PROJECT 1

DoS: Prof. Andrea Necchi

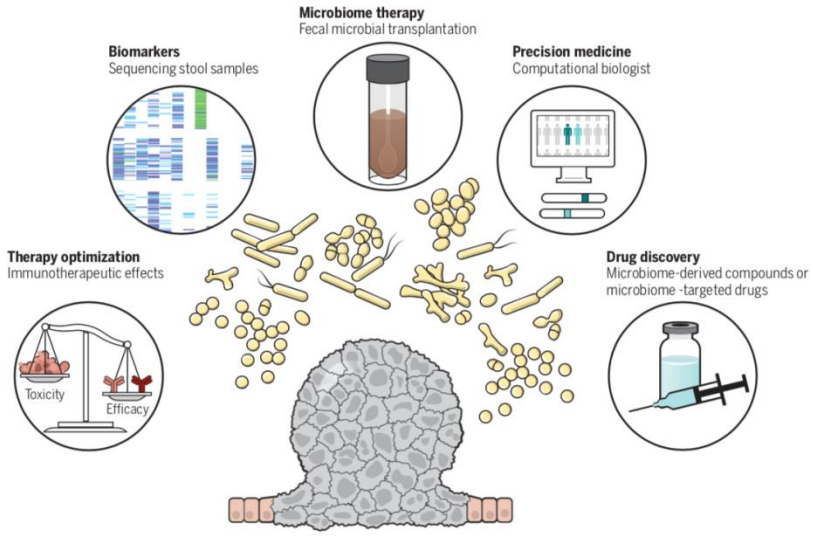
Title: Personalized immunotherapy in muscle-invasive bladder cancer

Curriculum: Experimental and Clinical Medicine

Link to OSR/UniSR personal page: https://www.unisr.it/docenti/n/necchi-andrea

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Background: Among the several mechanisms of tumor resistance to immunotherapy, host and environmental factors can have a major impact on the efficacy of cancer treatments (1). In this context, the composition of the mammalian microbiome (i.e. bacterial microbiome + archeal microbiome + virome + mycobiome + meiofauna) has emerged as a major factor that exerts a profound impact on the regulation of peripheral immune cells, including in the cancer microenvironment (2). Disrupting the microbiome, especially the gut microbiome, a condition that is referred to as "intestinal dysbiosis", can have systemic effects throughout the entire organism, including initiation/progression of various types of cancers (3), response to anticancer treatments (4,5) and onset and severity of treatment-related toxicities (4, 6).



It has been shown that the therapeutic efficacy of PD-1/PD-L1 blockade is associated with the presence of *Bifidobacterium* spp., which activate antigen-presenting cells (5). However, the most striking and provocative data on microbiome and immunotherapy have been derived from the sequencing of baseline stool samples from patients treated with anti-PD-1 therapies. 16S ribosomal RNA-based sequencing of gene amplicons and shotgun DNA sequencing of patient stool samples have identified subsets of bacteria more abundant in responding vs. nonresponding patients. It is even more impressive that bacteria associated with general health (e.g. *Clostridiales*, *Ruminococcacae*, *Faecalibacterium* spp., *B. fragilis*, *Akkermansia muciniphila* and *Bifidobacteria*) or immunogenicity (*Enterococci*, *Collinsella* and *Alistipes*) were found to be abundant in responding patients, regardless different dietary habits, geographic locations and tumor types (4,5,7-10). Microbiome studies are no longer relegated to microbiology labs, but they recently entered the clinical oncology departments as real trials. Several pharma and biotech companies have just launched microbiome-immunotherapy cancer clinical trials and the interest for such trials is growing steeply time after time.

Sponsors and collaborators	Checkpoint inhibitor	Microbiome intervention	Cancer	Status
The University of Pittsburg and Merck & Co.	Pembrolizumab	FMT from PD1 responders	Melanoma	Opens in March 2018
Evelo Biosciences and undisclosed partners	Undisclosed	At least two single-strain treatment arms	Melanoma, colorectal, renal and others	Opens in Q2 2018
Parker Institute for Cancer Immunotherapy, Seres Therapeutics and MD Anderson Cancer Center	PD1 blocker	FMT from PD1 responders, and/or SER401 (an oral consortium of spore-forming bacteria)	Melanoma	Opens in 2018
Vedanta Biosciences	Undisclosed	Clonal bacteria consortium	Undisclosed	IND filing in 2018, opens in 2018 or 2019

FMT, faecal microbiota transplantation; IND, investigational new drug; PD1, programmed cell death protein 1.

Methods:
The Aim of MIP-01 is to assess the role of microbiome in patients with muscle-invasive bladder cancer enrolled in the PURE-01 clinical trial and its influence on response to pembrolizumab. In particular, we aim to characterize the urinary microbiome, as we hypothesize is the most relevant for bladder cancer response to therapy.

All the samples from PURE-01 study are already available at our laboratory facility (Urological Research Institute, URI).

Paired urinary/fecal/vaginal/buccal microbiome in BCa patients, stratified for sex; urine from age-matched and sex-matched controls. Urinary meta-proteomic and meta-metabolomic might follow (i.e., biomarkers for responder and non-responder, for example for screening of BCa patients). Tissue-associated microbiome using pairwised non-neoplastic and neoplastic bladder from BCa patients treated with anti PD1 immunotherapy, responder and non-responder.

The primary goal will be to establish the association between anti-PD-1 responders/non-responders and the urinary microbiome, taking into account sex differences. Control group is represented by MIBC patients undergoing to standard neoadjuvant chemotherapy, treated at our clinical center.
Secondary objectives will be the following:

- To assess the association among several liquid tissues in BCa patients; this association will allow to establish if the dysbiosis is systemic or local (i.e., induced by the neoplasia)
- To assess the source of the urinary microbiome (gut vs. vagina)
- To establish if the urinary microbiome is representative of the bladder microbiome
- To associate all above features with clinical and anamnestic data

Study sponsor: IRCCS San Raffaele Hospital and Scientific Institute, Milan, Italy.

Skills to be acquired by the student:

The student will learn the basic techniques of DNA isolation and amplification, gene expression and biostatistical analyses aimed at achieving the study goals.

In addition, the student will be able to follow the entire process of tumor sample analyses, from the tissue harvest to the final biomarker report. He/she will be able to access the database we will generate from the planned analyses, and will have the possibility to independently propose original analyses after discussion with the DoS.

Finally, the student will work full time in a clinical research environment and will be able to learn the process of clinical trials submission, ethical discussion, approval and the start-up activities including the operational, management steps.

References (max. 3)

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