Bladder cancer (BC) is common worldwide and possess a significant public health challenge. Most BC cases are urothelial carcinoma (UC) in subtype and approximately 75% of these are non-muscle invasive bladder cancer (NMIBC). However, the low-grade NMIBC is characterized by ~70% recurrence rate leading to highly aggressive and metastatic bladder cancer. The effective therapeutic strategy requires repeated intravesical drug administrations with multiple catheter insertions into the urinary tract, which can cause discomfort or infection. Additionally, the majority of the instilled drug is easily eliminated via urination or diluted by urine production, which lowers drug bioavailability. There is a significant need for an intravesical sustained drug delivery. Therefore, the aim of the project is to develop monomethyl auristatin E (MMAE)-loaded nanospheres composed of bioresorbable polymer and coated with mucoadhesive layer for the intravesical administration in the treatment of bladder cancer (Fig. 1). This is a novel approach, because local delivery of MMAE or using biodegradable mucoadhesive nanospheres for intravesical administration of MMAE has not been reported so far. MMAE is dolastatin derivative, which exerts anticancer effects by inhibiting tubulin polymerization, inducing apoptosis and intratumoral vascular damage. It has significantly higher (100–1000 times) potency than doxorubicin. So far, a potential in nanoparticle formulation of MMAE has not been established due to the potential risk of immature release of extremely high cytotoxic dolastatin drugs during blood circulation**.** However, the intravesical administration of mucoadhesive nanospheres in a form of suspension may allow local action of the drug.

The poly(lactide-co-glycolide-co-trimethylene carbonate) will be used to form biodegradable and biocompatible drug carrier that will enable sustained and controlled drug release. The mucoadhesive coating of nanospheres, obtained from chitosan, thiolated chitosan or Pluronic will provide improved retention after intravesical administration and thus, increased internalization by cancer cells. Additionally, the nanospheres will be obtained by means of microfluidic technique, which offers controlled parameters of processing and thus high reproducibility, high drug encapsulation efficiency and narrow size dispersity.

The project involves synthesis of polymer, preparation of nanospheres by microfluidic technique and their characterization. The kinetics and mechanism of drug release and degradation will be evaluated under *in vitro* conditions. Mucoadhesive properties of the nanospheres will be tested *in vitro* (mucin assay) and *in vivo* using a mouse model. The cytocompatibility of the nanocarriers will be evaluated according to the ISO 10993-5 standard. Anticancer activity will be studied *in vitro* against human (T-24, RT-4 cells and UMUC-3) and murine (MB49) bladder cancer cell lines. Anticancer properties of mucoadhesive MMEA-loaded nanospheres will be also tested *in vivo*. For this purpose, two orthotopic models of bladder cancer will be used: human xenograft (UM-UC-3) and mouse syngeneic (MB49).

The project will increase the knowledge about novel polymeric formulation of MMAE, its loading properties and mechanism of release; understanding the relationship between the polymer properties and its processing into nanospheres by means of microfluidic method. Finally, the project will show the pharmaceutical potential and functionality of the developed nanosystem - the bladder cancer response to a local treatment. Such strategy may facilitate also the clinical translation of our project in future.

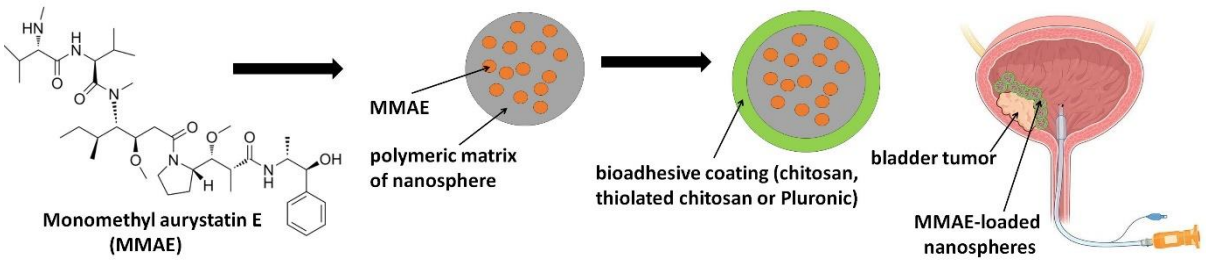

Figure 1. General concept of the project.