The aim of the project is to clarify whether and how the activity of human xenobiotic transport system (XTS) affects the efficacy of photodynamic therapy (PDT). This will be realized by finding the relationships between the structure of photosensitizers (derivatives of chlorophylls, Chls), their recognition by transport proteins (BCRP, P-glycoprotein, MRP1) and the strength of photodynamic effect in cells. The substrate-transporter interactions and structure-activity relationships will be analyzed in human cells *in vitro* and in model system consisting of human BCRP reconstituted in liposomes. My research hypothesis, that XTS determines the efficacy of PDT using Chls-derivatives and its interactions with the photosensitizers depend on their structure, is based on the results obtained earlier in our group. Therefore, the modifications of Chls will be done with the aim to affect the interactions with XTS. The realization of the project will contribute to a better understanding of cellular defense mechanisms against stresses induced by photocytotoxic xenobiotics/metabolites. In particular, it will allow us to clarify the role of xenobiotic transporters as factors limiting the outcome of photodynamic therapy and other therapies.

The main approach will be to compare the substrate specificity of xenobiotic transport at three different levels – uptake of photosensitizers by human cells, interactions of photosensitizers with reconstituted BCRP in model lipid system, and the outcome of photodynamic treatment of cells. The model photosensitizers will be obtained by chemical modification of chlorophylls. The modifications will affect the solubility, pharmacokinetics and the recognition by XTS. The *in vitro* studies will be performed in human cell lines MCF-7 (breast cancer), LoVo (colon adenocarcinoma) and MSU1.1 (*v-myc* immortalized fibroblasts). Expression of the transporters in the cells will be analyzed by the already established methods. The accumulation of the photosensitizers will be compared, varying the concentration and incubation period, in the presence/absence of inhibitors of specific transporters. The evaluation of photosensitizers' accumulation will be monitored fluorimetrically directly from the cells and from their extracts. Also, flow cytometry will be applied to follow the interactions of photosensitizers with intact cells, detached from their growth surface. Photodynamic effect will be induced by illumination of cells exposed to photosensitizers. The fraction of surviving cells will be determined, clones will be isolated from it, and after their multiplication, exposed to further photodynamic treatment. Several repetitions of this cycle will be performed and expression of xenobiotic transporters will be analyzed at each stage. In parallel, the interactions between the modified Chls and human BCRP will be analyzed in a model system based on BCRP expressed in yeast *S. cerevisiae*. The cell membrane fraction will be isolated from the yeast cells and then solubilized with mild detergent. The solubilized protein will be purified, and then reconstituted in liposomes. The ability of the reconstituted BCRP to hydrolyze ATP (ATPase activity) will be monitored after incubation with various photosensitizers and inhibitors. The lipid composition of the liposomes will be varied in order to assess its impact on BCRP activity.

The project will focus on BCRP, one of the most important xenobiotic transporters, which are among the main elements of the defense system against toxins in mammals, being at the same time the cause of unwanted multidrug resistance. The activity of analogous transporters in bacteria and fungi brings about their resistance against antibiotics and antifungal agents. Therefore it essential to understand the action of these proteins. The outcome of the project will contribute to a better understanding of the mechanisms of action of the ABC transporters, which is relevant also to the efficacy of photodynamic therapy and other therapies.