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ORIGINAL ARTICLE

Circulating SCCA–IgM complex is a useful biomarker to predict the outcome of therapy in hepatocellular carcinoma patients

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ABSTRACT

Introduction: Hepatocellular carcinoma (HCC) develops in about 3–4% of cirrhotic patients every year. The squamous cell carcinoma antigen (SCCA) has been found elevated in liver cancer specimens by immunohistochemistry, and detected in complex with IgM (SCCA-IgM) in the serum of patients with HCC. The aim of this study was to evaluate the ability of serological SCCA-IgM levels to predict the efficacy of HCC therapy.

Materials and methods: From April 2012 to April 2014, 131 patients with a new diagnosis of HCC were enrolled. The HCC diagnosis was made according to the EASL guidelines. The patients were staged and treated according to the BCLC Staging System: BCLC stages A and B were treated with locoregional therapy, and BCLC stage C was treated with Sorafenib. Response to therapy was evaluated according to the mRECIST criteria. Serum SCCA-IgM levels were determined by a commercially available ELISA kit at basal time (T_0) and after one month of treatment (T_1).

Results: At baseline and one month into therapy, SCCA-IgM levels were significantly lower (p value $<.05$) in patients who responded to therapy compared to those who did not respond (median SCCA-IgM level [25th + 75th percentile] at T_0:115.1 AU/mL [50.0 + 174.4] vs. 149.1 AU/mL [111.3 + 198.8]; median SCCA-IgM level [25th + 75th percentile] at T_1: 113.4 AU/mL [50.0 + 194.2] vs. 170.6 AU/mL [111.7 + 344.2]).

Conclusion: Our study suggests that the SCCA-IgM determination could be helpful in predicting the response to therapy in patients with HCC.

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KEYWORDS
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Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer death and the leading cause of mortality among cirrhotic patients. The major aetiologies are hepatitis B virus (HBV) or hepatitis C virus (HCV) chronic infections [1–3], even if in Western countries a rising proportion of HCCs is ascribed to alcohol abuse and metabolic disorders [3]. The prognosis depends mainly on the HCC stage [2,4]. The best strategy to survey cirrhotic patients is ultrasonographic follow-up, which is able to reveal hepatic lesions of about one centimetre in size; determination of serological alpha-fetoprotein (AFP) is also currently used in clinical practice, but its poor specificity and sensitivity raise concerns on its application as a screening tool [5,6].

Expression of the squamous cell carcinoma antigen (SCCA), a member of the high-molecular-weight family of serin protease inhibitors named Serpins [7–9], was recently described to be increased in liver cancer tissue. SCCA is physiologically expressed in the stratified squamous epithelium of tongue, tonsil, oesophagus, uterine cervix, vagina, thymus, skin and in the pseudo-stratified columnar epithelium of the conducting airways [10]. Normal hepatocytes do not express SCCA; however, the antigen levels increase with liver inflammation [11]. In cancer cells, SCCA has an antiapoptotic activity both related [12] and unrelated [13] to its protease inhibition function. Moreover, it protects cells from oxidative stress [14] and decreases proliferation and invasiveness [15], while reducing tumour infiltration by natural killer cells [16].

In different cancers, including HCC, it is possible to detect serological immunoglobulin-M (IgM) against tumour biomarkers [11,17–19]; it is hypothesized that these complexes represent the immune system's response to tumour antigens and/or to cancer cells. Detection of IgM–antigen complexes has been found to be more efficient at identifying liver damage [11,17,20], so a serologic assay detecting SCCA–IgM has been developed [11,20]. This assay reveals higher levels of SCCA–IgM in HCC patients [17,20,21] when compared to patients with cirrhosis and chronic hepatitis [22–24]. Increasing levels of circulating SCCA–IgM have been detected in cirrhotic patients at higher risk of HCC development [25,26]. Other studies have shown that

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successful HCV antiviral therapy is associated with a significant decrease in SCCA–IgM serum levels [27]. Moreover, it was shown that SCCA–IgM levels significantly correlate with progression-free and overall survival in HCC patients [28]. According to this background, we hypothesized that in HCC patients the immune-complex levels might be related to treatment response, and the aim of this study was to evaluate the ability of SCCA–IgM serum levels to predict response to HCC treatment.

Materials and methods

Patients

From April 2012 to April 2014, 131 adult patients with a new diagnosis of HCC were recruited in two referral centres in southern Italy (the Gastroenterology Unit of the University of Naples 'Federico II' and the Liver Unit of the 'A. Cardarelli' Hospital of Naples).

Demographic, clinical and biochemical parameters at baseline, including age, sex, body mass index (BMI), aetiology of cirrhosis, the presence of portal hypertension, Child-Pugh class, HCC gross pathology and extrahepatic diffusion and the presence of portal vein thrombosis were recorded.

The HCC diagnosis was performed according to the European guidelines of EASL [29] by imaging technique workup (dynamic computer tomography – CT, contrast-enhanced ultrasonography, dynamic magnetic resonance imaging – MRI), thereafter combining the diagnostic AFP increase (>200 ng/mL).

Tumour stage was assessed with both ultrasonography and CT features, according to BCLC-staging system [30]. Macroscopic types of HCC were classified as solitary nodular (<2 cm, between 2 and 3 cm, >3 cm), multifocal or diffuse. Bone scintigraphy and total body CT were performed in all patients to investigate extrahepatic spreading.

Treatment options were considered according to the BCLC staging system [29,30]. Patients were defined suitable for local ablation [radiofrequency ablation (RFA), laser thermal ablation (LTA), percutaneous ethanol injection (PEI)] according to the following criteria: solitary nodule or paucifocal with each node <3 cm, Child-Pugh A-B, no evidence of extrahepatic metastases, no general contraindication to the specific technique (BCLC stage A). Patients were candidates for transcatheter arterial chemoembolization (TACE) according to the following criteria: paucifocal HCC not treatable with local ablation or multifocal HCC, involving less than 40% of the liver volume, Child-Pugh A-B, small oesophageal varices, no portal vein infiltration or thrombosis, no extrahepatic metastases, no severe associated diseases, no general contraindications to TACE (BCLC stage B). Systemic therapy with sorafenib was indicated according to the following criteria: advanced HCC with or without extrahepatic metastases or portal vein infiltration/thrombosis or patients in whom TACE was contraindicated or considered useless due to the presence of a bilobar disseminated HCC or a huge non-resectable lesion or HCC unsuitable to locoregional therapy and an ECOG (Eastern Cooperative Oncology Group) performance status (PS) 0–1, a Child-Pugh score ≤7, adequate hematologic, hepatic and renal function, no severe associated diseases, no general contraindications for sorafenib (BCLC stage C). Patients showing an HCC terminal stage (BCLC D) were excluded from this study.

All patients underwent a 1-year follow-up including clinical, biochemical and imaging evaluation (ultrasonography, CT or MRI) performed at intervals of 4–24 weeks in relation to HCC stage and clinical needs of the single patient. Response to therapy was evaluated with imaging techniques performed one, three, six and 12 months after treatment, according to the mRECIST criteria [31]. In particular, a complete or partial radiological response to treatment was considered as a positive response; stable disease after treatment as stationary response; and progressive disease after treatment as negative response.

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices. Approval was obtained from the Institutional Review Board and Ethics Committee. All patients gave fully informed consent authorizing use of blood and diagnostic parameters for research purposes.

Measurement of SCCA-IgM

Blood samples were collected for SCCA–IgM levels determination at baseline (T_0) and one month after treatment (T_1); the serum was immediately separated by centrifugation and stored at −80 °C. SCCA–IgM serological levels were assessed by Hepa-IC ELISA kit (Xeptagen, Venice, Italy) according to the manufacturer's instructions. Briefly, plates pre-coated with oligoclonal anti-human SCCA antibodies were incubated with 100 µL of serum diluted 1:8 in dilution buffer. SCCA–IgM presence was revealed by the addition of a secondary antibody against human-IgM conjugated with horseradish peroxidase and ABTS chromogen solution. All reagents were provided in the kit and results were processed with Xerepro software (Xeptagen – available at http://www.xeptagen.com/software). SCCA–IgM levels were expressed as Arbitrary Units per millilitre (AU/mL) by interpolation of samples' absorbance on the calibration curves. The cut-off value for HCC diagnosis (previously assessed as the value of the 95th percentile of the distribution curve of the assay in 100 normal subjects) was 120 AU/mL.

Statistical analyses

Baseline characteristics were expressed as median and range for continuous and not normally distributed data, and as a percentage for categorical data. Statistical analyses were performed and algorithms were developed using R version 3.0.1 (The R Foundation for Statistical Computing, Wien, Austria) or SPSS v.16.0.1 (IBM Corp., Armonk, NY). For all statistical comparisons, a p value <.05 was accepted as statistically significant. The comparison among the different groups was performed using the Wilcoxon rank-sum test

(Mann–Whitney two sample statistic) and differences were shown using the Box–Whisker plot.

The relationship between two variables was assessed by Spearman's rank correlation coefficient. Receiver operator characteristic (ROC) curves were constructed for SCCA–IgM levels at T_0, T_1 and T_1–T_0 (ΔSCCA–IgM) and the area under the curve (AUC), a method to evaluate the diagnostic performance, was calculated. The ROC-AUCs relative to T_0, T_1 and T_1–T_0 were compared using a non-parametric method, which accounts for the correlation induced through the SCCA–IgM measurement at different time points [32].

Results

Baseline characteristics of the study population

A total of 131 patients with a new diagnosis of HCC was enrolled in this prospective study. Baseline demographics, clinical and biochemical characteristics of the patients were summarized in Table 1.

The median age of patients was 68 years and most of them (75.6%) were male. Viral infections (HCV and/or HBV) accounted for the majority (86.4%) of cases. HCC developed in a setting of well-compensated liver cirrhosis (Child-Pugh class A) in 84% of cases. The median serum AFP level was 23.0 ng/mL and only39 patients (29.8%) had AFP level >200 ng/mL. The HCC stage was evaluated according to BCLC-staging system: 40.5% of patients were included in early and very-early stages, 26.0% in intermediate stage, 33.6% in advanced stage and no patient in terminal stage.

Single nodule, multifocal and diffuse pattern were detected in 42.7%, 27.5% and 29% of cases, respectively. Portal vein thrombosis was observed in 39 patients (29.8%), and metastases were detected in 20 patients (15.3%). Loco-regional treatments (PEI, RFA, LTA) and TACE were performed in 64 patients (48.9% of cases), while systemic therapy with sorafenib was used in 67 patients (51.1% of cases) (Table 2).

Table 1. Baseline characteristics of the study population.

Patients	131
Age (range)[a] [yr]	68 (62 + 74)
Sex (%)	
Male	99 (75.6%)
Female	32 (24.4%)
Aetiology (%)	
Alcohol	13 (9.9%)
HBV	20 (15.3%)
HCV	45 (34.4%)
Viral coinfection	33 (25.2%)
Virus + Alcohol	15 (11.5%)
Other	5 (3.8%)
Child-Pugh class (%)	
A	110 (84.0%)
B	21 (16.0%)
C	0 (0.0%)
MELD <10 (%)	86 (65.6%)
BCLC class (%)	
A	53 (40.5%)
B	34 (26.0%)
C	44 (33.6%)
D	0 (0.0%)
AFP (range)[a] [ng/mL]	23.0 (7.5 + 305.5)
AFP >200 [ng/mL] (%)	39 (29.8%)
Tumor burden (%)	
Single nodule	57 (43.5%)
<2 cm	10 (7.6%)
2–3 cm	16 (12.2%)
>3 cm	31 (23.7%)
Multinodular	36 (27.5%)
Diffuse	38 (29.0%)
Portal thrombosis (%)	39 (29.8%)
Metastasis (%)	20 (15.3%)

[a]Data are expressed as median (25th percentile +75th percentile).

SCCA–IgM levels

Among the 131 treated patients, 78 (59.5%) showed a positive response, 29 (22.1%) remained stationary and 24 (18.3%) showed a negative response to therapy, according to mRECIST criteria (Table 2) [31]. Characteristics of the study population according to treatment response and SCCA–IgM levels are summarized in Table 2. Spearman's rank-order correlation (non-parametric test) and Pearson correlation (parametric test) showed that there is no correlation between clinical scores such as BCLC and SCCA–IgM T0 (p=.174 and .896, respectively).

Based on the cut-off value of 120 AU/mL, 36 (46.1%) and 37 (47.4%) positive responder patients showed detectable SCCA–IgM levels at T_0 and T_1, respectively, while 12 (41.4%) and 13 (44.8%) stationary patients showed detectable SCCA–IgM levels at T_0 and T_1, respectively. Finally, a higher percentage of negative responder patients showed detectable SCCA–IgM levels at T_0 and T_1 [15 (62.5%) and 17 (70.8%), respectively].

SCCA–IgM levels were significantly lower (p value <.05, Wilcoxon rank-sum test) in patients with positive (median: 115.1, 113.4 and 0.0 at T_0, T_1 and ΔSCCA–IgM, respectively) and stationary response (median: 106.5, 107.4 and 0.0

Table 2. Characteristics of the study population in relation to treatment options.

	Response			
	Positive	Stationary	Negative	Overall
Patients (%)	78 (59.5%)	29 (22.1%)	24 (18.3%)	131 (100.0%)
Treatment (%)				
TACE	10 (12.8%)	0 (0.0%)	2 (8.3%)	12 (9.2%)
RFA	25 (32.1%)	0 (0.0%)	1 (4.2%)	26 (19.8%)
Sorafenib	20 (25.6%)	27 (93.1%)	20 (83.3%)	67 (51.1%)
Other	23 (29.5%)	2 (6.9%)	1 (4.2%)	26 (19.8%)
BCLC class (%)				
A	49 (62.8%)	2 (6.9%)	2 (8.3%)	53 (40.5%)
B	20 (25.6%)	6 (20.7%)	8 (33.3%)	34 (26.0%)
C	9 (11.5%)	21 (72.4%)	14 (58.3%)	44 (33.6%)
D	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
SCCA-IgM T_0 [AU/mL]				
Median	115.1	106.5	149.1	116.3
25th +75th percentile	50 ÷ 174.4	50.0 ÷ 184.0	111.3 ÷ 198.8	50 ÷ 182.4
>120 AU/mL (%)	36 (46.1%)	12 (41.4%)	15(62.5%)	63 (48.0%)
p value[a]	—	.834	.044	—
SCCA-IgM T_1 [AU/mL]				
Median	113.4	107.4	170.6	122.9
25th +75th percentile	50.0 + 194.2	50.0 + 186.0	111.7 + 344.2	50 + 198.9
>120 AU/mL (%)	37 (47.4%)	13 (44.8%)	17 (70.8%)	67 (51.1%)
p value[a]	—	.779	.022	—
ΔSCCA-IgM				
Median	0.0	0.0	15.3	0.0
w25th +75th percentile	−16.4 + 19.6	−8.4 + 1.8	−4.7 + 110.6	−9.5 + 28.4
p value[a]	—	.585	.025	—

[a]Mann–Whitney p values versus positive response.

Table 3. Area under receiver operating characteristic curves (AUC), best value of separation, sensitivity and specificity between positive and negative response.

Biomarker	AUC (95% CI)	p^a	Best value of separation[b]	Sensitivity	Specificity
SCCA–IgM T_0 [AU/mL]	0.633 (0.512 + 0.754)	.050	107.0	83.3%	44.9%
SCCA–IgM T_1 [AU/mL]	0.649 (0.524 + 0.775)	.027	107.0	79.2%	47.4%
ΔSCCA–IgM [AU/mL]	0.649 (0.511 + 0.787)	.028	64.85	37.5%	92.3%

[a]Null hypothesis:true area 0.5.
[b]Best Youden Index = Sensitivity + Specificity −1.

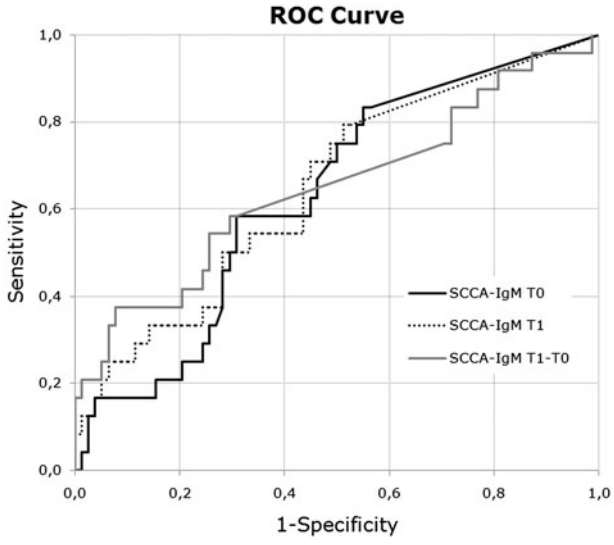

Figure 1. Receiver operating characteristic curves depicting the accuracy of SCCA-IgM measured at different time point of response (positive vs. negative).

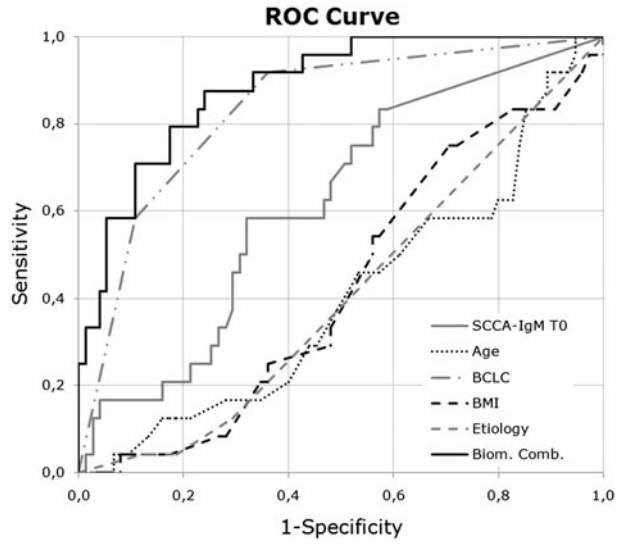

Figure 2. Receiver operating characteristic curves depicting the accuracy of SCCA-IgM, age, BCLC stage system, BMI, liver disease etiology and their combination (Biom. Comb.) of response (positive vs. negative).

at T_0, T_1 and ΔSCCA–IgM, respectively) in comparison to the negative responder ones (median: 149.1, 170.6 and 15.3 at T_0, T_1 and ΔSCCA–IgM, respectively) (Table 2).

The analysis of the SCCA–IgM levels in relation to treatment options revealed that patients with a positive response to sorafenib had ΔSCCA–IgM levels higher than patients treated with loco-regional therapies or TACE (p value =.012).

ROC curves for SCCA–IgM levels

ROC analysis was performed to evaluate whether SCCA–IgM serum levels can discriminate between patients with negative or positive response to treatment (Table 3). The performance of a test to separate patients with (sensitivity) and without (specificity) response to therapy is graphically expressed by the ROC curve (Figure 1). The area under the curve (AUC) allows a comparison of the diagnostic performance of the test: the greater the AUC, the better the ability to separate the two groups of patients.

As shown in Figure 1 and Table 3, the ROC curves of SCCA–IgM levels at T_0, T_1 and in ΔSCCA–IgM were very similar (AUC: SCCA–IgM $T_0 = 0.633$; SCCA–IgM $T_1 = 0.649$; ΔSCCA–IgM = 0.649) (all the p values of comparison between curves were not significant). The best separation SCCA–IgM values (determined with Youden index criterion) between positive and negative responders were 107.0 AU/mL at T_0 and T_1, and 64.9 AU/mL for the ΔSCCA–IgM. By adding baseline SCCA–IgM values to a biomarkers combination that includes BCLC score system, age, BMI and liver disease aetiology, the AUC increases to 0.887 (Figure 2). The addition of AFP does not improve the AUC. This indicates that SCCA–IgM is complementing the other baseline markers, adding predictive value.

Discussion

Currently, there is much debate as to which criteria should be used in order to predict and/or to assess HCC response to treatments, particularly for patients treated with targeted therapies, such as sorafenib [33,34]. The appropriate assessment of tumour response is critical to avoid an early treatment discontinuation because of a perceived absence of clinical benefit. The identification of serum biological markers is an exciting area of current translational research and holds the possibility of tailoring treatment and/or dosing to best effect in individual patients. However, no serum biomarker has yet been identified and validated to improve the selection of patients for the appropriate treatment options and/or to modulate the treatment schedule in relation to response. Recent studies have investigated plasma biomarkers as potential markers of response to sorafenib (and other antiangiogenic therapies), such as α-fetoprotein [35,36], angiopoietin-2 [37], hepatocyte growth factor [37], insulin-like growth factor-1 [38] and transforming growth factor-β1 [39], and additional research is ongoing. There are also some small studies suggesting that sorafenib adverse events (e.g. skin toxicity and arterial hypertension) may be on-treatment markers of clinical efficacy and predictors of survival in HCC patients treated with systemic therapy [40].

However, data from large, prospective studies are required in order to confirm this hypothesis. For these reasons, the possibility to predict the response to treatment in HCC patients would greatly help patient management, by directing specific efforts to the ones who will most probably show a beneficial effect and by reducing risk, stress and side effects in the ones who will probably not respond. In addition, a response-to-therapy prognostic tool would increase the effectiveness of HCC treatment and reduce the public health system's costs. Unfortunately, until now, no such means are available and serological markers used in clinical practice (i.e. alpha-fetoprotein) are not reliable enough for this purpose [5,6].

A recent study has shown that elevated SCCA–IgM serum levels in HCC patients correlate with reduced survival [28]. In particular, they subgrouped 78 patients in long- and short-term survivors (>36 and <36 months, respectively) showing that SCCA–IgM serum levels above 130 AU/mL were significantly associated with shorter survival. In line with these observations, this study suggests that SCCA–IgM serum levels can be a good predictor of response to HCC therapy. Indeed, SCCA–IgM levels were significantly lower in positive responder patients than in negative ones (p value <.05), both at basal time and 1-month post-treatment. This result confirms the ability of ΔSCCA–IgM to distinguish between good and bad HCC prognosis, and its promising use in the management of HCC systemic therapies.

Lower SCCA–IgM levels could reflect a reduced intrinsic immune response and/or tumour release activity in positive responder patients. Less circulating SCCA–IgM levels could reflect a reduced liver concentration or activity of SCCA and a reduced invasiveness, proliferation and resistance to apoptosis of tumour cells [16]. Moreover, according to literature [34], our study confirmed a higher percentage of therapeutic success in patients with early/intermediate HCC compared to the ones with advanced disease (88.4% vs. 11.5%). ΔSCCA–IgM was significantly higher in patients with advanced HCC confirming previous reports on the correlation between SCCA–IgM values and extension of liver disease [23–24]. For this reason, the SCCA–IgM serum determination could be more helpful in patients with advanced HCC, treated with systemic therapies, than in patients with early/intermediate HCC, treated with loco-regional approaches.

In this study, a direct comparison between SCCA–IgM and AFP with regard to prognostic value was not feasible, since a very high variability of AFP values was observed in this patient cohort, especially in the positive responder population (mean ± standard deviation = 885.7 ± 6303.5 ng/mL), dramatically reducing the statistical power during hypothesis testing [41]. Various groups already compared the diagnostic accuracy of AFP and SCCA–IgM in identifying HCC [11,20]. However, the focus of our study was the prognostic – not diagnostic – performance of SCCA–IgM. Whereas AFP and des-gamma-carboxy–prothrombin (DCP) can be useful in the diagnosis of HCC; to the best of our knowledge, these biomarkers have no prognostic value. The prognostic significance of SCCA–IgM has already been proven [28] and our report confirms this prognostic potential and provides novel data on how SCCA–IgM may help in predicting the response to therapy in HCC patients.

Due to conflicting results and small patients number, we could not draw reliable conclusions on the association between SCCA-IgM levels, therapeutic efficacy and treatment options. Based on the present study, serological SCCA–IgM could be used to predict the positive or negative response to treatment in HCC patients. More data from a larger group of patients are needed to offer a plausible answer and to define a more accurate cut-off for this specific application.

In conclusion, our findings suggest that SCCA–IgM serum levels in HCC patients can be useful in predicting and monitoring the response to treatment. The analysis of SCCA–IgM levels could greatly improve the management of HCC patients by guiding clinicians to focus some treatments only for patients who will respond positively, thus cutting costs and improving the quality of life of those patients who would not beneficiate from that therapy. Nevertheless, more studies with a larger number of patients are needed to provide a better evaluation of this therapeutic strategy.

Disclosure statement

AG, LP and GF are employees of XeptagenSpA. None of the other authors had any personal or financial conflicts of interest.

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