SUPPLEMENTARY MATERIALS AND METHODS

Tumor samples and mRNA/DNA extraction

Formalin-fixed paraffin-embedded (FFPE) tissue blocks from HCC patients resected between 2008 and 2010 and randomized into treatment in the STORM clinical trial (NCT00692770)[1] were collected from the highest enrolling centers after *ad-hoc* institutional review board (IRB) clearance (Supplementary Table 1). The design of the STORM trial is extensively described elsewhere.[1] The study protocol was approved at each center and specific informed consent for this biomarker study was obtained for all subjects. Out of the 202 collected samples, 188 were suitable for the study (hereinafter, BIOSTORM cohort) (Figure 1). An expert liver pathologist discerned tumor tissue from non-tumor liver parenchyma based on hematoxylin/eosin (H&E) staining. Tumor tissue was then macro-dissected from non-tumor adjacent tissue in freshly cut FFPE sections and total RNA and gDNA were isolated using, respectively, the miRNeasy FFPE and QiAmp DNA FFPE kits (Qiagen). The Qiacube system (Qiagen) was used to automatize the extraction process.

Immunohistochemistry

Immunostainings exploring the status of phospho-ERK (pERK) in tumor and endothelial cells, phospho-VEGFR2 (pVEGFR2), Ki67 and CD31 were performed. Stainings were carried out on 3µm-thick FFPE tissue sections after antigen retrieval using the Envision FLEX target Retrieval Solution (Dako) and the PT-Link Pre-Treatment Module (Dako). pERK was stained using a 1:100 dilution of anti-phospho-Thr202/Tyr204 ERK (Ref.#4370, Cell Signaling). pVEGFR2 was stained

using a 1:50 dilution of anti-phospho-Tyr1054/Tyr1059 VEGFR2 (Ref.#ab5473, Abcam). Proliferation was assessed by Ki67 immunostaining (Ref.#IR626, ClonMIB-1, Dako), and CD31 staining (Ref.#ab28364, Abcam) was used as a vascular marker. No avidin/biotin amplification was used to avoid cross-reaction with endogenous liver biotin. EnVisionTM+ System-HRP (3,3'-Diaminobenzidine, DAB) was applied as secondary antibody (Dako). All samples were counterstained with hematoxylin, and subsequently evaluated by independent expert pathologists (MS, SNT, LR-C). We followed either standardized scoring criteria or, when absent, published criteria to ensure analytical validity.[2–6] The pERK staining was assessed in tumor hepatocytes and endothelial cells, according to a previous study from our group.[6] pVEGFR2 staining and Ki67 were quantified as previously described.[2],[5]

Fluorescent *in situ* hybridization

VEGFA amplification was assessed by Fluorescent *in situ* hybridization (FISH). Centromeric probe against chromosome 6 (Vysis CEP6; Ref.#D6Z1) was from Abbott Molecular, and VEGFA-TexRed probe, from Abnova. Four-micrometer sections from FFPE HCC tissues were macro-dissected to remove the non-tumor adjacent tissue. Macro-dissection was conducted based on H&E staining analyzed by an expert liver pathologist. Slides were baked overnight at 65°C, deparaffinized and rehydrated. FISH procedure was conducted using the Histology FISH Accessory Kit (Dako), with 6 minutes of proteolytic digestion with pepsin (37°C), 5 minutes of FISH probe denaturation (75°C), and overnight hybridization (37°C). Slides were counterstained with ProLong Gold Antifade reagent containing DAPI

(Thermo Fisher Scientific), and quantified as previously reported.[3] Each sample was at least quantified by two independent investigators, scoring 100 cells with non-damaged DAPI-labeled nucleus each.

Whole transcriptome analysis

Transcriptome analysis was performed to assess the predictive/prognostic value of expression levels of previously reported individual candidate genes (HGF, c-Kit, bFGF/FGF2, VEGFA), gene signatures, and for generating a novel multi-gene signature predicting prevention of recurrence by sorafenib. Total RNA (350ng) was used for whole-genome gene-expression profiling (DASL Assay, Illumina). Samples displaying cycle threshold Ct>31 in quantitative real-time polymerase chain reaction (qRT-PCR) of RPLP13 were excluded from the analysis. Raw data was processed using the Illumina Expression File Creator Module from GenePattern. Samples with poor quality mRNA profiles[4] were also excluded. Data was normalized using cubic spline algorithm implemented in the GenePattern Illumina Normalizer Module. Microarray data were deposited in Gene Expression Omnibus database with the accession number GSE109211. Class predictions were performed using the GenePattern Nearest Template Prediction[4] Method. Each sample was assigned to a given class when the false discovery rate (FDR)<0.05. To generate a gene signature predicting benefit to sorafenib, individual association of each gene with clinical outcome according to treatment was assessed. Genes presenting a flat expression profile throughout the study cohort were considered not informative genes. Thus, the 5,191 genes with standard deviation (SD) within the upper quartile were ranked according to their Cox score using the

SurvivalGene[4] R package and default parameters (except for the number of sample permutations, which was increased to 10,000). The p-value associated with each Cox score was used to select genes significantly correlated with RFS (p-value <0.05). To reduce bias and gain robustness, we performed a leave-one-out cross validation (LOOCV) using the GenePattern LoocvSurvival Module. For each group of patients we obtained a set of genes associated with good and poor prognosis which combined, they originate the 146 gene signature. Gene Set Enrichment Analysis (GSEA)[7], ssGSEA[8], and INGENUITY® Pathway analysis (IPA) were used to assess enrichment of activated pathways.

Targeted exome sequencing and Sanger sequencing

Mutations from a set of 18 onco-driver genes described[9] to be relevant in HCC (Supplementary Table 2) were explored by targeted exome sequencing using TruSeq Custom Amplicon Low Input technology (Illumina). Libraries were sequenced with an average coverage of 500X using MiSeq (Illumina) (Supplementary Figure 4), and aligned to hg19 reference genome. Samples with very low average coverage (<20X) were excluded from the analysis. Mutations were called using the Genome Analysis Toolkit (GATK).[10] Variants present in the tumor and absent in the adjacent non-tumor tissue were kept as candidate somatic mutations, and were annotated with ANNOVAR.[11] Only variants reported in COSMIC v78 for HCC, with variant frequency >7% and read depth >10X were considered as potential biomarker candidates. All mutations were manually confirmed using Integrative Genomics Viewer. Targeted Exome Sequencing data were generated by IDIBAPS Genomic Facility.

The mutational status of TERT promoter was determined by Sanger sequencing as previously described[12].

End-points

The primary end-point of the BIOSTORM study was to define predictive biomarkers of response to sorafenib in terms of RFS. RFS was defined as the time from randomization to the first documented disease recurrence by independent radiological assessment or death by any cause, whichever happened first. The secondary end-point was to define prognostic biomarkers of RFS and/or validate those previously reported. In the BIOSTORM cohort all events were recurrences (70 RFS events in 188 patients), and thus time-to-progression (TTP) was equivalent to RFS. Because only 24/188 events of survival occurred in this cohort, prognostic/predictive biomarkers of overall survival (OS) were not explored.

Clinical data and statistical analyses

Clinical and outcome data used in these analyses were obtained from the STORM trial database, with a January 28th 2014 cutoff date for RFS. Median-follow up was 39.0 months (95%CI, 37.0–41.0). Clinical-pathologic variables tested in the univariate analysis included etiology, presence of satellites, presence of microscopic vascular invasion, histological grade, AFP, albumin, ALT, multinodularity, size of the tumor and presence of cirrhosis. Clinical-pathologic variables were binned and compared using Fisher exact test (categorical variables) and T test (continuous variables). To test prognosis and predictive value of previously studied candidate biomarkers (HGF, c-Kit, bFGF/FGF2, VEGFA)[13]

their mRNA levels were measured by microarray and data were dichotomized using fold change (tumor/adjacent) >2 as cutoff. Kaplan-Meier method, log-rank test, and univariate and multivariate Cox regressions were used to analyze the association of molecular and clinical-pathologic variables with RFS. The association between biomarkers and sorafenib treatment effect was evaluated using a Cox proportional hazards model with an interaction term. Biomarkers were considered predictive of sorafenib efficacy in preventing HCC recurrence when p of interaction was <0.05. Patients who had undergone less than one treatment cycle (4 weeks) were excluded from the predictive biomarker analysis (sorafenib: n=9 out of 83; placebo: n=6 out of 105). All statistical tests were two-sided. Data were analyzed with SPSS 23.0 (SPSS Inc., Chicago, IL, USA) and the R statistical package.

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