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Original Research

# Metformin and insulin impact on clinical outcome in patients with advanced hepatocellular carcinoma receiving sorafenib: Validation study and biological rationale

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**Abstract** ***Purpose:*** In 2015, we published a study on a small series of patients with hepatocellular carcinoma (HCC) treated chronically with metformin for type II diabetes mellitus (DM2) who showed a poorer response to sorafenib. The aim of the present study was to validate the prognostic significance of metformin in HCC patients treated with sorafenib, providing a biological rationale for the mechanism of resistance to sorafenib in patients on chronic metformin therapy, and to clarify the role of sirtuin-3 (SIRT-3), a protein involved in metabolic diseases and acknowledged as a tumour suppressor in HCC, in this resistance.
***Patients and methods:*** We analysed 279 patients consecutively treated with sorafenib for the clinical analysis. Of the 86 (30%) patients with DM2, 52 (19%) were on chronic treatment with metformin and 34 (12%) with insulin. We included 43 patients with HCC for the biological study: 19 (44.1%) were diabetic and 14 (73.7%) of these received metformin for DM2. SIRT-3 expression was investigated by immunohistochemistry (IHC) in formalin-fixed and paraffin-embedded (FFPE) samples.
***Results:*** In HCC patients undergoing chronic treatment with metformin, the use of sorafenib was associated with poor progression-free survival (PFS) and overall survival (OS) (1.9 and 6.6 months, respectively) compared to 3.7 months and 10.8 months, respectively, for patients without DM2 and 8.4 months and 16.6 months, respectively, for patients on insulin ($P < .0001$). We also observed that SIRT-3 protein expression was significantly higher in patients treated with metformin than in those not taking this medication (65% versus 25%, respectively) ($P = .013$).
***Conclusions:*** Our findings could be attributed to increased tumour aggressiveness and resistance to sorafenib caused by chronic treatment with metformin.


## 1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide in men and the second most frequent cause of cancer-related deaths [1–3]. Each year it is diagnosed in more than 500,000 people worldwide. The decrease in virus-associated HCC observed in Italy in recent years has been offset by an increase in HCC caused by non-alcoholic fatty liver disease (NAFLD) [4,5]. It has been seen that the use of sorafenib increases median overall survival of HCC patients (10.7 months for sorafenib group versus 7.9 months for placebo group), representing a 31% decrease in the relative risk of death [6]. However, there is still no validated biological or clinical marker that predicts response to treatment in these patients [7–12].

In 2015, we published a study on a series of HCC patients who showed a poorer response to sorafenib as a result of chronic treatment with metformin for type II diabetes mellitus (DM2) [13]. The patients who developed HCC whilst undergoing chronic therapy with metformin showed a median progression-free survival (PFS) of 2.6 months compared to 5.0 months for those not taking this medication. Overall survival (OS) was 10.4 months and 15.1 months, respectively.

Sirtuin-3 (SIRT-3), one of the evolutionarily conserved mammalian orthologues of the silent information regulator 2 (Sir2) is a nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylase involved in regulating mitochondrial metabolism [14]. Its regulatory effects and involvement in metabolic diseases are believed to have a strong impact on the development and treatment of HCC. Although the reported evidences suggest a putative bridge role of SIRT-3 between metabolic disorders and HCC, further studies are necessary to demonstrate such interconnection [15,16]. The aim of the present study was to validate the prognostic significance of metformin and insulin in HCC patients treated with sorafenib, and to establish a biological rationale for the mechanism involved in resistance to sorafenib in those undergoing chronic metformin therapy.

## 2. Patients and methods

### 2.1. Patient population for the clinical study

The present study was performed using the medical records from the databases of Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS (Meldola); Department of Medical Oncology, University of Cagliari; Università Campus Bio-Medico (Rome); National Cancer Institute 'Giovanni Paolo II' (Bari), University of Bari Medical School; and Sant'Orsola-Malpighi Hospital, University of Bologna. Data were entered into electronic data files by co-investigators from each centre taking part and checked at the data management centre for missing information and internal consistency. The study protocol was reviewed and approved by the local Ethics Committee. All patients gave their written informed consent.