***Combined urinary bladder cancer treatment with isothiocyanates-derived mercapturic acids and microtubule assembly modulators***

Urinary bladder cancer is currently the fourth on the incidence cancer in men in Poland and a very dynamic increase in the incidence and mortality in Poland is continuously observed. Over 80% of transitional cells carcinomas (TCCs) are at non-muscle invasive bladder cancer stage (NMIBC) at the time of diagnosis. Unfortunately, a risk of recurrence is high with 50-60% rate for grade 1/2 tumors and even 80% for grade 3. At least 20% tumors will progress to muscle invasive bladder cancer (MIBC) with poor overall prognosis and high risk of distant metastasis occurrence. The necessity of multimodality treatment (treatment comprising tumor electrocystectomy, systemic chemotherapy and radiotherapy) and careful surveillance, makes the urinary bladder malignancies one of the most expensive oncological diseases to treat. Moreover, bladder cancers are generally recognized as chemoresistant and in many cases failed first-line therapy based mainly on cisplatin leads to even more drug-resistant neoplasms. Since the elevation of drug doses is not a viable option, because of the toxicity and other adverse effects observed even for the most potent drugs like anti-microtubule cancer agents, efficient adjuvant therapy is desperately needed.

Naturally-occurring isothiocyanates receives a continuous scientific interest as chemopreventive and anticancer compounds. Every time when cabbage, broccoli, cauliflower, Brussel sprouts or bittercress is eaten, a portion of isothiocyanates is ingested by our organism. After a rapid metabolism isothiocyanates are converted into mercapturic acids, which accumulates in urine and urinary bladder tissue, thus this organ is recognized as the most exposed to these compounds, which makes it a perfect candidate for any therapy comprising mercapturic acids. Importantly, mercapturic acids express similar biological activity as the parental isothiocyanates being less toxic at the same time.

In last few years, sulforaphane-containing cruciferous vegetables extracts receive much attention as chemopreventive diet supplements. In case of isothiocyanates, this attention is justified – isothiocyanates' biological activity covers multiple interactions with several different proteins of which large amount is related to cell division, a process significantly enhanced and largely uncontrolled in cancer cells. Multimodal mode of action, low toxicity and natural origin makes mercapturic acids a perfect candidates for combined treatment as adjuvants enhancing chemotherapeutic activity, sensitizing cancer cell for these drugs but also limiting their toxicity.

Our preliminary results for the first time provides evidence, that selected isothiocyanate-derived mercapturic acids enhance tubulin polymerization inhibitors antiproliferative activity *in vitro*. Tubulin polymerization process is crucial for cell division and compounds used in our studies like vinblastine (an efficient constituent of M-VAC therapeutic regime – commonly used in urinary bladder cancer treatment) or vinflunine (semisynthetic *vinca* alkaloid recently approved for urinary bladder cancer treatment after failed first-line cisplatin-based therapy) are a very effective inhibitors of this process.

Further studies that will be performed during a recent project encompass further evaluation of the usability of combined treatment based on mercapturic acids and compounds like vinblastine, vinflunine, noscapine, combretastatins, colchicine and docetaxel in urinary bladder cancer treatment. To accomplish our goals a series of antiproliferative, proapoptotic and cytostatic assays *in vitro* were planned. Additionally, more complex assays like Western-blot, tubulin polymerization inhibition assay, glutathione level assessment, circular dichroism *etc*. were included to address important question about molecular mechanisms underlying synergistic interactions between two above mentioned group of compounds. Finally, an *in vivo* experiment were planned in order to evaluate combined therapy usability in murine models of human urinary bladder cancers. Advanced, USG-based ortotopic (cancer cells will be inoculated directly into bladder wall) models will be used, which will allow us to study a combined therapy potential in tumor growth inhibition, but also its antiangiogenic activity.

Ultimately, a project should provide evidence if mercapturic acids might be a useful adjuvant compounds for urinary bladder cancer treatment based on tubulin polymerization inhibitors. Indirectly we will try to answer the question if properly composed diet can impact and outcome of chemotherapy?