Tumors develop from a single cell that has lost the ability to control its growth and division. Observing the tumor tissue under a microscope, one can notice that the morphology of cells building this tissue is exceptionally diverse, even though it is a group of related cells derived from a common ancestor. This heterogeneity is also observed in vitro and does not only concern morphological differences, but rather the cell-to-cell heterogeneity at the molecular level that underlies the different sensitivity of individual cells to drugs.

We now know that clonal cancer cells can have identical genomes and still respond differently to therapy. Such heterogeneous responses can occur through many distinct outcomes including cell death, entering into quiescence or slowing down the mitotic events which altogether result in fractional killing of cancer cells. Moreover, cell resistance not acquired due to the emergence of a stable genetic mutation may be transient and reversible, and the clonal population of cells derived from a single resistant cell consists of both resistant and sensitive cells. The selective resistance of tumors and the failure of chemotherapy does not result, therefore, solely from genetic heterogeneity of cancer cells but has a tangible non-genetic component which includes in particular cell-to-cell differences in concentration and activities of proteins. It has particularly significant consequences for therapy responses in the case of cancers whose proliferation and survival highly depends on the activity of a single or few proteins. For example, the transcription factor STAT3 is constitutively active in many types of cancer, protecting cancer cells from apoptosis and promoting proliferation. Such tumors are often referred to as STAT3-dependent. Inhibiting STAT3 protein activity leads to a reduction in the rate of mitotic division of cancer cells and, ultimately, their apoptosis. However, also in this case, some cells show resistance to the drugs used.

Among the factors leading to the fractional killing of cancer cells, in addition to phenotypic variability at the molecular level (differences in the protein abundance), cell-to-cell heterogeneity at the organellar level seems to have a significant contribution, in particular, mitochondrial variability plays an essential role. Cells in the clonal population have been shown to differ in both the mitochondria mass and activity. Such mitochondrial variability can entail drastic alterations of a cell's gene expression program, ultimately leading to phenotypic variability, which may result in the fractional killing of cancer cells.

We hypothesize that the cell-to-cell heterogeneity of STAT3 levels and uneven segregation of mitochondria during cytokinesis, together with differences in their functionality, are the two major non-genetic components contributing to fractional killing in STAT3-dependent cancer cell lines. To verify this hypothesis, we will perform an analysis of the fate of individual cancer cells (dividing, quiescent, senescent, and dead cells) in response to treatment with STAT3 signaling pathway inhibitors. At the same time, we will determine the phenotypes of these cells, such as mitochondrial variability, level, and activity of STAT3 transcription factor and proteins regulating the processes of apoptosis and proliferation. To accomplish these tasks, we will use a high content automated fluorescence microscope, and cell tracking analysis. Quantification of the phenotypic features together with the defining of cell fates will be used for the comparative analysis to establish a relationship between a given parameter and cell fate. In particular, the fate of individual cells that survived the inhibitor treatment (senescence, quiescence or proliferative activity) will be linked to the mitochondrial parameters (mass, morphology, activity) and key protein levels. We are going to use the results of comparative analysis to plan the strategy of diminishing the fractional response of cancer cells to inhibition of STAT3 signaling pathway by targeting the mitochondrial variability and monitoring the response of individual cell for such treatment.

We expect that the use of compounds targeting various aspects of mitochondrial functioning (biogenesis, respiratory chain activity, production of reactive oxygen species) will reduce mitochondrial variability and translate into greater effectiveness of STAT3 activity inhibition in the entire cell population, leading to a reduction in the fractional killing of the STAT3-addicted cancer cells. Improved understanding of mitochondria variability contribution to fractional killing will be integral in developing better therapeutic strategies based on combinatorial targeting of the STAT3 pathway and mitochondrial functions.