Pharmacodynamic Biomarkers Predictive of Survival Benefit with Lenvatinib in Unresectable Hepatocellular Carcinoma: From the Phase 3 REFLECT Study

Richard S. Finn\textsuperscript{1}, Masatoshi Kudo\textsuperscript{2}, Ann-Lii Cheng\textsuperscript{3}, Lucjan Wyrwicz\textsuperscript{4}, Roger K.C. Ngan\textsuperscript{5}, Jean-Frederic Blanc\textsuperscript{6}, Ari D. Baron\textsuperscript{7}, Arndt Vogel\textsuperscript{8}, Masafumi Ikeda\textsuperscript{9}, Fabio Piscaglia\textsuperscript{10}, Kwang-Hyub Han\textsuperscript{11}, Shukui Qin\textsuperscript{12}, Yukinori Minoshima\textsuperscript{13}, Michio Kanekiyo\textsuperscript{14}, Min Ren\textsuperscript{14}, Ryo Dairiki\textsuperscript{13}, Toshiyuki Tamai\textsuperscript{15}, Corina E. Dutcus\textsuperscript{14}, Hiroki Ikezawa\textsuperscript{15}, Yasuhiro Funahashi\textsuperscript{13}, Thomas R. Jeffry Evans\textsuperscript{16}.

\textsuperscript{1}Division of Hematology/Oncology, Geffen School of Medicine, UCLA Medical Center, Santa Monica, CA, USA; \textsuperscript{2}Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, Osaka, Japan; \textsuperscript{3}National Taiwan University Cancer Center, Taipei, Taiwan; \textsuperscript{4}Centrum Onkologii-Instytut im., M. Sklodowskiej Curie, Warsaw, Poland; \textsuperscript{5}Queen Elizabeth Hospital, Kowloon, Hong Kong; \textsuperscript{6}University Hospital of Bordeaux, Bordeaux, France; \textsuperscript{7}California Pacific Medical Center, San Francisco, CA, USA; \textsuperscript{8}Hannover Medical School, Hannover, Germany; \textsuperscript{9}Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Kashiwa, Japan; \textsuperscript{10}General and University Hospital S. Orsola-Malpighi, Bologna, Italy; \textsuperscript{11}Severance Hospital, Yonsei University, Seoul, South Korea; \textsuperscript{12}Nanjing Bayi Hospital, Nanjing, Jiangsu, China; \textsuperscript{13}Eisai Co., Ltd., Tsukuba, Ibaraki, Japan; \textsuperscript{14}Eisai Inc, Woodcliff Lake, NJ, USA; \textsuperscript{15}Eisai Co., Ltd, Tokyo, Japan; \textsuperscript{16}Beatson West of Scotland Cancer Centre, University of Glasgow, Glasgow, UK.

Running title: Biomarker analysis following lenvatinib in unresectable HCC
**Corresponding Author:**

Richard S. Finn, MD  
UCLA Oncology  
2825 Santa Monica Blvd, Suite 200  
Santa Monica, CA 90404  
Phone: 310-586-2091; Fax: 310-586-6830  
Email: rfinn@mednet.ucla.edu

**Conflict of Interest Statements**

**Richard Finn** reports consulting or advisory roles for Bayer, Bristol Myers Squibb, Eisai, Genentech/Roche, Lilly, Merck, Novartis, Pfizer; institutional research funding from Bayer, Bristol Myers Squibb, Eisai, Lilly, Merck, Novartis, Pfizer.

**Masatoshi Kudo** reports honoraria from AbbVie, Bayer, Bristol Myers Squibb, EA Pharma, Eisai, Gilead Sciences, Merck Serono, MSD, Novartis, Pfizer, Taiho Pharmaceutical; consulting or advisory role for Bayer, Bristol Myers Squibb, Eisai, MSD; research funding from Abbvie, Astellas Pharma, Bristol Myers Squibb, Chugai Pharma, Daiichi Sankyo, Otsuka, Taiho Pharmaceutical.

**Ann-Lii Cheng** reports consulting or advisory fees from Bristol Myers Squibb, Ono Pharmaceutical Co., Ltd., Novartis International AG, Bayer AG, Merck Group, and Merck Sharp & Dohme.

**Lucjan Wyrwicz** reports honoraria from Eisai.

**Roger Ngan** reports honoraria from Novartis, AstraZeneca, Sanofi, Pfizer, ZaiLab, Lilly, MSD, Roche, and Eisai; research funding from Pfizer.

**Jean-Frederic Blanc** reports honoraria from Bayer, Ipsen, Eisai, Roche, Bristol Myers Squibb, and AstraZeneca.
Ari Baron reports speakers’ bureau fees for Amgen, Bristol Myers Squibb, Genentech/Roche, Lilly, Merck.

Arndt Vogel reports no conflicts of interest.

Masafumi Ikeda reports honoraria from Abbott, Bayer, Bristol Myers Squibb, Dainippon Sumitomo Pharma, Eisai, Lilly, Nobelpharma, Novartis, Otsuka, Taiho Pharmaceutical, Teijin Pharma, Yakult Honsha; consulting or advisory role for Bayer, Daiichi Sankyo, Eisai, Kyowa Hakko Kirin, MSD, NanoCarrier, Novartis, Shire, Teijin Pharma, Lilly; research funding from ASLAN Pharmaceuticals, AstraZeneca, Baxalta/Shire, Bayer, Bristol Myers Squibb, Chugai Pharma, Eisai, Kowa, Kyowa Hakko Kirin, Lilly, Merck Serono, MSD, NanoCarrier, Novartis, Ono Pharmaceutical, Taiho Pharmaceutical, Takara Bio, Yakult Honsha.

Fabio Piscaglia reports honoraria from: Alkermes, AstraZeneca, Bayer, Bracco, BMS, GE, EISAI, IPSEN, La Force Guerbet, Roche, Siemens Healthineers, Tiziana Life Sciences.

KH Han reports research funding and consulting or advisory fees from Eisai Co., Ltd. and KOWA Company, Ltd.; consulting or advisory fees from Bayer AG.

Shukui Qin reports no conflicts of interest.

Yukinori Minoshima reports stock ownership and is an employee of Eisai Co., Ltd.

Michio Kanekiyo reports stock ownership and is an employee of Eisai Co., Ltd.; reports stock ownership of Oncolys BioPharma Inc.

Min Ren is an employee of Eisai Inc.

Ryo Dairiki is an employee of Eisai Co., Ltd.

Toshiyuki Tamai is an employee of Eisai Co., Ltd.

Corina Dutcus is an employee of Eisai Inc.

Yasuhiro Funahashi reports stock ownership and is an employee of Eisai Co., Ltd.

Hiroki Ikezawa is an employee of Eisai Co., Ltd.
Thomas R Jeffry Evans reports honoraria from Bristol Myers Squibb, Bayer AG, Baxalta, Celgene Corporation, Eisai, GlaxoSmithKline, Immunova Therapeutics, Karus Therapeutics, Roche/Genentech, and TC BioPharm; speakers’ bureau fees from Eisai, Bristol Myers Squibb, Celgene; consulting/advisory fees from Eisai, Bristol Myers Squibb, Celgene, Karus; personal fees from Bristol Myers Squibb; research funding from ONO, Bristol Myers Squibb, Lilly, AstraZeneca, Celgene, Eisai, GSK, Roche, Basilea, Vestex, Merck, Daiichi, NuTide, Sierra, Immunocore, Verastem; support for sponsored clinical trials from Bristol Myers Squibb, GlaxoSmithKline, Roche/Genentech, Celgene Corporation, TC BioPharm, Merck Sharp & Dohme, Novartis, e-Therapeutics, Vertex, Verastem, Daiichi, AstraZeneca, Basilea, Immunocore, and Chugai; support to attend international scientific conferences from Bristol Myers Squibb, Roche/Genentech, Bayer AG, Merck Sharp & Dohme, and Eisai Co., Ltd.

Financial support: This study was sponsored by Eisai Inc., Woodcliff Lake, NJ, USA, and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Medical writing support was provided by Oxford PharmaGenesis Inc., Newtown, PA, USA, and was funded by Eisai Inc., Woodcliff Lake, NJ, USA, and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.
Abstract

Purpose: In REFLECT, lenvatinib demonstrated an effect on overall survival (OS) by confirmation of noninferiority to sorafenib in unresectable hepatocellular carcinoma. This analysis assessed correlations between serum or tissue biomarkers and efficacy outcomes from REFLECT.

Experimental Design: Serum biomarkers (VEGF, ANG2, FGF19, FGF21, and FGF23) were measured by ELISA. Gene expression in tumor tissues was measured by the nCounter PanCancer Pathways Panel. Pharmacodynamic changes in serum biomarker levels from baseline, and associations of clinical outcomes with baseline biomarker levels were evaluated.

Results: 407 patients were included in the serum analysis set (lenvatinib n=279, sorafenib n=128); 58 patients were included in the gene-expression analysis set (lenvatinib n=34, sorafenib n=24). Both treatments were associated with increases in VEGF; only lenvatinib was associated with increases in FGF19 and FGF23 at all timepoints. Lenvatinib-treated responders had greater increases in FGF19 and FGF23 versus non-responders at C4D1 (FGF19: 55.2% vs 18.3%, \( P=0.014 \); FGF23: 48.4% vs 16.4%, \( P=0.0022 \), respectively). Higher baseline VEGF, ANG2, and FGF21 correlated with shorter OS in both treatment groups. OS was longer for lenvatinib than sorafenib (median, 10.9 vs 6.8 months, respectively; HR, 0.53; 95% CI, 0.33–0.85; \( P=0.0075 \); \( P\)-interaction=0.0397) with higher baseline FGF21. In tumor tissue biomarker analysis, VEGF/FGF enriched groups showed improved OS with lenvatinib versus the intermediate VEGF/FGF group (HR 0.39; 95% CI 0.16–0.91; \( P=0.0253 \)).

Conclusions: Higher baseline levels of VEGF, FGF21, and ANG2 may be prognostic for shorter OS. Higher baseline FGF21 may be predictive for longer OS with lenvatinib compared with sorafenib, but this needs confirmation.
Statement of translational relevance

Biomarker identification in hepatocellular carcinoma (HCC) is challenging because of the molecular heterogenicity of HCC. Advanced disease is primarily diagnosed by radiological criteria rather than by tumor biopsy. This paucity of available tumor tissue further hampers biomarker discovery, leading to an increased focus on evaluating serum biomarkers in advanced HCC. Identifying biomarkers predictive of treatment effect could be clinically useful in guiding therapeutic decisions. The baseline serum level of FGF21 is a candidate predictive biomarker for longer overall survival with lenvatinib versus sorafenib. This analysis aimed to further elucidate pharmacodynamic differences in the mechanism of action of lenvatinib from that of sorafenib. In contrast to sorafenib, lenvatinib demonstrated evidence of inhibition of the FGFR family and a greater magnitude of VEGFR inhibition. These results suggest the inhibitory activity of lenvatinib against FGFR may contribute to its increased tumor response.
Introduction

Lenvatinib is a multikinase inhibitor targeting vascular endothelial growth factor (VEGF) receptors 1–3, fibroblast growth factor (FGF) receptors 1–4, platelet-derived growth factor receptor-α (PDGFRα), KIT, and RET, with a distinct in vitro kinase inhibitory profile and kinase binding mode compared with sorafenib (1-5). Until the approval of lenvatinib in 2018, sorafenib remained the only approved first-line systemic treatment for patients with unresectable hepatocellular carcinoma (uHCC) (6). Results from the IMbrave150 study showed that atezolizumab plus bevacizumab improved outcomes versus sorafenib (7), indicating that new options are widening the treatment landscape for uHCC. Presently, recommended and preferred first-line standard of care systemic therapies for hepatocellular carcinoma (HCC) include sorafenib and lenvatinib, and atezolizumab plus bevacizumab (8).

In the global, randomized, open-label, phase 3 REFLECT study, lenvatinib demonstrated noninferiority versus sorafenib for the first-line treatment of patients with uHCC in overall survival outcomes (hazard ratio [HR] 0.92; 95% confidence interval [CI] 0.79–1.06). The median overall survival was 13.6 months (95% CI 12.1–14.9) in the lenvatinib arm vs 12.3 months (95% CI 10.4–13.9) in the sorafenib arm. In addition, treatment with lenvatinib significantly (P<0.0001) improved progression-free survival (median: 7.4 months [95% CI 6.9–8.8] vs median: 3.7 months [95% CI 3.6–4.6]), time to progression (median: 8.9 months [95% CI 7.4–9.2] vs median: 3.7 months [95% CI 3.6–5.4]), and objective response rate (24.1% [95% CI 20.2–27.9] vs 9.2% [95% CI 6.6–11.8]) based on modified Response Evaluation Criteria in Solid Tumors (mRECIST) (9).

Lenvatinib is a distinct type V kinase-inhibitor; in pre-clinical models, it inhibits both VEGF- and FGF-driven angiogenesis and has demonstrated anti-proliferative activity against HCC cell lines dependent on the FGF-signaling pathway (4,10). Additionally, lenvatinib has been shown to induce HCC cell death via FGF receptor (FGFR) inhibition under nutrient-
and oxygen-starved conditions, which mimic the tumor microenvironment under angiogenesis inhibition (11). Lenvatinib has also demonstrated increased antitumor activity compared with sorafenib against HCC xenograft tumors overexpressing VEGF (12).

The molecular heterogeneity of HCC provides a challenge in biomarker identification (13). One of the main targets for treatment in HCC is the angiogenic activity of the disease (14). Therefore, prognostic and predictive biomarkers can be found not only in the cancer cell itself, but also in the tumor microenvironment or serum. Among many cancer-associated signaling pathways, the VEGF signaling cascade pathway has been implicated in the pathogenesis of HCC (15). Additionally, the FGF signaling pathway may be involved as FGFR4 is mainly expressed in liver tissue. Studies suggest overexpression of the FGFR4 receptor and amplification of FGF19 contribute to HCC progression (16,17). In this analysis, we evaluated serum and tumor tissue biomarkers using samples collected from the phase 3 REFLECT study and assessed correlations between those biomarkers and clinical outcomes, in order to identify potential biomarkers associated with clinical benefit and/or resistance, and to elucidate pharmacodynamic differences between sorafenib and lenvatinib.

Materials and Methods

Study design

The study design for the phase 3 open-label, multicenter, noninferiority REFLECT study (ClinicalTrials.gov identifier NCT01761266) has been reported previously (9). Briefly, from March 1, 2013, to July 30, 2015, 954 eligible patients with uHCC who had not received prior therapy for advanced disease were randomized (1:1) to receive oral lenvatinib (12 mg/day if bodyweight ≥ 60 kg or 8 mg/day if bodyweight < 60 kg) or sorafenib 400 mg twice daily in 28-day cycles. Patients provided written informed consent before undergoing any study-specific procedures. Relevant institutional review boards approved the study,
Serum biomarker analysis

Serum samples were taken from patients in the REFLECT study who consented to serum biomarker assessment. Samples were collected at baseline, cycle 1 day 15, and cycles 2–4 day 1; and were stored at -20°C or below until assayed. Biomarker assays for VEGF, angiopoietin-2 (ANG2), FGF19, FGF21, and FGF23 were performed on serum samples using enzyme-linked immunosorbent assays (ELISAs). VEGF, ANG2, FGF19, FGF21 and FGF23 were assayed at baseline and posttreatment (cycle 1 day 15, and cycles 2–4 day 1). Details on the ELISAs used can be found in the Supplementary Appendix.

Tumor tissue biomarker analysis

Archival tumor tissue samples were collected from patients in the REFLECT study who consented to tumor tissue biomarker assessment. Archival tumor samples from the most recent surgery or biopsy were collected at any time during the study, unless material was not available. If the entire tumor block could not be provided, 10 slides of 5-μm-thick tissue sections and 3–5 slides of 10-μm-thick tissue sections were provided. Procedures and medications that patients received between therapy initiation and tissue sample collection are listed in Supplementary Table S1.

Total RNA was isolated from tumor tissue samples for gene-expression analysis. Macrodissection was performed before extraction of total RNA if tumor content in the sample was less than 50%. Tumor samples for gene expression were analyzed using the
Supplementary Excel) with 29 custom genes (\textit{ANGPT2, TEK, KDR, FLT4, CDH2, KRT18, SNAI1, SNAI2, TWIST1, TWIST2, VIM, ZEB1, ZEB2, GAS6, AXL, SDF1 [CXCL12], CXCR4, CSF1, GLUT1 [SLC2A1], MCT1 [SLC16A1], MCT4 [SLC16A4], PDH [PDP1], PDK1, LDHA, NANOG, SOX2, NOTCH4, PD-L1 [CD274], and PD-1 [PDCD1]}). Pathway enrichment analysis was conducted on the sets of genes identified as having expression levels that were nominally significantly associated with overall survival in the lenvatinib and the sorafenib treatment arms. For this analysis, 12 canonical pathways and 1 cancer driver panel predefined by the PanCancer Pathways Panel, along with 1 additional angiogenic and growth factor pathway defined by our group, were used. Thirty-six genes were selected given their roles in the angiogenic and growth factor pathway (\textit{ANGPT1, ANGPT2, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23, FGFR1, FGFR2, FGFR3, FGFR4, FGF, FLT1, FLT4, KDR, PGF, TEK, VEGFA, and VEGFC}).

Clustering analysis was performed using baseline expression levels of these 36 genes, the predefined cancer driver panel and other genes involved in transcriptional regulation, Wnt and DNA repair pathways. In the clustering analysis, the distance matrix was calculated using the Manhattan method, and the dendrogram was generated using Ward’s method. From the dendrogram generated in the clustering analysis, 3 subgroups (VEGF-enriched, FGF-enriched, and intermediate) were identified in each treatment arm. The overall survival for each group identified in the clustering analysis were compared by plotting Kaplan–Meier curves. Baseline serum biomarker levels were also compared among subgroups identified by the clustering analysis in either lenvatinib, sorafenib, or combined arms.

\textbf{Statistical analysis}
The intent-to-treat (ITT) population included all patients randomized to treatment. The serum analysis set included all patients with at least 1 serum biomarker measurement at any time point. The gene-expression analysis set included all patients with a tumor gene-expression biomarker measurement.

All analyses were noted in the statistical analysis plan and all statistical analyses were performed using SAS (version 9.3). Clustering analysis of gene-expression profiles was performed using R version 3.3.2 by R Foundation for Statistical Computing (Vienna, Lower Austria, Austria). Percentage changes in serum biomarker levels from baseline to cycle 1 day 15 and cycles 2–4 day 1 were summarized using medians and analyzed using the 1-sample Wilcoxon signed-rank test for each treatment arm. The 2-sample Wilcoxon rank-sum test was used to compare the distribution of changes in levels between the treatment arms.

Correlation analyses of pharmacodynamic changes of serum biomarker levels from baseline with best overall response ([complete response/partial response] vs [stable disease/progressive disease/other]) based on mRECIST using independent imaging review assessments were performed using 2-sample Wilcoxon rank-sum tests for each treatment arm. Correlation analyses of baseline serum biomarker levels with overall survival were performed using univariate Cox regression for each treatment arm. HRs were expressed as an increase in 1 standard deviation in baseline values.

Overall survival was assessed using Kaplan–Meier estimates. Overall survival differences between treatment arms were examined using the univariate Cox regression model for subgroups divided on the basis of baseline and changes in biomarker levels: low (0–25%), middle (≥ 25–75%), or high (≥ 75–100%). Cutoffs were determined by visual inspection of Kaplan-Meier curves or minimum P-values from a log-rank test. Dichotomized analyses (cutoff at 3rd quartiles) of baseline levels of each serum biomarker and overall survival were performed using univariate Cox regression and log-rank test to investigate
possible prognostic biomarkers for overall survival. Correlation analysis of baseline levels of ANG2 and FGF21 with overall survival by HCC etiology was performed using the univariate Cox regression analysis, and subsequent dichotomized analysis using the third quartile cutoff. Also, multivariate Cox regression analysis with treatment arm, biomarker category, and their interaction, was conducted to explore predictive biomarkers for overall survival.

Gene lists were generated using the results of the univariate Cox regression for overall survival for the pathway enrichment analysis. The significance of pathway/panel enrichment was evaluated by Fisher’s exact test based on both the 13 pathways/panels in the PanCancer Pathways Panel and the additional angiogenic and growth factor pathway defined by our group. Correlation analysis of gene-expression levels of FGF ligands with progression-free survival was performed using univariate Cox regression for each treatment arm. False discovery rate adjustments (Benjamini-Hochberg) were utilized for all gene-expression analyses. Given the exploratory nature of our study, all statistical significance demonstrated and reported in this analysis was nominal.

Results

Patients

Eligible patients (N=954) were randomly assigned to receive lenvatinib (n=478) or sorafenib (n=476) in the ITT population of the REFLECT study. In the serum analysis set (n=407), 279 patients received lenvatinib and 128 patients received sorafenib (Fig. 1). In the gene-expression analysis set (n=58), 34 patients received lenvatinib and 24 patients received sorafenib; this sample size was decreased from 114 patients due to insufficient amounts of DNA/RNA extracted from available tumor tissue. Demographic and baseline characteristics of all patients are shown in Supplementary Table S2. There were differences in demographic and baseline characteristics between the serum analysis set and the ITT population.
Approximately 33% of the ITT population were from the Western region in both treatment arms whereas 48% and 56% of the serum analysis set were from the Western region in the lenvatinib and sorafenib arm, respectively. The percentage of white patients was lower (28% in lenvatinib and 30% in sorafenib vs 42% in lenvatinib and 48% in sorafenib), while the percentage of Asian patients was higher (70% in lenvatinib and 69% in sorafenib vs 55% in lenvatinib and 48% in sorafenib) in the ITT population compared to the serum analysis set, respectively. The percentage of patients with hepatitis B virus was higher in the ITT population than in the serum analysis set (53% in lenvatinib and 48% in sorafenib vs 36% in lenvatinib and 26% in sorafenib), respectively.

The proportion of Asian patients was larger in the ITT population versus the gene-expression analysis set (approximately two-thirds vs one-third), which, in turn, likely led to a difference in the percentage of patients with hepatitis B virus (approximately 50% in the ITT population vs approximately 30% in the gene-expression analysis set) as hepatitis B is highly prevalent in Asia (18). In addition, the percentage of patients with baseline alpha-fetoprotein (AFP) levels \( \geq 200 \text{ ng/mL} \) was approximately 39% to 46% in the ITT population in the sorafenib and lenvatinib arms, respectively, versus 17% to 24% in the gene-expression analysis set. Differences in median AFP levels were observed between the lenvatinib and sorafenib arms in the gene-expression analysis set (22.1 ng/mL vs 3.3 ng/mL, respectively), the serum analysis set (74.8 ng/mL vs 27.6 ng/mL, respectively), and the ITT population (131.1 ng/mL vs 71.2 ng/mL).

**Pharmacodynamic changes in serum biomarkers**

Overall, pharmacodynamic analysis confirmed different target engagement between lenvatinib and sorafenib. Both lenvatinib and sorafenib treatments resulted in increases in VEGF levels versus baseline, which reflect inhibition of the VEGF receptors (VEGFR) by
sorafenib. There was a median change from baseline of 57.8% at cycle 1, day 15 to 34.2% at cycle 4, day 1 with lenvatinib vs 35.7% to 18.1% at the respective time-points with sorafenib (Fig. 2; Supplementary Table S3). Conversely, only lenvatinib treatment resulted in increases in FGF19 (median change from baseline of 14.5% at cycle 1, day 15 to 31.8% at cycle 4, day 1), and FGF23 levels (median change from baseline of 15.8% at cycle 1, day 15 to 30.6% to cycle 4, day 1) from baseline during treatment at all timepoints, which highlights the differential activity of lenvatinib versus sorafenib against the FGFR family. Additionally, ANG2 levels were also seen to decrease from baseline only in the lenvatinib group which suggested that TIE-2 signaling was influenced by lenvatinib and not sorafenib. No significant changes in FGF21 levels from baseline were observed in either treatment arm (data not shown).

Associations of changes in levels of pharmacodynamic biomarkers and tumor response

In the lenvatinib arm, pharmacodynamic changes in FGF19 and FGF23 were associated with tumor responses (complete response/partial response vs stable disease/progressive disease/other) (Fig. 3, Supplementary Figure S1). Patients who achieved an objective response in the lenvatinib arm had a greater median percentage increase from baseline in FGF19 (55% vs 18%; $P=0.014$) and FGF23 levels (48% vs 16%; $P=0.002$) compared with patients who did not achieve an objective response at cycle 4, day 1. A weaker trend was observed at cycle 3, day 1 (Supplementary Table S4). There were no significant observable trends in FGF19 and FGF23 between patients with objective response compared to patients with no objective response in the sorafenib arm, with the exception of FGF23 at cycle 2 day 1 (-21% vs -7.5%; $P=0.0128$) and at cycle 4, day 1 (-20% vs 9.3%);
At these time-points, there was a decrease in FGF23 levels in the responders compared with the nonresponders. Larger decreases in ANG2 at cycle 3, day 1 and cycle 4, day 1 were observed in responders compared with nonresponders in the lenvatinib arm. There were no observable trends in ANG2 levels between responders and nonresponders in the sorafenib arm.

**Association of baseline biomarker levels and overall survival**

The distribution of baseline levels of serum biomarkers appeared similar, with only minor variations observed between the 2 treatment arms (Supplementary Fig. S2). Higher baseline levels of VEGF, ANG2, and FGF21 were associated with shorter overall survival in both treatment arms by Cox regression analysis (Table 1). Upon evaluation of 3 groups (low [0–25%]; middle [≥25–75%]; or high [≥75–100%]) with differing serum biomarker levels, a significant difference in overall survival was observed between the lenvatinib arm and sorafenib arm in patients with high FGF21 (HR 0.53; 95% CI 0.33–0.85; Fig. 4A). Additionally, a difference in overall survival was also observed between treatment arms in patients with high ANG2 (HR 0.64; 95% CI [0.41–1.00]; Fig. 4A). A cutoff level of 75% was used for the dichotomized analysis for baseline ANG2 and FGF21 levels because differences in overall survival were observed between the lenvatinib arm and sorafenib arm in patients with high FGF21 (≥75–100%; Fig. 4A). Median overall survival for patients with high baseline ANG2 levels was numerically longer in the lenvatinib arm vs the sorafenib arm (median 9.4 months [95% CI 7.0–13.6] vs 7.7 months [95% CI 6.1–9.6], respectively; Supplementary Figure S3; Fig. 4A). Dichotomized analysis also showed different associations between overall survival and treatment for baseline ANG2 levels (p-interaction=0.075; Supplementary Table S5); ANG2 levels trend as predictive of response, although not reaching statistical significance.
For patients with high baseline FGF21 levels, median overall survival was longer in the lenvatinib arm versus the sorafenib arm (median 10.9 months [95% CI 8.2–13.1] vs 6.8 months [95% CI 4.6–10.3], respectively; Fig. 4B). Multivariate analysis of baseline serum biomarker levels with overall survival demonstrated that high FGF21 levels are correlated with shorter median overall survival regardless of treatment arm (HR 2.475 [95% CI 1.565–3.922] with lenvatinib, \( P=0.0001 \) vs HR 2.475 [95% CI 1.279–4.808] with sorafenib, \( P=0.0072 \); Supplementary Table S6).

When serum biomarker analyses were conducted in subgroups of HCC etiology (hepatitis B virus [HBV], hepatitis C virus [HCV], and alcohol), both ANG2 and FGF21 were significantly correlated with overall survival in patients with an etiology of HBV (Supplementary Table S7). By dichotomized analysis at the third quartile cutoff point, high baseline FGF21 levels were associated with shorter median overall survival regardless of HBV or HCV etiology in the lenvatinib arm (Supplementary Fig. S4). Additionally, high baseline ANG2 levels were associated with shorter median overall survival in the HBV etiology subgroup, but not in the HCV etiology subgroup. In the sorafenib arm, high baseline ANG2 levels were associated with shorter median overall survival regardless of HBV or HCV etiology; high baseline FGF21 levels were associated with shorter median overall survival in the HBV etiology subgroup, but not in the HCV etiology subgroup.

**Tumor tissue gene-expression analysis of the angiogenesis and growth factor pathway**

Clustering analysis was performed on baseline expression profiles of all available tumor samples from lenvatinib and sorafenib combined arms (Supplementary Fig. S5), and 3 subgroups with unique patterns of gene expression were identified; 1 had high expression levels of a family of FGF ligands, another had high expression levels of VEGF-A, and the
groups, 1 subgroup (high FGF ligand) showed longer overall survival compared with the other subgroups, suggesting association of the gene-expression signature with overall survival. From this analysis, patients in the lenvatinib arm and patients in the sorafenib arm were divided into 3 groups by clustering analysis using the Manhattan method (19,20) based on expression levels of the same set of 36 genes consisting of the angiogenic and growth factor pathway: Group 1 (VEGF-enriched, lenvatinib arm [41.2%] and sorafenib arm [45.8%]), Group 2 (FGF-enriched, lenvatinib arm [20.6%] and sorafenib arm [20.8%]), Group 3 (intermediate expression of VEGF and FGF, lenvatinib arm [38.2%] and sorafenib arm [33.3%]; Supplementary Fig. S6). Although the groups were small, the distribution of patients among all groups was similar in both treatment arms. The Kaplan-Meier plot identified which of these subgroups had the longest overall survival in either the lenvatinib or sorafenib arm. In the lenvatinib arm, patients in Group 2 (high expression of FGF ligands at baseline) appeared to experience longer overall survival, followed by patients in Group 1 (the VEGF-enriched group). In patients treated with sorafenib, patients in Group 3 with intermediate expression levels of VEGF and FGF ligands appeared to experience longer overall survival. No statistical analyses were performed for each of the 3 groups due to the small group sizes. In an effort to generate larger groups, these 3 groups were condensed into 2 groups (eg, Group 1 and Group 2 [VEGF- and FGF-enriched] vs Group 3 [intermediate expression levels of FGF and VEGF]) and log-rank tests of overall survival between the 2 groups were conducted for each treatment arm (Fig. 5). The group enriched for higher expression of VEGF and FGF genes was associated with improved overall survival in the lenvatinib arm compared with the intermediate group (23.2 months vs 8.4 months; HR 0.39; 95% CI 0.16–0.91; P=0.0253). On the contrary, the VEGF and FGF enriched groups showed shorter overall survival compared with the intermediate group in the sorafenib arm (13.2
Baseline serum biomarker levels were compared among groups (VEGF-enriched, FGF-enriched, and intermediate) identified by the clustering analysis of tumor gene expression profiles in the lenvatinib and sorafenib combined arms (Supplementary Table S8). VEGF levels were high in VEGF-enriched group, and FGF19 levels were high in FGF-enriched group. In addition, both ANG2 and FGF21 levels in the VEGF-enriched group were higher among all 3 groups.

In addition, associations between FGF ligand gene-expression levels with progression-free survival as assessed by mRECIST using independent imaging review are shown as forest plots in Supplementary Fig. S7. In the lenvatinib arm, HRs for all FGF ligands except FGF2 and FGF7 were <1, suggesting high expression levels of FGF ligand genes were associated with prolonged progression-free survival.

**Tumor tissue gene-expression analysis using 13 canonical cancer pathways**

Pathway enrichment analysis was conducted for the sets of genes where expression levels were significantly associated with overall survival by Cox regression analysis in the separate lenvatinib and the sorafenib treatment arms. Associations with overall survival were identified in transcriptional regulation, Wnt, and DNA repair pathways for the lenvatinib arm, and in the cancer driver gene panel for the sorafenib arm (Supplementary Table S9). For the suggested associations identified in the pathway enrichment analysis, clustering analysis was conducted. Patient subgroups identified by clustering analysis in the lenvatinib arm appeared to have different resulting overall survival benefits (Supplementary Fig. S8; red, blue, and green bars on the heat map) in the Wnt and DNA repair pathways.

**Discussion**

The REFLECT study, which was the first positive phase 3 study in front-line uHCC since...
sorafenib was approved, demonstrated lenvatinib was noninferior to sorafenib in terms of overall survival (HR 0.92; 95% CI 0.79–1.06). This subsequent exploratory biomarker analysis suggests that the clinical activity of lenvatinib involves a distinct mechanism of action compared with sorafenib in patients with advanced uHCC, with unique inhibition of FGFR, and more potent inhibition of VEGFR with lenvatinib.

The identification of biomarkers with predictive value is crucial in guiding appropriate therapy selection in patients with uHCC. Biomarker studies are generally limited due to the lack of available tumor tissue samples since a tissue biopsy is typically not required to diagnose HCC (21). In our analysis, serum biomarker assays for VEGF, ANG2, FGF19, FGF21, and FGF23 were conducted both at baseline and post-treatment. No consistent changes in FGF21 levels from baseline were found in either treatment arm. The observed differences in serum biomarker changes between the lenvatinib and sorafenib arms support the distinct target kinase inhibitory profiles of each agent. Although both lenvatinib and sorafenib treatments resulted in increases in VEGF levels, the magnitude of change was greater with lenvatinib, which has been more potent against VEGFR in preclinical studies (1). Additionally, the lenvatinib arm showed decreases in ANG2 levels, as well as increases in levels of FGF19 and FGF23, which is supportive of lenvatinib inhibition of FGFR4 and FGFR1, respectively (22-24). FGF19 and FGF23 levels increased more in patients who achieved an objective response compared with those who did not achieve an objective response in the lenvatinib arm. A subsequent analysis, evaluating the relationship between overall survival and objective response in the REFLECT study, demonstrated that objective response was an independent predictor of overall survival in patients with HCC regardless of the treatment arm (25).

Results of this analysis were consistent with the antitumor activity of lenvatinib observed in HCC xenograft models (12) consisting of aggressive VEGF overexpressing tumors. In
addition, lenvatinib inhibited tumor FGF-signaling pathways in HCC xenograft models and suppressed proliferation of HCC cell lines with an activated FGF-signaling pathway (10). Also, lenvatinib induced cell death of HCC cell lines overexpressing FGF19 under a nutrient-depleted culture condition used to mimic the tumor microenvironment after angiogenesis inhibition (11).

In this analysis, results showed that higher levels of baseline VEGF, ANG2, and FGF21 may be prognostic for shorter overall survival, in patients with uHCC, regardless of treatment. Results from dichotomized analyses suggest that high baseline ANG2 and FGF21 levels were associated with shorter overall survival in both HBV and HCV etiologies for HCC in the sorafenib arm except for baseline FGF21 levels in the HCV subgroup. In the lenvatinib arm, high baseline ANG2 and FGF21 levels were associated with shorter overall survival in both HCC etiologies except for baseline ANG2 levels in the HCV subgroup. Additionally, high baseline ANG2 and FGF21 levels were associated with shorter overall survival in the HBV etiology subgroups across both arms. Clinical studies have demonstrated that ANG2 is an independent biomarker of poor prognosis in patients with HCC, with high ANG2 levels being associated with shorter overall survival (26,27). High levels of VEGF at baseline were also associated with shorter overall survival (26).

Median overall survival was numerically longer in patients who had high baseline levels of ANG2 with lenvatinib compared with sorafenib, which may be related to the decrease in ANG2 levels seen only with lenvatinib. In addition, higher baseline levels of FGF21 may be predictive of longer overall survival with lenvatinib compared with sorafenib. This observation was not seen with FGF19 or FGF23. While this may be due to a small sample size, it is important to note that FGF19, FGF21, and FGF23 each have distinct roles in biology and more specifically, the pathogenesis of HCC (28). FGF19 has been identified as a cancer driver in a subgroup of patients with uHCC (16) and FGF23 has been described as a
association was reported between baseline serum FGF19 levels and treatment response to lenvatinib in a biomarker analysis of uHCC patients treated with lenvatinib or sorafenib (30). However, the sample size (n=27) included in the study is small, emphasizing the importance for further biomarker analysis. In another HCC study involving lenvatinib treatment (31), median serum FGF19 levels at baseline were similar between patients with objective response (n=35) and patients with no objective response (n=39). However, increases in serum FGF19 levels during treatment were associated with a response in patients receiving lenvatinib. These results are consistent with our findings that pharmacodynamic increases in FGF19 levels were associated with tumor responses in the lenvatinib arm. The details of FGF21’s interactions in HCC are largely unknown (28). FGFR inhibition by lenvatinib may contribute to improved overall survival in patients with high baseline levels of FGF21 compared to sorafenib. Multivariate analysis of baseline FGF21 levels with overall survival suggests that FGF21 may be an independent prognostic factor for overall survival. These results are hypothesis generating and warrant further investigation to evaluate the role of FGF21 in HCC.

Analysis of gene-expression patterns with an emphasis on angiogenesis and growth factor signaling pathway genes identified 3 subgroups in HCC. Interestingly, the longest median overall survival amongst the 3 subgroups in the lenvatinib arm was observed in patients with high expression levels of FGF ligands, followed by those with high expression levels of VEGF. Conversely, the longest median overall survival in the sorafenib arm was observed in patients with intermediate expression levels of VEGF and FGF ligands. Although these results should be interpreted with caution given the small number of evaluable samples, they support the distinct mechanism of action of lenvatinib from sorafenib and are consistent with the changes in levels of pharmacodynamic biomarkers (VEGF, FGF ligands, and ANG2) in
These results further support that targeting the FGF and VEGF signaling pathways is important in the treatment of HCC.

This analysis was limited by the small sample of patients available for the biomarker analyses. Moreover, more patients in the lenvatinib arm than in the sorafenib arm were included in the biomarker analysis set. The number of patients was particularly small in the gene expression analysis set, which was further complicated by any procedures and medications that patients may have received between therapy initiation and sample collection. These procedures and medications were not controlled for and could have impacted results, as intra-arterial therapies can alter the microenvironment and biomarker expression patterns. The efficacy of lenvatinib may involve additional signaling pathways, and it is important to continue to investigate its mechanism of action. Of note, lenvatinib has demonstrated immunomodulatory activity and potentiation of the antitumor activity of programmed death receptor-1 (PD-1) inhibitors in syngeneic HCC mouse models, which supports combination treatment with pembrolizumab, a PD-1 inhibitor (32). This observation is supported by clinical data demonstrating a significant response rate (36% by RECIST 1.1) with the combination of lenvatinib and pembrolizumab in advanced HCC (33). Further hypotheses could be raised from the differences in tumor gene-expression patterns of genes involved in transcriptional regulation, Wnt, and DNA repair pathways in the lenvatinib arm, and the cancer driver gene panel in the sorafenib arm; these factors may impact overall survival benefits for each drug treatment and may incite future study of novel combinations.

In conclusion, serum biomarker and gene-expression levels appeared to correlate with survival outcomes among patients with evaluable samples from REFLECT. This analysis was limited by the small number of patients with evaluable samples, and the variation in baseline characteristics between the gene-expression analysis set and the ITT population. However, the differences in baseline characteristics between these groups were understandable and
limitations, multivariate analysis of important clinical prognostic factors in HCC supports our results. Of note, data were analyzed by mRECIST per a blinded independent review. In patients with unresectable HCC who had not received prior systemic therapy for advanced disease, lenvatinib demonstrated clinical activity based on a mechanism of action that is distinct from sorafenib. Specifically, lenvatinib demonstrated clinical evidence of FGFR inhibition and stronger inhibition of angiogenesis pathways (VEGFR and TIE-2/ANG2). ANG2 and TIE-2 are selectively expressed on endothelial cells and are increased with enhanced tumor angiogenesis. Lenvatinib could lead to decreases in both ANG2 and TIE-2, without direct TIE-2 inhibition, based on its potent angiogenesis inhibition and resultant decrease in endothelial cells.

These results suggest that the inhibitory activity of lenvatinib against FGFR may contribute to the increased tumor response, and FGF21 may be a candidate biomarker predictive of longer overall survival with lenvatinib. Interestingly, it appears that lenvatinib may perform better in the poor prognosis subgroups independent of the specific pathway, due to its increased activity overall and similarity to the overall study population results. These results are hypothesis-generating and warrant further study. The ongoing Phase 3 LEAP-002 study (NCT03713593), evaluating lenvatinib versus lenvatinib and pembrolizumab in advanced HCC will provide further material for investigation.
Acknowledgements

Medical writing support was provided by Oxford PharmaGenesis Inc., Newtown, PA, USA, and this support was funded by Eisai Inc, Woodcliff Lake, NJ, USA, and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

References


Table 1. Association of baseline biomarker levels with overall survival.\(^a\)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lenvatinib</th>
<th>Sorafenib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>VEGF</td>
<td>259</td>
<td>1.181 (1.055–1.321)</td>
</tr>
<tr>
<td>ANG2</td>
<td>266</td>
<td>1.436 (1.283–1.607)</td>
</tr>
<tr>
<td>FGF19</td>
<td>260</td>
<td>1.103 (0.946–1.286)</td>
</tr>
<tr>
<td>FGF21</td>
<td>261</td>
<td>1.275 (1.130–1.438)</td>
</tr>
<tr>
<td>FGF23</td>
<td>265</td>
<td>0.861 (0.738–1.005)</td>
</tr>
</tbody>
</table>

\(^a\)Cox proportional hazard model including standardized baseline value was used for each treatment group. Hazard ratios are based on comparison between high and low baseline biomarker levels and are expressed as an increase in 1 standard deviation in baseline values.

ANG2, angiopoietin-2; CI, confidence interval; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor.
A total of 41.7% (n=398) of patients in the overall study (n=954) had tissue confirmation of HCC histology. In the overall study, 95.0% (n=378) of patients who had tissue confirmation of HCC histology, consented for tissue analyses.

Fewer blood serum samples were obtained from the sorafenib arm until the clinical study protocol was amended to clarify that samples were to be collected uniformly across both arms.

Baseline samples were unavailable for 12 patients in the lenvatinib arm, who were therefore excluded from the correlation analyses.

Only tissue available (n=114) at the cutoff date for sample collection (July 15, 2016) were used for biomarker analyses.

Sample sizes were decreased due to the insufficient amounts of DNA/RNA extracted from tumor tissues.

HCC, hepatocellular carcinoma; ITT, intent-to-treat.

Figure 2. Percentage changes in levels of serum biomarkers from baseline.

* \( p < 0.05 \) vs baseline; \( \dagger p < 0.01 \) vs baseline; \( \ddagger p < 0.0001 \) vs baseline; \( \S p < 0.05 \) between the LEN and SOR arms.

ANG-2, angiopoietin-2; C, cycle; D, day; FGF, fibroblast growth factor; LEN, lenvatinib; SOR, sorafenib; VEGF, vascular endothelial growth factor.

Figure 3. Percentage change in pharmacodynamics biomarker levels and association with objective response.*

Median percentage changes of biomarker levels at cycle 4 day 1 from baseline are shown at the top of each box plot.

Objective response was assessed by mRECIST using independent imaging review.

*Figures exclude outliers outside the y-axis range: VEGF (lenvatinib complete response/partial response \( n = 4; 358.4, 366.1, 393.2, 642.5 \); lenvatinib noncomplete response/partial response \( n = 7; 391.9, 407.7, 467.7, 469.9, 482.7, 526.3, 692.5 \)); sorafenib complete response/partial response \( n = 3; 436.0, 475.7, 803.1 \); sorafenib noncomplete response/partial response \( n = 4; 399.9, 461.0, 505.1, 524.3 \)); angiopoietin-2 (lenvatinib noncomplete response/partial response \( n = 2; 132.9, 193.5 \); sorafenib noncomplete response/partial response \( n = 1; 184.4 \)); FGF19 (lenvatinib complete response/partial response \( n = 6; 614.3, 640.1, 831.4, 1097.6, 1233.3, 1462.5 \)); lenvatinib noncomplete response/partial response \( n = 5; 532.8, 533.2, 555.7, 606.7, 843.4 \)); sorafenib noncomplete response/partial response \( n = 2; 773.4, 776.6 \); FGF23 (lenvatinib CR/PR \( n = 1; 258.1 \); lenvatinib non-CR/PR \( n = 1; 207.7 \)).

All other groups had 0 outliers.

ANG-2, angiopoietin-2; C, cycle; D, day; FGF, fibroblast growth factor; LEN, lenvatinib; SOR, sorafenib; VEGF, vascular endothelial growth factor.
Figure 4. Associations between baseline serum biomarkers and overall survival. (A)

Associations between treatment arms and overall survival by baseline serum biomarker-level group (B) Kaplan–Meier plot showing associations by high or low baseline serum biomarker levels of FGF21 (cut-off level of 0.75 [= 688.0 ng/L]) and overall survival.

ANG-2, angiopoietin-2; C, cycle; CI, confidence interval; D, day; FGF, fibroblast growth factor; HR, hazard ratio; LEN, lenvatinib; OS, overall survival; SOR, sorafenib; VEGF, vascular endothelial growth factor.

Figure 5. Association between molecular subgroups (FGF- and VEGF-enriched groups vs FGF and VEGF-intermediate groups) identified using the angiogenic and growth factor gene-expression profile and overall survival in the lenvatinib (A) and sorafenib (B) arms.

ANG-2, angiopoietin-2; C, cycle; CI, confidence interval; D, day; FGF, fibroblast growth factor; HR, hazard ratio; LEN, lenvatinib; OS, overall survival; SOR, sorafenib; VEGF, vascular endothelial growth factor.
Total patients on study
N = 954

ITT population

Consent for blood (n = 540)
Lenvatinib (n = 281); sorafenib (n = 259)

Consent for blood samples and tumor tissue (n = 119)
Lenvatinib (n = 68; sorafenib: n=51)

Serum sample available (n = 407)
Lenvatinib (n = 279); sorafenib (n = 128)

Tumor tissue available (n = 114)
Lenvatinib (n = 65); sorafenib (n = 49)

Histology review passed (n = 80)
Lenvatinib (n = 47); sorafenib (n = 33)

Biomarker analysis set

Serum assay completed (n = 407)
Lenvatinib: (n=279); sorafenib (n = 128)
Serum analysis set

Gene expression assay completed (n = 58)
Lenvatinib (n = 34); sorafenib (n = 24)
Gene expression analysis set

Analyzed (n = 395)
Lenvatinib (n = 267); sorafenib (n = 128)

Analyzed (n = 58)
Lenvatinib (n = 34); sorafenib (n = 24)
Figure 2

### VEGF

<table>
<thead>
<tr>
<th>Group</th>
<th>C1D1</th>
<th>C1D15</th>
<th>C2D1</th>
<th>C3D1</th>
<th>C4D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td>57.8</td>
<td>40.6</td>
<td>39.7</td>
<td>34.2</td>
<td></td>
</tr>
<tr>
<td>SOR</td>
<td></td>
<td>35.7</td>
<td>18.7</td>
<td>32.8</td>
<td>18.1</td>
</tr>
</tbody>
</table>

n = 259 242 231 208 191

### ANG-2

<table>
<thead>
<tr>
<th>Group</th>
<th>C1D1</th>
<th>C1D15</th>
<th>C2D1</th>
<th>C3D1</th>
<th>C4D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td></td>
<td>4.9</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOR</td>
<td>-29.4</td>
<td>-30.5</td>
<td>-34.4</td>
<td>-35.4</td>
<td></td>
</tr>
</tbody>
</table>

n = 266 253 240 218 202

### FGF19

<table>
<thead>
<tr>
<th>Group</th>
<th>C1D1</th>
<th>C1D15</th>
<th>C2D1</th>
<th>C3D1</th>
<th>C4D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td>14.5</td>
<td>25.6</td>
<td>40.8</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>SOR</td>
<td>-13.6</td>
<td>-16.3</td>
<td>-0.6</td>
<td>-3.9</td>
<td></td>
</tr>
</tbody>
</table>

n = 260 242 233 210 195

### FGF23

<table>
<thead>
<tr>
<th>Group</th>
<th>C1D1</th>
<th>C1D15</th>
<th>C2D1</th>
<th>C3D1</th>
<th>C4D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td>15.8</td>
<td>20.5</td>
<td>24.1</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>SOR</td>
<td>-13.4</td>
<td>-11.9</td>
<td>-9.1</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

n = 126 122 121 104 83
**Figure 3**

### VEGF

<table>
<thead>
<tr>
<th></th>
<th>CR/PR</th>
<th>non-CR/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN P = 0.460</td>
<td>33%</td>
<td>36%</td>
</tr>
<tr>
<td>SOR P = 0.194</td>
<td>44%</td>
<td>13%</td>
</tr>
</tbody>
</table>

### ANG-2

<table>
<thead>
<tr>
<th></th>
<th>CR/PR</th>
<th>non-CR/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN P = 0.022</td>
<td>-38%</td>
<td>-32%</td>
</tr>
<tr>
<td>SOR P = 0.712</td>
<td>2.2%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

### FGF19

<table>
<thead>
<tr>
<th></th>
<th>CR/PR</th>
<th>non-CR/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN P = 0.014</td>
<td>55%</td>
<td>18%</td>
</tr>
<tr>
<td>SOR P = 0.545</td>
<td>0.3%</td>
<td>-5%</td>
</tr>
</tbody>
</table>

### FGF23

<table>
<thead>
<tr>
<th></th>
<th>CR/PR</th>
<th>non-CR/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN P = 0.002</td>
<td>48%</td>
<td>16%</td>
</tr>
<tr>
<td>SOR P = 0.027</td>
<td>-20%</td>
<td>9%</td>
</tr>
<tr>
<td>Biomarker / Level</td>
<td>Events / Patients, n (LEN / SOR)</td>
<td>HR (95% CI) LEN vs SOR</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>351 / 478 350 / 476</td>
<td>0.92 (0.79–1.06)</td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>34 / 65 22 / 31</td>
<td>0.62 (0.36–1.07)</td>
</tr>
<tr>
<td>Middle</td>
<td>95 / 130 42 / 64</td>
<td>1.04 (0.72–1.51)</td>
</tr>
<tr>
<td>High</td>
<td>54 / 64 28 / 33</td>
<td>0.86 (0.54–1.36)</td>
</tr>
<tr>
<td><strong>ANG-2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>31 / 65 15 / 32</td>
<td>0.84 (0.45–1.57)</td>
</tr>
<tr>
<td>Middle</td>
<td>106 / 139 43 / 58</td>
<td>1.10 (0.77–1.57)</td>
</tr>
<tr>
<td>High</td>
<td>52 / 62 33 / 37</td>
<td>0.64 (0.41–1.00)</td>
</tr>
<tr>
<td><strong>FGF19</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>45 / 66 16 / 30</td>
<td>1.47 (0.83–2.60)</td>
</tr>
<tr>
<td>Middle</td>
<td>88 / 127 56 / 68</td>
<td>0.66 (0.47–0.93)</td>
</tr>
<tr>
<td>High</td>
<td>50 / 67 20 / 30</td>
<td>1.00 (0.59–1.69)</td>
</tr>
<tr>
<td><strong>FGF21</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>38 / 64 20 / 32</td>
<td>0.83 (0.47–1.45)</td>
</tr>
<tr>
<td>Middle</td>
<td>91 / 127 44 / 67</td>
<td>0.97 (0.67–1.39)</td>
</tr>
<tr>
<td>High</td>
<td>55 / 70 26 / 27</td>
<td>0.53 (0.33–0.85)</td>
</tr>
<tr>
<td><strong>FGF23</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>47 / 64 26 / 33</td>
<td>0.89 (0.55–1.45)</td>
</tr>
<tr>
<td>Middle</td>
<td>94 / 135 44 / 61</td>
<td>0.89 (0.62–1.28)</td>
</tr>
<tr>
<td>High</td>
<td>47 / 66 20 / 32</td>
<td>0.94 (0.55–1.60)</td>
</tr>
</tbody>
</table>

**HR and 95% CI**

Low (0–25%), middle (≥ 25–75%), high (≥ 75–100%)

**B**

**Median OS, months (95% CI)**

- LEN / FGF21-High: 10.9 (8.2, 13.1)
- LEN / FGF21-Low: 18.0 (14.1, 20.9)
- SOR / FGF21-High: 6.8 (4.6, 10.3)
- SOR / FGF21-Low: 17.8 (13.7, 22.9)

**Number of patients at risk:**

- LEN / FGF21-High: 70 61 51 39 30 19 16 9 6 4 3 1 0 0 0
- LEN / FGF21-Low: 191 182 155 130 117 100 89 70 52 40 26 15 7 2 0
- SOR / FGF21-High: 27 24 17 10 4 3 2 1 1 1 1 0 0 0 0
- SOR / FGF21-Low: 99 95 85 73 61 55 42 31 18 6 4 3 1 0 0
Figure 5

A

Median OS, months (95% CI)

- LEN / Group 1+2: 23.2 (8.6, NE)
- LEN / Group 3: 8.4 (5.5, 17.6)

HR (95% CI): 0.39 (0.16, 0.91)

Log-rank Test: $P$-value: 0.0253

Number of patients at risk:

<table>
<thead>
<tr>
<th>LEN / Group 1+2</th>
<th>21</th>
<th>20</th>
<th>18</th>
<th>15</th>
<th>13</th>
<th>12</th>
<th>11</th>
<th>9</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN / Group 3</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

B

Median OS, months (95% CI)

- SOR / Group 1+2: 13.2 (6.1, 22.0)
- SOR / Group 3: NE (19.4, NE)

HR (95% CI): 14.55 (1.87, 113.14)

Log-rank Test: $P$-value: 0.0009

Number of patients at risk:

<table>
<thead>
<tr>
<th>SOR / Group 1+2</th>
<th>16</th>
<th>15</th>
<th>13</th>
<th>12</th>
<th>10</th>
<th>8</th>
<th>7</th>
<th>4</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOR / Group 3</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
Pharmacodynamic Biomarkers Predictive of Survival Benefit with Lenvatinib in Unresectable Hepatocellular Carcinoma: From the Phase 3 REFLECT Study

Richard S. Finn, Masatoshi Kudo, Ann-Lii Cheng, et al.

Clin Cancer Res  Published OnlineFirst June 9, 2021.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-20-4219

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2021/06/05/1078-0432.CCR-20-4219.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/early/2021/06/08/1078-0432.CCR-20-4219. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.