A majority of solid tumors have a monoclonal origin and are derived from one cell. Progenitors of this cell accumulate genetic alterations (mutations) which causes the appearance of a tumor phenotype. Many of somatic mutations found in cancer are clonal events, which occur in the founding cell at the time of tumor initiation, and then are propagated during clonal expansion. While the majority of driver gene mutations are present in nearly all cancer cells, some other genetic mutations are sub-clonal. As tumors accumulate genetic alterations, an evolutionary process occurs in which genetically distinct sub-clonal populations of cells co-exist and interact. As a result solid cancers evolve to mosaic entities composed of a mixture of cells with different genomes. Altogether, the repertoire of mixed clonal and sub-clonal genetically distinct cancer cell populations is referred to as **intra-tumor heterogeneity** (**ITH**). Tumor heterogeneity observed at the level of a cancer cell phenotype could be "hereditary" in nature and would result from genetic heterogeneity. Moreover, a substantial component of phenotypic tumor heterogeneity could be related to "non-hereditary" factors, which include phenotypic plasticity induced by interactions between cancer cells and different local microenvironments. Tumor heterogeneity is further increased by the presence of heterotypic elements (immune cells, connective tissues, etc.). In addition to ITH observed within a primary tumor one should also consider evolutionary divergence between a primary tumor and a metastatic outgrowth. Importantly, distant metastasis is the most harmful aspect of a cancer because it is responsible for the majority of cancer-related deaths. Currently, the majority of therapeutic decisions is made based on an analysis of a primary tumor, which approach could be justified only in the cases where genetic and molecular composition of a metastasis and a primary tumor is similar. Therefore, understanding of clonal heterogeneity of metastatic cancers is a burning problem of current molecular oncology.

The intra-tumor heterogeneity facilitates and accelerates natural evolution of a cancer that drives the process of tumor progression. Moreover, ITH could influence effectiveness of an anti-cancer treatment due to selection of resistant sub-clones initially present in a tumor, or owing to induction of new resistant sub-clones upon a treatment. Despite of fundamental relevance of ITH for cancer progression and response to a treatment, their clinical implications remain poorly defined. Although it has been hypothesized that ITH would be associated with poorer clinical outcomes in cancer patients, yet supportive evidence remains rather limited. The fact that ITH has been apparently under-researched is related to serious limitations of analytical approaches that could be implemented in this field of research.

The major hypothesis of this proposal states that a risk of cancer spread and long-term outcome of a treatment is associated with the degree of ITH observed in a primary tumor and/or in metastases to local lymph nodes. To verify this hypothesis directly we plan to assess molecular ITH using original methods based on mass spectrometry imaging, and to compare observed degree of ITH in patients sub-groups with different status of lymph nodes and with different treatment outcomes. The proposal is based on a retrospective clinical model involving two groups of patients treated radically for breast cancer, and for head and neck cancer. Phenotypic heterogeneity of molecular profiles of cancer cell sub-clones will be assessed using MALDI Mass Spectrometry Imaging approach.

We expect that the project will deliver original knowledge expanding our understanding of tumor heterogeneity and the role of ITH in progression and response to treatment of malignant solid cancers.