Research Article

JOURNAL OF HEPATOLOGY

Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma – The SHELTER study

Michael Bitzer[1,*], Marius Horger[2], Edoardo G. Giannini[3], Tom M. Ganten[4], Marcus A. Wörns[5], Jens T. Siveke[6], Matthias M. Dollinger[7], Guido Gerken[8], Max E. Scheulen[9], Henning Wege[10], Vittorina Zagonel[11], Umberto Cillo[12], Franco Trevisani[13], Armando Santoro[14], Vincenzo Montesarchio[15], Nisar P. Malek[1], Julia Holzapfel[16], Thomas Herz[16], Astrid S. Ammendola[16], Stefano Pegoraro[16], Bernhard Hauns[16], Anna Mais[16], Ulrich M. Lauer[1], Stefan W. Henning[16], Bernd Hentsch[16]

[1]*Department of Internal Medicine I, Eberhard Karls University, Tuebingen, Germany;* [2]*Department of Diagnostic & Interventional Radiology, Eberhard Karls University, Tuebingen, Germany;* [3]*Gastroenterology Unit, Department of Internal Medicine, University of Genoa, Genoa, Italy;* [4]*Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany;* [5]*First Department of Medicine, Johannes Gutenberg-University, Mainz, Germany;* [6]*Second Department of Internal Medicine, Technical University, Munich, Germany;* [7]*Internal Medicine I, University Ulm, Ulm, Germany;* [8]*Center for Internal Medicine, University Clinic, Essen, Germany;* [9]*Department of Medical Oncology, West German Cancer Center, Essen, Germany;* [10]*University Hospital Hamburg-Eppendorf, Hamburg, Germany;* [11]*Medical Oncology Unit 1, Istituto Oncologico Veneto, IRCCS, Padova, Italy;* [12]*Hepatobiliary Surgery and Liver Transplant Unit, Azienda Università di Padova, Padova, Italy;* [13]*University of Bologna, Bologna, Italy;* [14]*Department of Oncology, Humanitas Cancer Center, Rozzano, Italy;* [15]*UO Oncologia Ospedale Cotugno Napoli, Napoli, Italy;* [16]*4SC AG, Planegg-Martinsried, Germany*

See Editorial, pages 243–244

Background & Aims: No established therapies for patients with hepatocellular carcinoma (HCC) and progression on first-line sorafenib treatment currently exist. This phase I/II trial investigated safety, pharmacokinetics and potential biomarkers of the histone deacetylase inhibitor resminostat and a combination therapy with resminostat and sorafenib.

Methods: Patients with HCC and radiologically confirmed progression on sorafenib were treated in an exploratory, multicenter, open-label, uncontrolled, non-randomized, parallel group phase I/II study. In the combination group (n = 38) four dose levels ranged from daily 200 to 600 mg resminostat plus 400 to 800 mg sorafenib. The monotherapy group (n = 19) received 600 mg resminostat.

Results: 57 patients received treatment. Most common adverse events were gastrointestinal disorders, thrombocytopenia and fatigue. Median maximal histone deacetylase inhibition and highest increase in H4-acetylation matched T_{max} of resminostat. Sorafenib or the Child-Pugh score did not affect typical pharmacokinetics characteristics of resminostat. Efficacy assessment as progression-free survival-rate after 6 treatment cycles (12 weeks, primary endpoint) was 12.5% for resminostat and 62.5% for resminostat plus sorafenib. Median time to progression and overall survival were 1.8 and 4.1 months for resminostat and 6.5 and 8.0 months for the combination, respectively. Zinc finger protein 64 (ZFP64) baseline expression in blood cells was found to correlate with overall survival.

Conclusions: The combination of sorafenib and resminostat in HCC patients was safe and showed early signs of efficacy. Sorafenib did not alter the pharmacokinetic profile of resminostat or its histone deacetylase inhibitory activity *in vivo*. A prognostic and potentially predictive role of ZFP64 for treatment with resminostat should be further investigated in HCC and possibly other cancer indications.

Lay summary: No established therapy for patients with advanced hepatocellular carcinoma and progression under first-line systemic treatment with sorafenib currently exists. Epigenetic modulation by inhibition of histone deacetylases might be able to overcome therapy resistance. This exploratory phase I/II clinical study in patients with radiologically confirmed progression under first-line treatment with sorafenib investigated the histone deacetylases inhibitor resminostat as single agent or in combination with continued application of sorafenib.

Clinical trial registration: The clinical trial has been registered at www.clinicaltrials.gov as NCT00943449.

Keywords: Histone deacetylase inhibitor; Cancer epigenetics; Epigenetic treatment; Drug resistance; Zinc finger protein 64.

Received 18 September 2015; received in revised form 23 December 2015; accepted 24 February 2016; available online 4 March 2016

* Corresponding author. Address: Medical University Clinic, Eberhard Karls University, Otfried-Müller-Str. 10, 72076 Tübingen, Germany. Tel.: +49 7071 29 80583; fax: +49 7071 294402.

E-mail address: michael.bitzer@uni-tuebingen.de (M. Bitzer).

Abbreviations: HCC, hepatocellular carcinoma; HDAC, histone deacetylase; $PFSR_6$, PFS-rate after 6 treatment cycles (12 weeks); TTP, time to progression; OS, overall survival; ZFP64, zinc finger protein 64; SD, stable disease; PR, partial response; CR, complete response; DLT, dose limiting toxicity; PK, pharmacokinetics; CI, confidence interval; MTD, maximum tolerated dose; AE, adverse events; ITT, intention-to-treat; PP, per protocol; DCR, disease control rate; CP, Child-Pugh; FIM, first-in-man study.

Introduction

With globally approximately 750,000 new cases of hepatocellular carcinoma (HCC) per year, primary cancer of the liver is the second leading cause of cancer-related deaths worldwide [1]. For HCC patients with advanced disease, the kinase inhibitor sorafenib (Nexavar®) is currently the only approved drug, providing a median time to radiologic progression (TTP) of 5.5 months and an overall survival (OS) of 10.7 months [2]. However, many tumors are either primarily resistant to sorafenib or develop drug resistance during therapy. Since a number of other kinase inhibitors including sunitinib [3], linifanib [4], and brivanib [5,6] have shown limited benefit in clinical studies, a high medical need exists to identify new drugs that employ alternative mechanisms of action in HCC.

Epigenetic changes play an important role in the pathogenesis of HCC [7,8]. High histone deacetylase (HDAC) expression correlates with higher incidences of HCC, cell invasion into the portal vein, poorer histological differentiation, a more advanced TNM stage and lower survival rates after surgical resection [9]. Recent evidence suggests that acquisition of drug tolerance and resistance against anti-cancer agents is at least partly mediated by epigenetic mechanisms, such as histone deacetylation [10]. Inhibition of HDACs may revert chromatin modifications in tumor cells and restore sensitivity towards previously inefficient drugs. HDAC inhibitors have promising preclinical and clinical antitumor activity in HCC [11–14] and their further exploration might prove an attractive concept to overcome drug tolerance and therapy resistance in sorafenib pretreated patients.

In this exploratory phase I/II study advanced HCC patients with radiologically confirmed progression on first-line treatment with sorafenib were recruited. The study investigated safety, pharmacokinetics and early evidence of efficacy of the HDAC inhibitor resminostat [15] as single agent or in combination with sorafenib (phase II part of the study) and included a dose escalation phase for the combination (phase I part). The study was accompanied by an exploratory biomarker program, including gene expression analyses in peripheral blood cells, to identify biomarkers that might offer correlation to clinical responsiveness to resminostat, that could guide a personalized treatment approach for patients with HCC.

Patients and methods

This exploratory, non-randomized, open-label clinical phase I/II study evaluated safety, pharmacokinetics, efficacy and potential biomarkers of resminostat and the combination of resminostat and sorafenib after progression on treatment with sorafenib. The study involved 8 centers in Germany and 5 in Italy. The study protocol and amendments were approved by national regulatory authorities and independent ethics committees for each center. Patients provided written informed consent before study entry. Prior to the start of the study, eligible patients were without sorafenib therapy for at least 2, but not more than 10 weeks.

Patients

Eligible patients had measurable, unresectable, locally advanced or metastatic HCC (BCLC class B or C; Child-Pugh stage A or B of not more than 7, exclusion if hepatic encephalopathy >grade 1) that was either histologically proven or clinically diagnosed according to the American association for the study of liver diseases (AASLD) criteria [16]. Patients had documented progressive disease on first-line sorafenib (400 mg or higher) after at least 8 weeks' treatment, which had to be confirmed by central radiological image evaluation prior to study entry. According to RECIST guideline 1.1 progressive disease was defined as an increase of the sum of diameters of target lesions of at least 20% and an absolute increase of at least 5 mm from the smallest value on study. The appearance of one or more new lesions during treatment with sorafenib was also considered progression [17]. Further inclusion and exclusion criteria are provided online in the Supplementary material and the study protocol.

Treatment plan

Patients were centrally assigned to either resminostat plus sorafenib (group A) or resminostat alone (group B). Prior to recruitment to group A, a dose escalation phase I part was conducted in 18 patients. Cohorts of 3 to 6 patients received escalating doses of resminostat and 400 mg or 800 mg sorafenib. Details are presented in Fig. 1 and Supplementary material. The main study phase consisted of 6 treatment cycles of 14 days each with resminostat treatment for 5 consecutive days followed by a 9-day rest period. In combination treatment sorafenib was given every day. Patients with stable disease (SD), partial response (PR) or complete response (CR) after 6 treatment cycles were eligible to enter follow-up treatment until progressive disease (PD). Treatment was discontinued in the event of unacceptable or intolerable toxicity, evidence of PD or withdrawal of consent.

Safety analysis

Patients were assessed at baseline and at 11 scheduled visits during the main study phase. Central ECG analysis was performed on days 1, 5, 33 at predose and 1, 2, 3, 4, 5, 6 h post-dose. On the remaining visits at least one control ECG was recorded. Toxicity was graded according to National Cancer Institute common toxicity criteria version 3.0. A dose limiting toxicity (DLT) was defined as any drug-related hematological grade 4 or non-hematological grade 3 or 4 toxicity occurring in cycle 1.

Pharmacokinetic analysis

Blood samples for pharmacokinetic (PK) analysis were collected predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h post-dose on day 1 of cycle 1 (C1D1), C1D5 and C3D5. Additionally, a predose sample was collected on C1D8. Quantitative analyses on plasma levels of resminostat and sorafenib were performed using a validated HPLC-MS/MS method.

Biomarker analysis

Blood-based assays for monitoring of the pharmacodynamic activity of resminostat included measurement of cellular HDAC enzyme activity and histone acetylation levels in all patients. Samples were collected predose, 2 and 5 h postdose during cycle 1 and 3. The expression of the following 10 genes previously identified to respond to resminostat treatment in cell culture studies, including HepG2 cells, were assessed (relative to housekeeping genes 18sRNA, TATA-box binding protein and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) in all patients: general transcription factor IIIC subunit 6 (*GTF3C6*), coiled-coil domain containing 43 (*CCDC43*), dipeptidyl-peptidase 3 (*DPP3*), MICAL-like 1 (*MICALL1*), KDEL (Lys-Asp-Glu-Leu) containing 2 (*KDELC2*), 2′-5′-oligoadenylate synthetase 2 (*OAS2*), zinc finger protein 64 (*ZFP64*), exostosin-like glycosyltransferase 2 (*EXTL2*), histone cluster 2, H4 (*HIST2H4*), and DEP domain containing 7 (*DEPDC7*). Additionally, the following exploratory genes, based on literature or biological relevance to HDAC inhibition were measured in a limited number of patients: baculoviral IAP repeat containing 5 (*BIRC5*), cadherin 1, type 1 (*CDH1*), secreted phosphoprotein 1 (*SPP1*). Statistical calculations and figures were generated using open source software package R (http://www.R-project.org/). For detailed assay information refer to Supplementary material.

Efficacy methods

Efficacy to treatment was evaluated using TTP, progression-free survival (PFS) and OS, defined as the time from the date of the first administration of resminostat to the date of the event: death (OS), radiologically confirmed objective tumor progression (TTP) or progression or death from any cause (PFS). The median time to event with its 95% confidence interval (CI) was calculated using the Kaplan-Meier method [18]. Patients who had a missing radiological assessment at the end of the study or who were lost to follow-up prior to tumor progression were censored at the time of their last tumor assessment. Patients for whom no date of