Endometriosis is a very widespread gynecological disease affecting up to 15% of reproductive-aged women. It is characterized by the presence of endometrial tissue outside the uterine cavity, e.g. in the peritoneum, at the surface of ovaries, of bowels and of the bladder, as well as at sites distant from the uterus, like the pleura or pericardium. Despite the common belief that it is a relatively harmless disease it represent a significant social and health care problem, because of its chronicity and accompanying severe pain symptoms it very often significantly compromise the social activity of a large group of young women and is one of the leading causes of infertility. In spite of its widespread nature and the progress in research about the mechanisms leading to endometriosis development, so far its cause has not been explicitly determined. One of the most prevailing theories is the theory of so called retrograde menstruation, when the menstrual blood reaches through the fallopian tubes into the pelvic cavity, allowing the implantation of the in this way co-transported endometrial cells at sites distant from the uterus (ectopic endometrium). The phenomenon of retrograde menstruation occurs however in most females of reproductive age and it is not known why only some women develop endometriosis. Accumulating research studies are indicating a connection between the development of endometriosis and a dysfunction of the immune system, which is eliminating ectopic endometrial cells at normal conditions. Changes in numbers and dysfunction of different immune cell populations, like cytotoxic T-cells, NK cells and macrophages, have been observed in women with endometriosis.

A well know immunosuppressive factor, especially within the tumor microenvironment, is arginase (ARG), one of the enzymes of the amino acids metabolism. We have recently discovered elevated levels of the two arginase isoforms, ARG1 and ARG2, as well as increased arginase activity in serum of endometriosis patients compared to healthy women. Furthermore, in previous studies we provided the first evidence for the presence of ARG1 in tumor-derived EVs – nano-sized double-membrane-enclosed particles released by tumor cells, in ovarian cancer. Hereby we identified a new mechanism of tumor-induced immunosuppression, based on the delivery of ARG1 by EVs to local lymph nodes, where the enzyme blocks T-cell activation and proliferation. A similar, however only temporary, down-regulation of T-cell activity as in tumors has been observed in the female reproductive tract (FRT) during the menstrual cycle and normal pregnancy. This T-cell inhibition correlated with an increased expression of ARG in the endometrial tissue. Furthermore, evidence is accumulating that analogous to the key role of EVs in pre-metastatic niche formation and metastatic organotropism in cancers, also EVs in the uterine and peritoneal microenvironment may support implementation and growth of ectopic endometrial cells outside the uterus. Therefore, we hypothesize that a similar immunosuppressive mechanism mediated by ARG+ EVs, as described by us in OvCa, may play a role in the promotion of endometriosis and may be responsible for the observed dysfunction of several types of immune cells in this disease.

The aim of the project is to identify arginase (ARG)-carrying extracellular vesicles (EVs) in the serum and peritoneal fluid (PF) of endometriosis patients and to decipher their impact on the dysfunction of the immune system in endometriosis and to verify their diagnostic or prognostic potential. Basing on our previous profound expertise in studying EVs, we will use state-of -the-art methods for phenotyping and functional analysis of EVs isolated from serum and PF. We will try to answer the question if the clinically relevant differences in the EV profile and the ARG-EV content found in PF are also reflected in the EV profile from peripheral blood. If yes, this EV profile could be translated into a clinically useful diagnostic test in the future. We will determine the effects of EV-associated ARG on selected types of immune cells like cytotoxic T-cells, NK cells and macrophages in functional cell co-cultures. Since exosomal arginases, as we have shown in OvCa, are more stable, easily cross tissue barriers and are transported over long distances, they may exert systemic biological effects and significantly contribute to niche formation for ectopic lesions and disease progression. Therefore, we believe that they can be a more significant and reliable potential biomarker than soluble arginases. Besides, ARG is a promising therapeutical target and has already entered clinical trials in cancer treatment. One can assume, that similar therapeutic options may become available for endometriosis, once the role of arginases in the progression of the disease will be confirmed. We believe, that this project will be a first step in this direction.