

To the Academic Board of the Research Doctorate in Molecular Medicine

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Cycle XXXVI Tutor Prof. Federica Gemignani

Scientific activity carried out in the 2st year of PhD, Academic Year 2021/2022

Introduction

Multiple myeloma (MM) is a malignant tumor of differentiated plasma cells and is still incurable. Progression from MGUS/SMM to MM can be determined by a change in genetic alterations. Genetic abnormalities found in bone marrow biopsies were also found from circulating tumor DNA without cells (cfDNA). Liquid biopsies (LB) are a minimally invasive alternative to tissue biopsies. The purpose of my project is to investigate by next generation sequencing (NGS) the increase in genetic mutations charged on cfDNA by LB of patients with different stages of disease.

Methods

Collection of biopsies samples from patients affected by MGUS, SMM, MM.

Extraction and quality control of cfDNA from biopsies samples.

Library preparation with UMI adapter system for NGS Illumina.

Analysis of NGS data through UMI-specific pipeline in BASH.

Design primers and optimization of specific protocols for ASO PCR and ASO RealTime PCR.

Results

During this second year I have continued the collection and processing of LBs samples.

I concentrated my activities on the investigation of the mutational profile of patient samples at various stages, and of MGUS and SMM stage samples of the same patient with pathological evolution. The cfDNA of 16 MGUS, 2 SMM, 2 MM was qualitatively analyzed and processed by NGS techniques using UMI adapters and a custom panel of 84 MM recurrent genes. The library preparation protocols have been optimized, and the optimization of the steps in the pipeline for UMI for the analysis of results in BASH language is still in progress. An effort was dedicated to the preparation of a method for investigating relevant SNVs in MM in KRAS and NRAS genes, designing specific primers used in ASO PCR and ASO RealTime PCR. The techniques have been validated on mutation-specific cell line gDNAs and will be used on cfDNA from MGUS and MM patients.

- Abstracts and participation in congresses and courses: authors, title of the presentation, name, and dates of the congress
 - Participation in Congress – Virtual Edition: “La risposta cellulare al danno al DNA e l'integrità del genoma: le nuove frontiere della ricerca biomedica e ambientale”; Authors: ISTITUTO SUPERIORE DI SANITÀ in collaboration with SIMAG (Società Italiana Mutagenesi Ambientale e Genomica) ; 23-25 November 2021.
 - Participation in a conference – Virtual Edition: “Rapid Next-Generation Sequencing for the Study of Acute Myeloid Leukemia”; Authors: Genomeweb; 27 January 2022.
 - Participation in a conference – Virtual Edition: “Genomics approaches for characterizing germline mutations in humans and animal model”; Speaker: Dr. Francesco Marchetti; Authors: SIMAG (Società Italiana di Mutagenesi Ambientale e Genomica); 3 May 2022.
 - Participation in the advanced training course – Virtual Edition: “CAR-T cells”; Authors: Ghislieri Foundation Center for University Research and Teaching; 4-6 May 2022.
 - Participation in a Congress – In Presence in Cortona (Italy): “SISTEMI MODELLO COME STRUMENTO DI ANALISI GENETICA”; Authors: AGI (Associazione Genetica Italiana); 30-31 May 2022.
 - Participation in a conference – Virtual Edition: “Computer-assisted functional precision medicine in cancer”; Authors: EMBL (European Molecular Biology Laboratory) in collaboration with PerMedCoE; 01 June 2022.
 - Participation in a conference – Virtual Edition: “Take Quantification to Absolute perfection with Digital PCR”; Authors: AppliedBiosystem, Thermo Fisher Scientific; 25 July 2022.
 - Participation in a Conference – In Presence in Mandelieu-La Napoule (France): “6th Translational Research Conference: Multiple Myeloma”; Authors: ESH (European School of Haematology); 7-9 October 2022.
 - Participation in Soft skills courses for PhD at the University of Siena – Virtual Edition:
 - o “Creating value from large archive and big data”; 13 June 2022 9.00-13.00/14.00-16.00.
 - o “Comunicare in ricerca”; 20 June 2022 09.00-13.00 / 14.00-18.00.
 - o “Possibili percorsi formativi post PhD”; 15 September 2022 10.00-13.00.
 - o “Il ruolo della standardizzazione nei processi di innovazione”; 7 and 8 September 2022 9.00-13.00.
 - Participation in the English course B2 Level: “Inglese: Corsi Generali A.A. 2021-22”; Authors: CLI (Centro Linguistico, Università di Pisa); 120 hours from October 2021 to June 2022.
- Scientific publications, authors, title of publication, name and number of the journal, year of publication
 - ❖ Morelli M, Lessi F, Barachini S, Liotti R, et al. Metabolic-imaging of human glioblastoma live tumors: A new precision-medicine approach to predict tumor treatment response early. *Front. Oncol.* 12:969812. (2022) doi: 10.3389/fonc.2022.969812
 - ❖ Parisi, F., Lessi, F., Menicagli, M. et al. Presence of a mouse mammary tumour virus-like in feline lymphomas: a preliminary study. *Infect Agents Cancer* 17, 35 (2022).
<https://doi.org/10.1186/s13027-022-00449-9>

- Possible stays in other Italian or foreign laboratories

- Internship, 3 months in GENARTIS - Castel D'azzano (VR) - from 25/03/2022 to 26/06/2022. Investigation and data analysis of the recurrent mutational landscape in Multiple Myeloma (MM) using library preparation with UMI targets for NGS sequencing on cfDNA extracted from liquid biopsies of patients with MM and pre-pathological stages and set BASH pipeline for data analysis.
- Research and training course for the protection and genotyping of the *Caretta caretta* sea turtle in Calabria, from 08.28.2022 to 09.03.2022, concerning the application of biological tissue sampling methods in the field, the analysis of the stages of embryonic development and the study of laboratory techniques for the genotyping of nesting *C. caretta* females.