Colorectal cancer (CRC) was the third most common cancer worldwide while In Poland, nearly 11 500 new cases are noted each year. Despite significant progress in early detection and treatment the morbidity is still as high as 62%. Thus, the management of CRC patients is a great burden to health care system and budget. New efficient drug combinations are constantly being sought because currently approved therapies fail in a substantial number of CRC patients.
Epigenetic modification e.g. hypermethylation of cancer genome often leads to reduced expression of tumor suppressor genes and makes it attractive target for cancer treatment itself or as a sensitization stage for other chemotherapeutics. Two the most extensively studied inhibitors of DNA methylation are 5-azacytidine (5-aza-C) and 5-aza-2'-deoxycytidine (5-aza-dC, decitabine), analogs of cytidine and 2'-deoxycytidine. In 2004 (5-aza-C) and 2006 (5-aza-dC) they were approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with myelodysplastic syndromes (MDS) (FDA 2017). After cellular uptake, 5-azanucleosides are phosphorylated and incorporated into DNA during replication as normal nucleotides (or also to RNA in case 5-aza-C). In preclinical studies, 5-azanucleosides have been demonstrated to be effective against various cancer cell lines. However, the early clinical studies have indicated that treatment of solid tumors with these DNMT inhibitors leads to only limited responses, especially in solid tumors. There are many reports that cytidine analogs may increase the effectivity of other chemotherapeutics for example cisplatin in prostate cancer or nuroblastoma, erlotinib and gefitinib in AML and CRC, or finally etoposide and doxorubicin in neuroblastoma. Although the results and proposed mechanisms are often contradictory.

In our studies, we demonstrated that low concentration of 5-aza-dC, 5-aza-C induce long-lasting sensitization of CRC cells to whole class of chemotherapeutic namely inhibitors of topoisomerase (TOP). This effect was valid in wide spectrum of CRC cells regardless their genetic backgrounds and can be applied to the cells which were initially sensitive or resistant to TOP inhibitors. Those findings strongly encourage *in vivo* studies on combinatorial use of DNA demethylating agents and TOP inhibitors.
In this regard the main aim of this project is to evaluate the synergy between the azanucleosides and topoisomerase inhibitors in animal model of human colorectal cancer model.
Usage of therapeutic scheme where there is a long gap between the treatments as well as opportunity for significant decrease of effective doses can result in reduction of adverse reactions caused by chemotherapy. Regarding the molecular mechanism of 5-aza-dC and TOP inhibitors we found that:
1. Only 5-aza-dC, 5-aza-C, which need to be incorporated to DNA molecule for its activity are able to enhance effectivity of TOP inhibitors.
2. At least 2-day gap between the treatments is required to observe an enhanced effect of the drugs. It suggests the 5-aza-dC or 5-aza-C incorporation into DNA as crucial element of the sensitization.
The next aim of our project is to study the nature of cellular response and the long-term effects of azanuclosides and TOP inhibitors on cancer cells *in vitro* and *in vivo* models.
In heart of proposed mechanism of action are the direct interaction drug-stabilized enzyme-DNA complexes. We will take advantage of our unique instrumentation optical trap, atomic force microscope and 2D magnetic resonance we could address the questions of direct interaction of 5-aza-dC and TOP inhibitors on transcription and replication complexes in straightforward way.
Considering this fact, the third aim of this project will be a single-molecule study of direct mechanism of immediate toxicity of 5-aza-dC and TOP inhibitors related to DNA molecule.
Our findings strongly suggest that the combination of these two drug classes represents a general and promising therapeutic approach for the treatment of CRC and possibly other cancers.
We believe that our project:
1) Will answer the question of efficacy of sequential therapy with 5-aza-dC and TOP inhibitors. Prior exposure to 5-azanucleosides could potentially reduce TOP inhibitors dosing and therefore decrease their side effects. This could impact the cost of cancer patient care.
2) Resolve the DNA-related mechanisms of 5-aza-dC and TOP inhibitors. Regardless the results of the first part of the project our interdisciplinary approach will shed the new light on mechanism of action of widely used chemotherapeutics allowing its more rational use in the future.