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Erik H.C.J. Buster
Harry L.A. Janssen
Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, 's Gravendijkwal 230, 3015, CE Rotterdam, The Netherlands
Tel.: +31 10 4635942; fax: +31 10 4365916
E-mail address: h.janssen@erasmusmc.nl (H.L.A. Jans)

HBV genotype must be included in treatment algorithm

Reply to Buster and Janssen:
We thank Drs. Buster and Janssen for their appreciative comments on our review article recently published in the Journal. We entirely share their views on the important role of HBV genotype in a modern treatment algorithm of patients with chronic HBV infection irrespective of the medicament used, even though, as the authors also reiterated, its effects are indisputably evident only in interferon-treated patients. We also think that a correct algorithm must include histology as an additional variable and that future trials must be powered to detect the effects of other factors, such as age, ALT, and HBV DNA.

We apologize for not citing their abstracts on the role of HBV genotype as predictor of sustained off treatment responses in HBeAg positive patients, but the criteria we set for our review were such that we considered for analysis only full papers reporting randomized clinical trials with any information provided on HBV genotypes, baseline characteristics of study subjects, response to antiviral therapy, and interaction with the type of therapy. Finally, we fully support Buster and Janssen's recommendation that HBV genotyping should be part of a diagnostic work-up at least in all tertiary referral centers where specialized treatment is actually instituted.

Conflict of interest

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Mario U. Mondelli[1,*]
Sara Raimondi[2]
Savino Bruno[3]
Patrick Maisonneuve[2]
[1]*Research Laboratories, Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy*
[2]*Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy*
[3]*Liver Unit, Department of Medicine, Azienda Ospedaliera Fatebenefratelli e Oftalmico, Milan, Italy*
[*]*Address: S.C. di Infettivologia Molecolare, Dipartimento di Malattie Infettive, Fondazione IRCCS Policlinico San Matteo, p.le Golgi 19, 27100 Pavia, Italy*
Tel.: +39 0382 502639; fax: +39 0382 526450
E-mail addresses: .mario.mondelli@unipv.it, m.mondelli@smatteo.pv.it (M.U. Mondelli)

mTOR inhibitors and sorafenib for recurrent heptocellular carcinoma after orthotopic liver transplantation

To the Editor:
With interest we read the recent publication of the Milan group by Bhoori et al., describing a patient with a late recurrent hepatocellular carcinoma (HCC) after orthotopic liver transplantation who was successfully treated with a combination of everolimus and sorafenib after initial failure of sorafenib [1]. In general, the prognosis of recurrent HCC after orthotopic liver transplantation is fatal, especially in those patients presenting with early recurrence [2]. The majority of these patients present with metastatic disease and treatment options are very limited [3].

Sorafenib, a tyrosine kinase-inhibitor with anti-proliferative and anti-angiogenic activity, is currently the only approved systemic treatment in patients with advanced Barcelona Clinic Liver Cancer (BCLC) stage HCC [4]. It inhibits downstream signaling of VEGFR-2 and -3, Flt-3, PDGFR, and FGFR-1 and blocks the Ras-Raf-MEK-ERK cascade by targeting the serine–threonine kinase Raf. Rapamycin and its analogues, e.g. sirolimus and everolimus, inhibit *mammalian target of rapamycin* (mTOR),

Table 1
Patients with recurrent HCC after liver transplantation treated with sorafenib and everolimus or sirolimus.

Patient	1	2	3
Etiology of cirrhosis	alcohol	alcohol	HBV
HCC growth pattern	multifocal	multifocal*	multifocal
Bridging therapy	TACE	-	-
MELD score at time of transplantation	28**	30	22
Time until recurrence	16 months	20 months	78 months
Localization of recurrence	liver, skin, adrenal glands, mesenteric lymph nodes	liver, mesenteric lymph nodes	liver
Survival after recurrence	>7 months***	16 months	1 month
Immune suppression	everolimus (1 mg twice daily)	sirolimus (2 mg daily)	sirolimus (2 mg daily)
Start of mTOR inhibitor	after recurrence	after recurrence	66 months before recurrence
Start of sorafenib	concomitant with everolimus	4 weeks later	after recurrence
Baseline creatinin level	0.86 mg/dl	1.23 mg/dl	1.23 mg/dl
Baseline total bilirubin level	0.7 mg/dl	0.5 mg/dl	0.9 mg/dl
Duration of treatment with mTOR inhibitor	>7 Months	16 months	5 weeks
Duration of treatment with sorafenib	5 months	14 weeks	3 weeks
Best documented tumor response	stable disease	progressive disease	not done

*Incidental diagnosis in explant; **standard exception MELD; ***alive.

a downstream target of phosphoinositide 3-kinase (PI3K) signal transduction. mTOR inhibitors exhibit anti-tumor activity probably through reduced expression of hypoxia-inducible factor and subsequently VEGF and PDGF expression. The Milan group suggested a strong phosphorylation of the S6 protein, a downstream signal of mTOR, as a potential predictive biomarker for everolimus [1], an inhibitor of mTOR approved as an immunosuppressant after solid organ transplantation as well as targeted therapy in renal cell cancer. The critical role of mTOR signaling for hepatocarcinogensis is well-documented preclinically [5] and inhibition of the mTOR pathway after liver transplantation for HCC leads to higher survival rates in some groups of patients [6]. The results of the SILVER study, comparing relapse-free survival rates in this population either treated with sirolimus or mTOR-inhibitor-free immunosuppression are anticipated in the near future [7]. Targeting the Ras pathway with the multikinase inhibitor sorafenib in addition to mTOR inhibition has synergistic effects on tumor growth in xenograft mice [8]. These data form the current rational to switch patients with recurrent HCC after liver transplantation from calcineurin inhibitors to mTOR inhibitors like sirolimus or everolimus, and additionally to start sorafenib. However, even in patients without liver transplantation, the toxicity of sorafenib can severely impact quality of life [9,10] and up to now only anecdotal data on the use of sorafenib after liver transplantation are available [2]. Although sorafenib and mTOR inhibitors have different targets, side-effects of both drugs overlap (e.g. fatigue, diarrhea, skin toxicity) and therefore can lead to unacceptable toxicity. Dose finding studies for the combination of sorafenib with mTOR inhibitors have not been published and it remains speculative if side-effects of either drug could be boosted by the other. Herein, we present our experience with recurrent HCC after liver transplantation in three patients who were treated with sorafenib and an mTOR inhibitor (Table 1). Hepatic recurrence occurred in all three patients, and additionally two presented with extrahepatic metastasis. After diagnosis of recurrence, two out of the three patients were switched from cyclosporine to everolimus (1 mg twice daily) or sirolimus (2 mg daily). Patient 3 was already switched to sirolimus before because of cyclosporine-induced nephrotoxicity. Dose titration with sorafenib (starting dose 200 mg twice daily) was initiated in patient 1 concomitantly and in patient 2 after one month. In patient 1 the sorafenib dose was elevated to 400 mg twice daily after three weeks but had to be reduced to the starting dose because of the development of grade 3 hand–foot-syndrome and fatigue. Upon follow-up CT scanning after 3 months, a stable disease was documented; however, after 5 months progression was documented and sorafenib was stopped permanently. Patient 2 had to temporarily stop sorafenib because of grade 3 hand–foot-syndrome. Since side-effects re-occurred after re-exposure even in a very low dose (200 mg every second day) sorafenib was permanently stopped. Patient 3 developed grade 3 fatigue and his performance status worsened rapidly. He was admitted to the hospital three weeks after the initiation of sorafenib treatment with a sepsis. Although sorafenib was stopped immediately, he died of multi-organ-failure one week later.

In summary, our preliminary experience indicates that combination treatment with an mTOR inhibitor and sorafenib is possible only in selected patients after liver transplantation. Even under close monitoring and careful dose titration of an mTOR inhibitor followed by sorafenib, major toxicity, predominantly severe hand–foot-syndrome, occurred in two of three patients.

Conflict of interest

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Oliver Waidmann
Wolf-Peter Hofmann
Stefan Zeuzem
Joerg Trojan*
Medizinische Klinik 1, Klinikum der Johann-Wolfgang, Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany
**Tel.: +49 069 6301 7860; fax: +49 069 6301 83776*
E-mail address: trojan@em.uni-frankfurt.de (J. Troja)

Magnetic resonance spectroscopy of bile in the detection of cholangiocarcinoma

To the Editor:

We would like to draw your attention to some of the drawbacks in a recent article published by Wen et al. in your journal [1], on NMR-based metabolomics of bile samples in distinguishing cholangiocarcinoma from benign biliary diseases. The authors performed orthogonal partial least square discriminant analysis (OPLS-DA) of ^{1}H NMR spectra of bile samples obtained from patients with cholangiocarcinoma and benign biliary diseases. They classified both groups with a sensitivity of 88% and a specificity of 81%. We are currently working on the utility of magnetic resonance spectroscopy in the study of hepatopancreatobiliary diseases [2–6] and have noticed some shortcomings in the above study.

The authors have mentioned that their work was the first metabolomics approach reported in the diagnosis of human hepatobiliary diseases [1]. However, a similar metabolomic study using bile samples for the detection of cholangiocarcinoma was published by our group about two years ago [2]. In our study, we performed multivariate analysis of ^{1}H NMR spectra of bile samples obtained from patients with cholangiocarcinoma and other benign biliary diseases (primary sclerosing cholangitis/choledocholithiasis) with a comparable sample size as that of Wen et al. [1]. We reported a sensitivity of 88.9% and a specificity of 87.1% in classifying cancer and control groups. Khan et al. [7] had also previously reported a study in which they analyzed bile samples from cholangiocarcinoma, pancreatic cancer, and other hepatobiliary diseases using ^{1}H NMR spectroscopy. Although our study [2] and the one by Khan et al. [7] were undertaken with similar objectives as that of Wen et al., they were not cited.

We also have concerns regarding some of the metabolites quantified in the targeted metabolic profiling. Wen et al. [1] compared the levels of choline in both groups and the difference was not found to be statistically significant ($p = 0.85$). However, in our study, we quantified the predominant choline-containing phospholipid, phosphatidylcholine (PC) which was decreased in cancer patients compared to the benign group with the difference being statistically significant ($p = 0.02$). Khan et al. had also reported a reduced phosphatidylcholine signal in cancer patients (cholangiocarcinoma/pancreatic cancer) compared to the non-cancer patients ($p = 0.007$) [7]. In a similar study, Nagana Gowda et al. observed decreased levels of phospholipids in cholangiocarcinoma patients compared to the non-liver disease control group ($p = 0.001$) [8]. Moreover, in a recent study, Sharif et al. made similar observations in comparing patients with cholangiocarcinoma and gallstone disease ($p = 0.01$) [9]. The above observations are consistent with the fact that phosphatidylcholine is an important component of bile protecting bile ducts from the harmful effects of bile acids and its depletion can lead to bile duct injury [3]. Second, the authors reported that citrate levels were elevated in cancer patients compared to the benign subjects with the difference being statistically significant [1]. Although citrate is a component of bile acting as a calcium chelator in gallbladder disease [10], its levels are considerably low (0–406 μM). As a result, detection of citrate in bile using ^{1}H NMR spectroscopy would be difficult. Moreover, they have associated citrate with a signal resonating at 1.5 ppm (one of the signals found to be discriminatory in the OPLS-DA). This assignment is not correct as the $-CH_2-$ protons in citrate resonate at totally different chemical shift (in the region 2.55–2.75 ppm). Furthermore, the authors have misas-