Breast cancer is the most common type of cancer in woman, and despite the significant research effort that has been made in several past decades, this disease is still responsible for millions of deaths worldwide. The treatment usually includes the combination of surgery, radiation and chemotherapy, however more safe and efficient are targeted therapies, for example the application of prodrugs. Prodrugs are currently the fastest growing segment in oncology. These molecules contain a chemotherapeutic drug (payload) that is conjugated with a chemical moiety, specifically recognized by the enzyme. In principle, the prodrug displays no or only negligible biological activity in its native state, and becomes fully active once cleaved by an enzyme. Most of such prodrugs are activated by cancer-upregulated proteases, which significantly reduces their systemic off-target toxicity, while maintaining high anticancer efficiency. Nevertheless, there is mounting evidence, that the expression and activity level of these proteases varies from cell to cell, and from patient to patient, stressing the need for the simultaneous analysis of multiple proteases at a single cell level.

In this project, we aim to use the mass cytometry technology to dissect the expression and distribution of lysosomal proteases (cathepsins and legumain) in breast cancer tissues. These enzymes are known to be involved in cancer progression, but their context-specific role remains to be elucidated. The proteolytic landscape we will obtain, will be further used to develop new generation of prodrugs, composed of cytotoxic moiety conjugated with a peptide, that will be selectively recognized only by one protease of interest. These prodrugs will be furhter tested *ex vivo* on breast cancer tissues from oncological patients. We claim, that the use of mass cytometry technology for the muliparametric analysis of proteases in combination with selective prodrugs development is an optimal strategy for seeking of new, more safe and efficient anticancer therapeutics.

Knowledge of the lysosomal proteases landscape, combined with current breast cancer classification will: (1) allow for more informed and effective drug regiments; (2) help to explain why some therapies display limited efficiency, and (3) help to identify new protease targets for the development of selective prodrugs. Moreover, the correlative analysis will be performed with of machine learning algorithms, which are nowadays frequently used for cancer risk prediction, diagnosis and classification. This will be of a great importance, as the multiparametric analysis of proteases, their inhibitors and cancer markers by mass cytomery may provide completely new insight into their biological functions.

Economic and societal impact: Understanding the exact role of proteases and their inhibitors in terms of cancer clinical settings is paramount for the appropriate disease managemet. By utilizing mass cytometry for the proteolytic analysis of breast cancer we will create a novel research platform and propose a new paradigm for more accurate cancer subtypes classification, which may help to understand anticancer therapy outcome. Moreover, by developing truly protease-selective prodrugs we will create a new research framework for the development of personalized therapeutics.