Epithelial ovarian cancer (EOC) is the most commonly fatal gynecologic malignancy. Most EOC patients are diagnosed at advanced stage when disease has spread beyond the ovary. Such a high fatality rate is linked to the complexity of making an accurate diagnosis. Most cases are diagnosed at the third or fourth stage of clinical advancement of the disease. Important risk factors include a family history of ovarian cancer and a genetic predisposition such as BRCA1/BRCA2 mutations. Currently, only a few FDA (Federal Drug Agency)-approved drugs (carboplatin, anthracyclines and taxane-based chemotherapy) are available but they can cause side effects and are effective temporarily only in patients with mild to moderate ovarian cancer. Therefore, there is clearly a great need for new therapies that target the underlying cause(s) of ovarian cancer, reverse symptoms or reduce completely. Olaparib (AZD2281, PARPi), the first approved DNA repair inhibitor, remains one of the most important anticancer drugs in the clinic. DNA repair enzyme called PARP has shown promise killing cancer cells with defects in DNA repair due to mutations in the DNA repair genes BRCA1 and BRCA2. Combining olaparib with ATR or CHK1 kinase inhibitors could potentially deliver a lethal effect in cancer cells.

The DNA damage response pathway is a very complex signaling network in which many proteins are involved. We are proposing a novel kind of approach to obtain a superior effect at lower doses. Combination of olaparib with ATRi (ataxia telangiectasia and Rad3-related kinase inhibitor, AZD6738) or CHK1i (checkpoint kinase 1 inhibitor, MK8776) may therefore represent an effective strategy to sensitize ovarian cancer cells to olaparib. ATR and CHK1 could repair cells with damage DNA by a mechanism called homology repair (HR). This occurs when a healthy strand of DNA is used as a template to repair the identical, but damaged DNA strand.

We assume that PARPi increases dependence on protein activity in the ATR/CHK1 pathway to repair DNA damage and maintain genomic stability, so that the combination of PARPi with ATRi /CHK1i will result in increased cell death *in vitro* and tumor regression induced in mice (*in vivo*). We also expect that the tested compounds will be effective in both ovarian cancer cells, with and without BRCA mutation.

Thus, the aim of the research is to show that PARPi treatment results in activation of ATR /CHK1 pathway. The next step of the study will be to check whether combination of PARPi (AZD2281) with either CHK1i (MK8776) or ATRi (AZD6738) improve cell growth inhibition of HR-proficient/deficient EOC with/without BRCA mutation. The cytotoxic and genotoxic effects of tested compounds will be determined *in vitro* (resistant and sensitive ovarian cancer cell lines) and *in vivo* by using mice model. The research methods that will be used in the project are spectrophotometry, fluorescence, flow cytometry, western blot, immunofluorescence tests, immunohistochemistry, protein electrophoresis (SDS-PAGE), real-time PCR and cytogenetic analysis.

We want to confirm that PARPi-ATRi or PARPi-CHK1i will change miRNAs expression, significantly increase the level of chromosomal aberrations and will cause improper DNA double strand break (DSB) repair before entering mitosis. Inhibition of ATR-CHK1 and PARP together increases the DSB generation, resulting in either amplified apoptosis or mitotic catastrophe due to synthetic lethality. This process is characterized by the simultaneous lack of activity of at least two protein products of specific genes, resulting in cell death, while each of them silenced individually does not cause a lethal effect. We expect tumor growth suppression or regression after CHKi and ATRi as single agents and much stronger changes in combination of ATRi/CHK1i with olaparib. We look forward to an insignificant or no side effects due to the use of small doses of compounds, although we cannot rule them out. Confirmation of hypothesis will provide the basis for the continuation of studies on AZD6738 (ATRi), MK-8776 (CHK1i) as compounds for widespread use in ovarian cancer.

Presented project places itself in the mainstream of fundamental researches. Therefore, study carried out in the project will significantly increase the knowledge on the mechanism of action of ATR and CHK1 kinase inhibitors administered singly and combined with olaprib in ovarian cancer.