Multicenter prospective study of angiogenesis polymorphism validation in HCC patients treated with sorafenib. An INNOVATE study protocol

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ABSTRACT

Introduction: Although sorafenib is the upfront standard of care for advanced hepatocellular carcinoma (HCC), molecular predictors of efficacy have not been identified yet. In the ALICE-1 study, rs2010963 of VEGF-A and VEGF-C proved to be independent predictive factors for progression-free survival (PFS) and overall survival (OS) in multivariate analysis. The ALICE-1 study results were confirmed in the ALICE-2 study, in which VEGF and VEGFR SNPs were analyzed. In the ePHAS study we analyzed the SNPs of eNOS. In univariate analysis, patients homozygous for an eNOS haplotype (HT1: T-4b at eNOS-786/eNOS VNTR) had significantly shorter median PFS and OS than those with other haplotypes. These data were confirmed in the validation set.
Methods: This nonpharmacological, interventional, prospective multicenter study aims to determine whether eNOS, HIF-1, VEGF, Ang2 and VEGFR polymorphisms play a role in predicting the objective response rate, PFS, and OS of advanced HCC patients treated with sorafenib. The study will involve 160 advanced HCC patients with

Child-Pugh class A disease. The primary aim is to validate the prognostic or predictive roles of eNOS, Ang2, HIF-1, VEGF and VEGFR polymorphisms in relation to the clinical outcome (PFS) of HCC patients treated with sorafenib.
Conclusions: Overall, our data may suggest that polymorphism analysis of the VEGF, VEGFR-2, HIF and eNOS genes can identify HCC patients who are more likely to benefit from sorafenib.

Keywords: Hepatocellular carcinoma, Sorafenib, Biomarkers, Trial, VEGF, eNOS

Introduction

Molecular predictors of sorafenib efficacy have not yet been identified (1-5). Sorafenib is a multikinase inhibitor acting on vascular endothelial growth factor (VEGF) receptors and platelet-derived growth factor receptor beta (PDGFR-β), which are involved in tumor cell proliferation and tumor angiogenesis (6). Data have suggested that hepatocellular carcinoma (HCC) is dependent on angiogenesis (7) and VEGF is an important pathway in hepatocarcinogenesis (7).

In the ALICE-1 study (8), in univariate analysis VEGF-A alleles C of rs25648, T of rs833061, C of rs699947, C of rs2010963, VEGF-C alleles T of rs4604006, G of rs664393, VEGFR-2 alleles C of rs2071559 and C of rs2305948 were significant predictors of progression-free survival (PFS) and overall survival (OS). In multivariate analysis, allele C of rs2010963 and allele T of rs4604006 of VEGF-A proved to be independent factors influencing both PFS and OS.

Hypoxia-inducible factor (HIF)-1α plays a role in tumor angiogenesis and its overexpression is correlated with tumor angiogenesis, invasion, metastasis, treatment resistance and poor prognosis (7). In the ALICE-2 study (9), single nucleotide polymorphisms (SNPs) of HIF-1α were analyzed. In particular, the GG genotype of rs12434438 characterized a population with a particularly poor outcome regardless of the clinical effect of either VEGF SNP (PFS: 2.6 months, p<0.0001; OS: 6.6 months, p<0.0001).

Sorafenib inhibits VEGF receptor 2 (VEGFR-2), resulting in decreased endothelium-derived nitric oxide synthase (eNOS) and lower production of nitric oxide (NO). NO seems to play a proangiogenic role in tumor angiogenesis (10-13).

In the ePHAS study (14), a training cohort of 41 HCC patients and a validation cohort of 87 patients receiving sorafenib were analyzed. At univariate analysis, patients homozygous for an eNOS haplotype (HT1: T-4b at eNOS-786/eNOS VNTR) had significantly shorter median PFS (2.6 vs. 5.8 months, p<0.0001) and OS (3.2 vs.14.6 months, p = 0.024) than those with other haplotypes. These data were confirmed in the validation set for median PFS (2.0 vs. 6.7 months, p<0.0001) and OS (6.4 vs.18.0 months, p<0.0001).

On the basis of these preliminary results, our aim is to validate in a prospective study the potential role of eNOS, VEGF, VEGFR, HIF-1 and Ang2 polymorphisms in patients with HCC treated with sorafenib (15).

Methods

This nonpharmacological, interventional, prospective multicenter study is aimed at determining whether eNOS, HIF-1, VEGF, Ang2 and VEGFR polymorphisms play a role in predicting the objective response rate, PFS and OS of advanced HCC patients treated with sorafenib. The study will involve 160 patients.

The primary aim of the study is to validate the prognostic or predictive roles of eNOS, Ang2, HIF-1, VEGF and VEGFR polymorphisms in relation to the clinical outcome (PFS) of HCC patients treated with sorafenib; its secondary aim is to verify the prognostic value of eNOS, Ang2, HIF-1, VEGF and VEGFR polymorphisms in relation to clinical outcome (OS) and the basal level of lactate dehydrogenase, blood pressure, MELD, Ang2 plasma levels, VEGF and Ang2 in relation to the clinical outcome (PFS and OS) of HCC patients treated with sorafenib.

Study population

The study population consists of patients with advanced stage HCC and patients with intermediate HCC who are ineligible for locoregional treatments (surgical resection, percutaneous ablation, transarterial chemoembolization) or liver transplantation.

The inclusion criteria are as follows:

1. HCC diagnosed according to the AASLD and/or EASL criteria
2. Age >18 years
3. Eastern Cooperative Oncology Group (ECOG) performance status score ≤2
4. Child-Pugh liver function class A
5. Life expectancy ≥12 weeks
6. Adequate hematological function
7. Patients are required to have at least 1 untreated target lesion measurable in 1 dimension, according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1
8. Concomitant antiviral systemic therapy is allowed. Patients are excluded if they have previously received molecularly targeted therapies or any other systemic treatment
9. Resolution of all acute toxic effects of any prior local treatment according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 grade ≤2.

The exclusion criteria are as follows:

1. Previous or concurrent cancer that is distinct in primary site or histology from HCC, except for cervical carcinoma in situ, treated basal cell carcinoma, and superficial bladder tumors. Any cancer curatively treated >3 years prior to entry is permitted
2. Patients eligible for curative treatments (transplantation, surgical resection, percutaneous treatment)
3. Renal failure requiring hemodialysis or peritoneal dialysis
4. Presence of recent (<6 months) or current cardiac failure (class II, III or IV NYHA classification), baseline LVEF <LLN by either cardiac ultrasound or cardiac scintigraphy; recent

(<6 months) acute coronary syndrome, clinically significant ECG abnormalities or recent (<6 months) acute vascular diseases (stroke, myocardial infarction, etc.); active clinically serious infections grade >2 (CTCAE version 4.03)

5. Known history of human immunodeficiency virus (HIV) infection

6. Known central nervous system tumors including metastatic brain disease

7. Clinically significant gastrointestinal bleeding within 30 days prior to study entry.

Ethics approval

The IRST-IRCCS-AVR ethics committee approved the study (approval number 1524).

Sample collection

Blood samples will be collected from each patient at baseline, 14 days after sorafenib, 28 days after sorafenib, 60 days after sorafenib and at the time of disease progression. For each patient we will collect 1 sample tube (3 mL of blood) containing EDTA, 1 sample tube (5 mL of blood) without anticoagulant, and 1 sample tube (2.5 mL of blood) of PAXgene.

Sample evaluation

All genotyping analyses will be performed using DNA extracted from corpuscular components of whole blood. Genomic DNA will be extracted from 200 µL of corpuscular components by a QIAamp DNA Minikit (Qiagen) according to the manufacturer's instructions. DNA quantity and quality will be assessed with a NanoDrop 1000 spectrophotometer (Celbio). DNA will be extracted at our center (Biosciences Laboratory at IRST, Meldola, Italy).

Genotyping analyses of eNOS and Ang2 will be performed at the Biosciences Laboratory at IRST, while HIF-1, VEGF and VEGFR genes will be analyzed at the University of Cagliari.

Genotyping analyses of eNOS-786 will be performed by TaqMan technology using SNP genotyping assays. Polymerase chain reaction (PCR) will be performed and genotypes will be analyzed on the 7500 Real-Time PCR System (Applied Biosystems) using the 7500 software, version 2.3. PCRs will be performed starting from 20 ng of genomic DNA.

eNOS intron 4 VNTR and Ang2 polymorphisms will be instead determined by standard PCR and direct sequencing analysis on an ABI 3130 Genetic Analyzer (Applied Biosystems). The PCR conditions and primer sequence for eNOS VNTR were reported in our previous study (16). We will choose the most interesting Ang2 polymorphisms after the results of the retrospective study.

Analysis of the HIF-1, VEGF and VEGFR genes will be performed by TaqMan technology using an SNP genotyping assay (Applied Biosystems). PCR will be performed and genotypes will be analyzed on the 7300 Real-Time PCR System (Applied Biosystems) using the ABI Prism 7300 Sequence Detection System software (version 1.3.1; Applied Biosystems). Each reaction will contain 0.2 µl of total genomic DNA. Laboratory personnel blinded to patient status will perform the genotyping and a random 10% of the samples will undergo repeat genotyping to validate the genotyping procedures. All genotyped SNPs will have to present an overall call rate of ≥90% to be included in our analysis.

The plasma/serum levels of HIF-1 and VEGF will be measured by enzyme-linked immunosorbent assay (ELISA).

Blood mRNA will be extracted with a PAXgene blood RNA kit (PreAnalytiX-Qiagen). mRNA will be treated with DNAse I (Qiagen) and reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad). Real-time PCR will be performed using the 7500 Applied Biosystems and Taqman assay chemistry.

Study monitoring

The principal investigator (PI) agrees to perform the study in accordance with ICH Good Clinical Practice and to provide all information requested in the case report form/study database, in an accurate manner according to the instructions provided. The PI has responsibilities to the health authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms/study database. According to the guidelines on ICH Good Clinical Practice, the trial monitoring aim is to check subject informed consent and the study database entries against the source documents. Trial staff will check data for compliance with the protocol, data consistency, missing data and timing. The PI will be sent requests for missing data or clarification of inconsistencies or discrepancies.

Statistical considerations

Population size

On the basis of the results obtained from our previous studies, we assume a 0.50 prevalence of the polymorphisms to be validated. When the sample size in each group is 80 (160 total sample size), an exponential maximum likelihood test of equality of survival curves with a 0.05 2-sided significance level will have 90% power to detect the difference between a group 1 exponential parameter, λ_1 of 0.1155 (median PFS of 6 months) and a group 2 exponential parameter, λ_2 of 0.198 (median PFS of 3.5 months) (a constant hazard ratio of 0.58). Patient enrolment will take about 24 months. We will consider a follow-up period of at least 12 months for each patient.

Data analysis

A specific database will be created to prospectively collect personal, clinical, histological, and treatment data.

PFS is intended as the time from entry into the study to first observation of documented disease progression or death due to any cause, whichever occurs first. Patients without tumor progression at the time of analysis will be censored at the time of their last tumor evaluation. OS is defined as the observed length of life from study entry to death of any cause or date of last contact with patients lost to follow-up. OS and 95% confidence intervals will be estimated with the Kaplan-Meier product-limit method. Further analyses of PFS/OS will be performed using a multivariable Cox model in which gender, age, center and other prognostic factors will be entered

as covariates. All tests will be 2-sided at a significance level of 0.05. No interim analyses are planned.

Conclusions

Sorafenib represents the standard of care for HCC but molecular predictors of efficacy have not yet been identified. Overall, our previous findings indicate that polymorphism analysis of the VEGF, VEGFR-2, HIF and eNOS genes may represent a valuable asset to better identify those HCC patients who are more likely to benefit from sorafenib treatment. The aim of our large multicenter study is to prospectively validate these observations.

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Abbreviations

CTCAE	Common Terminology Criteria for Adverse Events
ECOG	Eastern Cooperative Oncology Group
HCC	Hepatocellular carcinoma
HIF	Hypoxia-inducible factor
NYHA	New York Heart Association
OS	Overall survival
PCR	Polymerase chain reaction
PDGFR	Platelet-derived growth factor receptor
PFS	Progression-free survival
PI	Principal investigator
RECIST	Response Evaluation Criteria in Solid Tumors
SNP	Single nucleotide polymorphism
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

Disclosures

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