Targeting extracellular NAMPT in cancer: generation and validation of NAMPT neutralizing antibodies

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Extracellular nicotinamide phosphoribosyltrasferase (eNAMPT) represents a secreted form of intracellular NAMPT (iNAMPT), a pleiotropic protein involved in NAD biosynthesis.
eNAMPT was first described as an active protein in the extracellular space and reported on its secretion from pre-B-cells and its ability to synergize with stem-cell factor and IL-7 to promote colony formation. Indeed, this was the basis of its classification as a cytokine, and demonstrated its biological potential as a putative paracrine and autocrine factor, however the mechanism of action is still unknown and only recently it has been proposed TLR4 as eNAMPT receptor.
eNAMPT circulating levels are increased in several pathologies, included inflammatory diseases, metabolic disorders and cancer. Now eNAMPT could be defined as a 'metabokine' in cancer, namely a cytokine-like protein involved in immune and metabolic functions, and the different targeting strategies that may benefit future therapeutics.
In order to shed light on the relevance of eNAMPT in cancer and thanks to a SIF-Takeda prize, we have generated 12 murine neutralizing antibodies against eNAMPT, which recognized different epitopes of the protein. First, we have performed an initial screening of validation of the target protein and we have chosen 5 clones. Then, the hybridoma cells were used to purified the respective antibodies. These 5 antibodies have been further characterized for the ability to recognize eNAMPT (using an ELISA method and western blot), moreover the isotype of each antibody was determined using ouchterlony assay. In literature, it has been described that eNAMPT stimulation of cancer cells induce proliferation and metastatic potential. We found that eNAMPT increases the viability of mammary carcinoma cells *in vitro* and STAT3-activation. Now, we are focusing on the determination of the neutralization potential of these new class of antibodies in two cancer models, a fibrosarcoma and a mammary carcinoma.