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Angiopoietin-2/Tie2 inhibition by regorafenib associates with striking response in a patient with aggressive hepatocellular carcinoma

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Conflict of interest

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List of abbreviations:

AFP: alfafetoprotein

Ang: angiopoietin

AST: aspartate aminotransferase

BMI: body mass index

CLD: chronic liver disease

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

CSF1R: Colony stimulating factor 1 receptor

ECOG PS: Eastern Cooperative Oncology Group performance status

GGT: gamma glutamyl transferase

FGFR: fibroblast growth factor receptor

HCC: hepatocellular carcinoma

IRB: Institutional Review Board

MKI: multi-kinase inhibitor

PDGFR: Platelet-derived growth factor receptor

RET: REarranged during Transfection

TS: transcriptomic signature

VEGF: vascular endothelial growth factor

Despite surveillance, hepatocellular carcinoma (HCC) often presents at such an advanced stage that only systemic therapy with sorafenib is feasible. However, aggressive HCCs bearing the neoangiogenic transcriptomic signature (TS)(1) are scarcely sensitive to sorafenib, which has no activity against the leading gene of the signature Angiopoietin-2 (Ang-2). The recently approved HCC drug regorafenib could instead possess some pharmacologic activity against it (2,3). We report a case of massive aggressive TS-positive HCC had a striking response to regorafenib.

Presentation of the case

A 62-year old teetotaler male, without previous history of CLD was seen in November 2017. Viral markers were negative. He was obese (BMI 32,5), had type 2 diabetes, and COPD. General conditions were fair (ECOG PS 1), although he complained of severe asthenia. A massive HCC was an incidental finding during a routine echocardiographic examination. At computed tomography (CT), a highly vascular HCC, measuring 22x19x20 cm, encompassing the whole right lobe was present (Figure 1A). Several other lesions, ranging from 1 to 4.5 cm, were present in the left liver lobe.

Ultrasound-guided liver biopsy showed a moderately differentiated Edmondson-Steiner grade 2 HCC. Peri-tumoral liver tissue showed F3 fibrosis but no cirrhosis. Liver TS analysis indicated a full-blown neoangiogenic 5-genes signature, with a 10-fold higher Ang-2 expression than surrounding non-tumoral liver. As data from our group indicate that aggressive TS-bearing HCCs are scarcely responsive to sorafenib, we started oral regorafenib, 160 mg once daily during weeks 1–3 of each 4-week cycle, after obtaining approval from IRB to use it in first line. Already after one month, CT scan showed great decrease of vascularization of the tumor lesions

in both lobes (Figure 1B). Subsequent radiological controls showed complete inhibition of tumoral vascularization. After 7 months from starting treatment, the patient is still well with no active hepatic lesion (Figure 1C). The drug was well tolerated. Liver function and inflammatory status have improved from baseline to May 2018, with bilirubin decreasing from 2.2 mg/dL to 1.3 mg/dL, AST from 50 IU/L to 18 IU/L, GGT from 230 IU/L to 93 IU/L, CRP from 11 mg/dL to 1.5 mg/dL, and AFP from 47.4 ng/mL to 10.7 ng/mL (Table 1). Circulating Ang-2 went from 25.685 pg/mL at baseline to 19.080 pg/mL in May 2018,while VEGF levels remained stable throughout.

Discussion

Regorafenib, an oral MKI that potently blocks multiple protein kinases, involved in tumor angiogenesis (VEGFRs 1–3, TIE2), oncogenesis (KIT, RET, RAF-1, BRAF), metastasis (VEGFR3, PDGFR, FGFR) and tumor immunity (CSF1R), significantly improves survival in HCC patients progressing on sorafenib (2). Regorafenib has no direct inhibitory action on Ang-2 but has a potent activity (30–150 nmol/L) against Tie2, the vascular receptor tyrosine kinase for Angiopoietin-1 (Ang-1) and Ang-2 (3). The role of the Ang-2/Tie2 axis in tumoral angiogenesis is not straightforward. Ang-2 was initially described as a Tie2 antagonist, which would make Tie2 inhibition undesirable, when seeking for anti-angiogenic activity. However, it has become evident that Ang-2 can act differently depending on the organ context and on different associated conditions (4). Transcriptomic analysis of tumoral and non tumoral liver tissue of prospective HCC cohort (described in ref. 1) shows that while Ang-2 level are increased in tumoral tissue vs. non tumoral tissue, Ang-1 levels are not significantly different between tumoral and non tumoral tissue. Level of

expression of Ang-1 in aggressive HCC was about half of that of Ang2. In non-inflamed endothelium and in certain vascular beds that have a low level of Ang-1 signaling, it has been shown that Ang-2 can act as a Tie2 agonist (5). The striking response to regorafenib in our patient, with remarkable and protracted decrease of tumoral vascularization and of circulating Ang-2 levels and marked improvement of clinical and biochemical parameters, is a proof of concept that regorafenib can efficiently block non-canonic Ang-2 driven angiogenesis. This could suggest the opportunity of a larger study evaluating the role of Ang/Tie2 signaling pathway as an anti-angiogenic target, most specifically in aggressive cases with activated neoangiogenesis. VEGF levels did not decrease during the 6-month observation. As VEGF action is critical in maintaining the pro-angiogenic effect of the Ang-2/Tie2 axis, the association with VEGF signaling inhibition could lead to a more profound antiangiogenic effect. On the same line, as the activation of angiogenesis in TS+ HCCs is striking, it would be logical to test combination therapies with selective Ang-2 inhibitors.

On the whole, this clinical observation suggests that it is time to explore in detail the utility of the prognostic and response-predicting HCC signatures especially now that many different molecular-targeted drugs have become available.

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Legend to Figure

Representative CT scans at Baseline (A, B), after 1 (C, D) and 6 months (E, F) from starting regorafenib. A striking decrease of tumor vascularization is evident throughout the observation period, with almost no abnormal vascularization at the last control.

Table 1 - Course of clinical scores and blood biochemistry at beginning of therapy (November 2017) and throughout the treatment period.

	November 2017	**February 2018**	**May 2018**
Child-Pugh score	A6	A5	A5
MELD score	12	10	8
Bilirubin (mg/dl)	2.2	1.6	1.3
INR	1.24	1.14	1.09
Albumin (g/dl)	3.6	3.7	3.8
AST (UI/l)	50	24	18
ALT (UI/l)	41	20	12
GGT (UI/l)	230	164	93
FA (UI/l)	359	302	193
VEGF (pg/mL)	643	1116	654
ANG-2 (pg/ml)	25.685	22.940	19.080
CRP (mg/dl)	11.0	6.7	1.5
CEA (ng/mL)	3.4	-	-
CA 19.9 (U/mL)	30.1	-	-
AFP (ng/ml)	47.4	21.6	10.7

Normal ranges

Bilirubin (mg/dl)	0.16 - 1.10
INR	0.84 - 1.25
Albumin (g/dl)	3.5 - 5.0
AST (UI/l)	1 - 37
ALT (UI/l)	1 - 40
GGT (UI/l)	1 - 55
FA (UI/l)	38 – 126

VEGF (pg/ml)	10–310
ANG-2 (pg/ml)	95-1010
CRP (mg/dl)	0 - 0.7
CEA (ng/mL)	0 - 5
CA 19.9 (U/mL)	0 - 37
AFP (ng/ml)	0 - 8