The knowledge of the biology of cancer remains unsatisfactory. Due to the absence of this full knowledge, anticancer therapies are not completely effective. Personalized cancer therapy may allow for effective treatment and for minimizing its toxic effects to a minimum. One of the promising approaches for cancer treatment is personalized cancer therapy based on the synthetic lethality (SL) phenomenon. SL is defined as a combination of genetic mutations in two or more genes leading to cell death, while the mutation in each gene individually does not produce this effect. In this scheme, the cell loss of a gene involved in important cellular metabolic process, allowing for its survival is compensated by a second gene involved in the alternative pathway for this process. In the tumor cell, where the loss of one of these genes is very likely due to the magnitude of various types of rearrangements in the genome of the cell, its survival depends on the alternative gene. This second gene is a target for inhibitors. Disabling this alternative gene by the use of an inhibitor is a target for novel anticancer therapies. Tumors are often defected in DNA repair, suggesting that inhibition of the alternative DNA repair pathways may lead to eradicate them by SL effect. There are many DNA repair pathways, where one replaces another, e.g. homologous recombination repair (HRR) may be compensated by non-homologous end joining (NHEJ). Both of these types of repairs are the mechanisms repairing a DNA double-strand breaks (DSBs). In proliferating cells DSBs, the most lethal DNA lesions, are usually repaired by two major mechanisms, BRCA1/BRCA2-RAD51 (BRCA) dependent homologous recombination (HR) and DNA-PKcs-mediated non-homologous end-joining (D-NHEJ), whereas PARP1-dependent back-up NHEJ (B-NHEJ) serves as an alternate mechanism. HRR usually depends on BRCA, and RAD52-RAD51 serves as back-up and targeting RAD52 in cancer cells defected in main pathway, should kill them, by SL inducing. Also, PARP1 exerts an important impact on DSB repair rate, because this is a protein that binds to both single- and double-strand DNA breaks and later on modifies proteins involved in their repair. Because of SL, PARP1 inhibitors, like Olaparib, may be highly effective drugs in patients whose tumors have germline or somatic defects in DNA damage and repair genes. Recent studies demonstrated that POLQ becomes essential in cells deficient in factors facilitating canonical DSB repair mechanism (BRCA1, BRCA2, Ku70) indicating backup function of POLQ-dependent DNA repair processes. This discovery led to the increasing attention to Polθ as a novel therapeutic target. Now, new genes involved in DNA damage repair, chromatin structure maintenance and DNA metabolism are being identified as Polθ synthetic lethality partners. In our project we plan to utilize synthetic lethality (SL) and dual synthetic lethality (DSL) approach to eliminate DSB-repair-deficient primary cancer cells. According to TCGA database glioblastoma, melanoma and pancreatic cancer cells express increased POLQ mRNA levels when compared to normal counterparts. In our project we plan to determine if survival of given primary cancer cell line carrying specific DSB-repair-deficiency depends on Polθ mediated-MMEJ (microhomology-mediated end-joining). It is anticipated that pharmacological inhibition of Polθ will selectively kill cancer cells which depend on Polθ mediated-MMEJ. Moreover, recent studies suggest, that secondary mutations restoring BRCA1/2 function are caused by activity of Polθ mediated-MMEJ and Polθ inhibition can prevent development of PARPi resistance.

The research will be carried-out at 4 stages. At the first stage we plan to establish primary cancer cell lines from surgical specimens obtained from patients diagnosed with melanoma, glioblastoma and pancreatic cancer. At stage 2, established cell lines and tumor samples will undergo DSB repair component profiling. We will analyze gene and protein expression of the factors which participate in DSB repair mechanisms what will allow us to identify DSB repair-deficient and -proficient cells. At stage 3 in stage III A we plan to treat normal and established primary cancer cell lines with specifically selected DSB-repair inhibitor for used alone or in combination with standard cytotoxic drug used in the treatment of given tumor types. We propose that selecting specific inhibitor/cytotoxic compound cocktail we will be able to trigger synthetic lethality and double synthetic lethality in cancer cells with no harm to normal cell lines. In in this stage we will also test our hypothesis using genetic tools (including shRNA Polθ silencing). Stage III B focuses on identification of the pathways which deficiencies are synthetic lethal with Polθ deficiency. We plan to use CRISPR/Cas9 and dominant-negative mutant carrying plasmids to generate PolθLow cancer cell lines deficient in various DSB-repair mechanisms. We plan to analyze different cell response aspects after treatment with cytotoxic compounds. At stage 4, we plan to injected DSB repair -deficient and -proficient patient-derived cancer cell lines (2 of each tumor type) into murine NSG model.

It is expected that this study will increase the knowledge of cancer biology, based on DNA repair mechanisms, which is very incomplete to this day. Correlating of the DSB repair genes profiles, in different types of cancers, with response of cells to chemicals targeting the DNA repair proteins, causing SL-mediated death, may allow for better understanding mechanism of this process. Additionally, creation of the library of specific profiles of DSB repair components, associated with DNA repair-inhibiting effect of chemical compounds on the growth and development of various types of tumor cells, may allow for use of this model in the personalized therapy in the future, as a long-term goal.