

List of Conference Abstracts

- Adam Nasreldeen Mohamed Karshom, Eszter Vámos, Arshi Arshi, and Ferenc Gallyas Jr., "Dual Inhibition of AKT and PARP As a Mitochondria-Targeted Therapeutic Approach in Triple-Negative Breast Cancer Cell Line," Mitochondrial Medicine and Therapeutic Development, Wellcome Genome Campus, UK, November 10–12, 2025.

- Adam Nasreldeen Mohamed Karshom, and Ferenc Gallyas Jr., "Combination of Olaparib and Capivasertib Induces Metabolic Collapse and Apoptosis in MCF7 Breast Cancer Cells", NAD for Health, Royal Danish Academy of Sciences and Letters, Copenhagen, March 23–25, 2026.

- Arshi Arshi, Geofrey Ouma Maloba, Adam Nasreldeen Mohamed Karshom, and Ferenc Gallyas Jr., "Effects of 2-Deoxy Glucose and Diclofenac Sodium on MCF7 Cell Lines," FEBS3+ Meeting 2025 in Belgrade.

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