Con il patrocinio di

INTERNATIONAL WORKSHOP NO-CANCER 2024

from cancer cell biology to personalized therapy

The workshop focuses on **basic and translational cancer research** with emphasis on **cancer cell biology** and **advanced diagnostic** and **personalized therapeutic approaches**

Novara, May 27-28, 2024

ABSTRACT BOOK

https://nocancercongress.uniupo.it

Droplet Digital PCR Development to Quantify the DNA Methylation Levels of *SEPT9* and *SHOX2* in Plasma from Patients with Head and Neck Squamous Cell Carcinoma

Ilaria Grossi[1], Claudia Assoni[2], Luigi Lorini[2], Davide Smussi[2], Cristina Gurizzan[3,4], Salvatore Grisanti[2], Alberto Paderno[5], Davide Mattavelli[5], Cesare Piazza[5], Iulia Andreea Pelisenco[1], Giuseppina De Petro[1], Alessandro Salvi[1], Paolo Bossi[3,4]

[1]Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; *email: ilaria.grossi@unibs.it*
[2]Unit of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, ASST Spedali Civili of Brescia, University of Brescia, Brescia, Italy
[3]Department of Biomedical Sciences, Humanitas University, Milan, Italy
[4]IRCCS Humanitas Research Hospital, Rozzano MI, Italy
[5]Unit of Otorhinolaryngology-Head and Neck Surgery, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, ASST Spedali Civili of Brescia, University of Brescia, Brescia, Italy

Methylation of septin 9 (*SEPT9*) and short stature homeobox 2 (*SHOX2*) in circulating cell-free DNA (ccfDNA) has emerged as a promising biomarker in many cancers. In head and neck squamous cell carcinoma (HNSCC), data obtained with qPCR suggested the valuable role of *SEPT9* and *SHOX2* gene methylation as non-invasive tool for diagnosis and prognosis. However, a precise method would improve detection at low circulating gene methylation levels. Therefore, we have developed highly sensitive assays using droplet digital PCR (ddPCR) for the absolute quantification of *SEPT9* and *SHOX2* methylation. The methylation-specific ddPCR (MS-ddPCR) assays were first set up using commercial methylated/unmethylated DNA set, and then used to quantify *SEPT9* and *SHOX2* methylation in the plasma of 20 HNSCC patients before treatment and longitudinally during follow-up. The MS-ddPCR efficiency was demonstrated. Methylated *SEPT9* and *SHOX2* levels were significantly reduced in patients at first follow-up time points *versus* T0. Interestingly, different trends of longitudinal DNA methylation variation were found in small groups of stratified patients. In summary, we present successful MS-ddPCR assays for detecting *SEPT9* and *SHOX2* methylation in ccfDNA; a prospective multicenter study is ongoing to validate the ability of *SEPT9/SHOX2* ccfDNA methylation for recurrence/second malignancy detection in HNSCC (IDENTIFY project).

The present study is supported by Fondazione Spedali Civili (Brescia).