Background
Among several mechanisms of immune tolerance, metabolic rewiring is gaining consideration as a strategy used by cancer cells to escape the immune response. Indeed, metabolic remodeling induces changes of gene expression through epigenetic and post-transcriptional regulation and modifies tumor microenvironment, thus creating a loop in which the modified microenvironment induces further metabolic alterations and/or sustains aberrant metabolism. In such a perspective, this interplay between cancer metabolic remodeling and gene expression reprogramming sustains immune escape mechanisms. Colorectal cancer (CRC) is a frequently lethal disease with heterogeneous outcomes and drug responses. Pembrolizumab, an immune checkpoint inhibitor (ICI), is a new standard of care only for Microsatellite Instability High (MSI-H) metastatic colorectal cancers (mCRCs), being, conversely, inactive in the vast majority of human CRCs with MSS status. Thus, novel strategies to improve/expand ICI activity are needed.
CRC is classified into four consensus molecular subtypes (CMSs) with distinct biologic features, being the CMS1 hypermutated, frequently MSI and with higher likelihood to respond to ICI and the CMS3 epithelial, with evident metabolic dysregulation and unresponsiveness to ICI.

Hypothesis
The present study aims at demonstrating that the differences between immune-responsive (CMS1) and immune-resistant (CMS3) cancer cells are, at least in part, due to metabolic alterations in immune-resistant cells.

Aims
Objectives of the project are:

- To identify metabolic differences between CMS1 and CMS3 tumor cells and relevant metabolites characterizing tumor microenvironment;
- To characterize molecular mechanisms underlying the above-mentioned metabolic alterations, by exploring the epigenetic and the gene expression profile of the two tumor subtypes, focusing on the enzymes and signaling mechanisms likely involved in relevant metabolic pathways;
- To explore the hypothesis that metabolic alterations may affect gene expression programs at transcriptional and/or post-transcriptional levels, thus inducing a vicious loop, which progressively worsen the immune resistance;
- To evaluate the effect of tumor metabolic modulation on immune response *in vitro* and in humanized mice engrafted with human CMS3 CRCs;
- To highlight the clinical relevance of epigenetic/metabolic modifications in mCRC treated with ICIs.

Experimental Design
This will be achieved by novel multiomics and traditional cell and molecular biology approaches and using *in vitro, ex vivo* and *in vivo* human CRC cell models.

Expected Results
These studies will allow us to identify: i) epigenetic/metabolic biomarkers predictive of resistance/response to ICIs in human mCRC; ii) possible new targets for therapeutic approaches aimed at reverting immune escape mechanisms.

Impact on cancer
The novel experimental approach described in this proposal and the validation of metabolic regulation in intra- and inter-cellular signaling adaptation mechanisms will help to achieve milestones towards personalized medicine.