Project 4

Name/title of the PhD course	**Molecular Medicine and Medical Biotechnology**
Name of the PhD coordinator	Prof. Massimo Santoro
Name/Title of the PhD project	***Evaluation of intestinal microbiota as a determinant for the efficacy of immunovirotherapy***
Department of reference	Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II (http://dmmbm.dip.unina.it/)
Working conditions, research team, infrastructures, equipment	The Department of Molecular Medicine and Medical Biotechnology (DMMBM) was established in January 2013 to implement the new organizational structure of the University of Naples Federico II, in accordance with the Law 240/2010. The Department include about one hundreds scientists and PhD students. The facilities for research and teaching of the DMMBM are mostly located within the campus of the Medical School, Via S. Pansini, 5. The DMMBM carries out research activities in the following fields: structure and function of biological molecules and their involvement in the pathogenesis of human diseases, development of innovative biotechnological approaches for prevention, diagnosis and treatment of human diseases. Teachers and researchers of the DMMBM contribute to the pre- and post- graduate teaching and training in Medicine and in Medical Biotechnology, through the organization of Bachelors, Masters, Specialization and PhD Courses. To carry out research and teaching activities, scientists and teachers of the DMMBM also contribute to the activity welfare of the University Hospital Federico II, in particular via the Departments of Integrated Activities (DAI) such as the Departments of Laboratory Medicine and Transfusion Medicine. The DMMBM promotes the dissemination of research results, lifelong formation, transfer of knowledge and technology as a factor of socio-economic development, scientific cooperation. To this end, the DMMBM collaborates with numerous organizations and research institutions, in particular with the Institute of Endocrinology and Experimental Oncology (IEOS) of the CNR, the research centers CEINGE and Biogem, the District high-tech Campania Bioscience.
Scientific context	The participant will be involved in two main research lines: a) how intestinal microbiota can affect immunotherapy outcome b) how gut microbiota may impact on epigenetic profiles. In the last decade, immunotherapy has delivered tremendous advances in the treatment of melanoma and other cancers. Understanding the molecular mechanisms that tumors adopt to impair immune response, has allowed the development of immunotherapy drugs that have dramatically changed patients' life expectancy. Among these immunotherapies, immune checkpoint inhibitors (ICIs) T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are particularly successful (1,2). However, efficacy has not been consistent probably because of additional environmental and genetic factors influencing therapy outcome. Gut microbiota plays an important role in shaping systemic immune response (3-5); furthermore, a role for intestinal microbiota in mediating immune activation in response to chemotherapeutic agents has been demonstrated (6,7). Impact of the gut microbiota on ICIs response has also been investigated: composition of gut microbiota, in fact, influences response to ICIs targeting CTLA-4 and the PD-1 in mouse models (8-9).In particular, Bifidobacterium seems to have a role as a positive regulator of antitumor immunity in vivo by promoting pro-inflammatory signals in innate immune cells. We have hypothesized that modulation of host microbiota could also synergize with active immune therapy, such as oncolytic viruses. Oncolytic viruses can infect and lyse tumor cells, causing the release of tumor-associated antigen, therefore, stimulating an antitumoral immune response. About the second line of research project, the participant will be involved in the challenging investigation related to the potential impact of gut microbiota manipulation on epigenetic memory of immune and brain cells.
Project Research plan	Does the microbiome composition affect the anti-tumor response elicited by oncolytic vaccines? To address the question, we will carry out a first experiment in vivo, using an oncolytic vaccine a modified version of the Ad5D24 vector that include an insertion of 18 immunostimulatory CpG islands (Ad5D24-CpG), in order to increase adenovirus adjuvancy previously described (10). We will investigate whether the effect of this vector on a mouse model of melanoma could be enhanced by the administration of a mix of *Bifidobacterium* (*B. longum, B. breve, B. fragilis*) We will administer syngeneic B16 melanoma cells (3x105 cells/tumor) in both sides of four groups consisting of 8 C57BL/6J female mice. We will treat the animals as follows: • Group 1: Ad5D24-CpG • Group 2: Ad5D24-CpG and a mix of three strains of Bifidobacterium • Group 3: a mix of three strains of Bifidobacterium • Group 4: PBS The first dose of vector will be injected in mice at day 0 (when tumor lesion diameter reached 5 mm). We will treat groups 1 and 2 with three doses of the virus (1 x 10^8 vp/tumor each), every two days after day 0; treatment with the *Bifidobacterium* mix (200µl of Bifidobacterium mix consisting in 1x10^9 CFU/mouse) will start the day after the last virus injection and lasted until the endpoint of experiment.Mice treated with *Bifidobacterium* supplements and oncolytic viruses should show a reduction of tumor size, compared to control groups, on the basis of our previous results. By using 16S rRNA sequencing of stool sample, we will indirectly observe *Bifidobacterium* sp. and *Faecalibaculum* sp. abundance-featured gut microbiota, in mice that effectively respond to the viral therapy. Because of possible correlations between perturbation of gut microbiome and systemic immune responses to the different treatment, we will determine the CD4- and CD8+ T cell dependent IFN-γ production, obtaining specific antigen expression pattern correlated to therapies. We

	will also analyze, in the laboratory of Prof. Chiariotti, upon microbiome manipulation, the methylation patterns (genome wide and at gene-specific levels) of CD4+ and CD8+ T cells and brain derived cells, in order to identify eventual signature and stable changes in the expression program.
Research and Training Innovative aspects	Intestinal microbiota has been demonstrated to show influence i has been identified as a modulator of cancer chemotherapy and classic immunotherapy with anti-PD-1 and anti-CTLA4 antibodies. Viral-based immunotherapy is emerging for the potential of turning "cold" tumors (i.e, with lack of T-lymphocytes infiltration) into "hot" tumors, thereby sensitive to checkpoint inhibitors. To date no data are available on the influence of microbiota on the sensitivity to viral immunotherapy. We have preliminary data showing that Bifidobacterium presence correlates with response to immunotherapy; we plan to characterize the bacterial components involved in this effect and modulate intestinal microbiota to increase sensitivity to therapy in a syngeneic mouse model of melanoma. Regarding the second research project (b), the participant will be involved in the challenging investigation involving the potential impact of gut microbiota manipulation on epigenetic memory of immune and brain cells.
Inter-Multidisciplinary aspects	This project involves competences in microbiome analysis as well as oncoviral vector development and analysis of murine model of cancer. In addition, analysis of immune system is also extremely relevant to this project. Epigenetic analysis of T-cell will provide us information about an additional layer (methylome) involved in stable changes induced by microbiome manipulation. Chemical characterization of bacterial components (glycans and metabolites) will be fundamental to define the type of influence of microbiota on tumor therapy. .
Secondment opportunities	A period of about 6 months will be spent by PhD in **Sanofi laboratories (Italy; www.sanofi.com)** to acquire specific expertise in development of therapeutical probiotics. An international and intersectoral secondment of between 3-6 months is also foreseen at BIoArte (**Malta**; https://thebioarte.com/), an innovative player in the field of biomolecular research, highly specialized in researching the human microbiota and microbial communities in other environments, under the co-supervision of the company founder **Dr Manuele Biazzo.**
Main Supervisor: Prof. Lucio Pastore (https://www.docenti.unina.it/lucio.pastore	
Brief CV	Full Professor of Clinical Biochemistry and Molecular Biology, University of Naples Federico II, Naples Italy. Lucio Pastore, MD, PhD, is Professor of Clinical Biochemistry and Molecular Biology at the Dipartimento di Medicina Molecolare e Biotecnologie Mediche of the Università di Napoli Federico II and group leader at CEINGE-Biotecnologie Avanzate. He is the coordinator of the Master degree (Laurea Magistrale) in Medical Biotechnology of the Università d Napoli Federico II and Manager of R&D and startup at CEINGE-Biotecnologie Avanzate. He is currently the Director of the Centro Interuniversitario di Studio della longevità, delle malattie genetiche e multifattoriali e dei loro modelli animali e cellulari of the Università di Napoli Federico II and founder of Kimera, a biotech startup company. Lucio Pastore is author of more than 80 original articles on peer-reviewed journals and has carried most of his research activities on gene therapy of metabolic diseases with a particular focus on genetically-determined forms of atherosclerosis using chemically modified-adenoviral vectors; he has also identified the role of a number of genes and protein in osteoblast differentiation of bone marrow stromal cells for possible applications to regenerative medicine. His clinical diagnostic activity is focused on the identification of genomic alterations using techniques such as comparative genomic hybridization array. More recently he has been involved in the development of novel oncolytic vaccines based on adenoviral vectors. Oncolytic viruses have been demonstrated to stimulate antitumoral immune response. Many studies have shown that intestinal microbiota can affect immunotherapy outcome: in fact, stimulator interactions between microbiota and host immune system point to Bifidobacterium as a positive regulator of antitumor immunity in vivo by promoting pro-inflammatory signals in innate immune cells. We are evaluating the role of gut microbiota in modulating oncolytic virus-based immunotherapy and its possible role as prognostic marker**. Both Lucio Pastore and the co-supervisor Lorenzo Chiariotti** have mentored several PhD students for over 15 years.
Publications	The **5 main/latest** publications are: -Capasso C, Magarkar A, Cervera-Carrascon V, Fusciello M, Feola S, Muller M, Garofalo M, Kuryk L, Tähtinen S, **Pastore L**, Bunker A, Cerullo V. A novel in silico framework to improve MHC-I epitopes and break the tolerance t melanoma. Oncoimmunology. 2017 May 11;6(9):e1319028. doi: 10.1080/2162402X.2017.1319028. - Feola S, Capasso C, Fusciello M, Martins B, Tähtinen S, Medeot M, Carpi S, Frascaro F, Ylosmäki E, Peltonen K, **Pastore L,** Cerullo V. Oncolytic vaccines increase the response to PD-L1 blockade in immunogenic and poor immunogenic tumors. Oncoimmunology. 2018 May 7;7(8):e1457596. doi: 10.1080/2162402X.2018.1457596. - Paparo L, Tripodi L, Bruno C, Pisapia L, Damiano C, **Pastore L**, Berni Canani R. Protective action of Bacillus clausii probiotic strains in an in vitro model of Rotavirus infection. Sci Rep. 2020 Jul 28;10(1):12636. doi: 10.1038/s41598 020-69533-7. PMID: 32724066; PMCID: PMC7387476. -Tripodi L, Vitale M, Cerullo V, **Pastore L**. Oncolytic Adenoviruses for Cancer Therapy. Int J Mol Sci. 2021 Mar 3;22(5):2517. doi: 10.3390/ijms22052517. PMID: 33802281; PMCID: PMC7959120. -Scialo F, Amato F, Cernera G, Gelzo M, Zarrilli F, Comegna M, **Pastore L,** Bianco A, Castaldo G. Lung Microbiome in Cystic Fibrosis. Life (Basel). 2021 Jan 27;11(2):94. doi: 10.3390/life11020094. PMID: 33513903; PMCID: PMC7911450.
Projects participation	Lucio Pastore has obtained several grant funds for research and awards for his scientific activity, including the 2001 Lyndon Johnson Award from American Heart Association. Fondo SATIN (PON regione Campania) 120,000. Fond Centro Interuniversitario di Studio della longevità, delle malattie genetiche e multifattoriali e dei loro modelli animali cellulari between Università di Napoli "Federico II", l'Università di Roma "Tor Vergata" and of Chieti-Pescara