



PROJECT

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1) Project title **Next-generation NSAIDs to tackle chronic inflammation-associated diseases**

2) Abstract (max 500 words)

Platelets have been recognized to play a central role in intestinal and liver inflammation contributing to the risk of cancer development. This is also suggested by the finding that the use of the antiplatelet agent low-dose aspirin reduces the risk of the incidence and mortality for colorectal and liver cancer, nevertheless the mechanism is still unclear.

It has been hypothesized that activated platelets can trigger chronic inflammation via the release of soluble mediators, such as TXA₂ and VEGF and extracellular vesicles (EVs) containing genetic material (including microRNAs), which can be transferred to target cells. These events may contribute to the induction of cyclooxygenase (COX)-2 involved in the enhanced biosynthesis of prostaglandin (PG)E₂ in cancer cells as well as in cells of the tumor microenvironment, such as endothelial cells, thereby promoting tumorigenesis and metastasis. Differently from low-dose aspirin, selective COX-2 inhibitors, such as celecoxib, have the potential to restrain inflammation and tumor progression by direct inhibition of COX-2-dependent PGE₂. Novel anti-inflammatory compounds endowed with inhibitory COX-2 activity and antagonism of the receptor for TXA₂ (TP) could mitigate the side-effects of COXIBs on the cardiovascular system.

Based on this background, in the setting of a multidisciplinary project involving clinicians and pharmaceuticals, **the first specific aim** will be to evaluate the capacity of EVs from cancer patients to educate endothelial cells towards a pro-inflammatory phenotype. For this purpose, we will incubate EVs with endothelial cells of different origin (human umbilical endothelial cells, HUVECs, microvascular endothelial cells, and human lymphatic endothelial cells) to assess: i) phenotypic and functional changes related to barrier integrity (permeability assay, cytoskeletal protein, rearrangement/phalloidin-rhodamine staining), ii) migratory capacity, iii) immune function (expression of PD-L1 by flow-cytometry), iv) expression of inflammatory and permeability/adhesion markers (COX-2, VE-Cadherin, CAMs, P-selectin), and v) secretome (VEGF, PGE₂ in endothelial cell culture medium).

In the **second specific aim**, endothelial cells, exposed to EVs from cancer patients, will also be assessed for the capacity of modulating the trans-endothelial migration of platelets, monocytes, and tumor cells. Selected experiments of trans-endothelial migration will be performed in collaboration with the University of Verona using 3D microfluidics apparatus, a particularly promising approach to provide functional models of human organs with high predictive power in preclinical tests.

Furthermore, we will evaluate the effect of novel COX-2 inhibitors /TP antagonists provided by Prof Sala's group on their capacity of patient's EVs to affect phenotypic changes and cellular functions in coculture experiments as described above. The main output of this project is to provide new

therapeutics that disrupt cell-cell interactions involved in colorectal cancer progression