Synopsis

Title	A phase-II open-label study of lenvatinib and gefitinib in patients with advanced stage hepatocellular carcinoma with positive EGFR immunohistochemistry
Short title	LEGEND-HCC
Trial type	Single arm, multicenter, open label, phase II trial
Investigational medicinal products (IMPs)	Lenvatinib Gefitinib
Objectives / endpoints	The aim of this phase II study is to determine evaluate the efficacy and safety of lenvatinib in combination with gefitinib in patients with EGFR-positive hepatocellular carcinoma (HCC). **Primary Objective** Efficacy of lenvatinib in combination with gefitinib Corresponding endpoint • Objective response rate (ORR) according to mRECIST **Secondary Objectives** a) To further characterize the efficacy of the lenvatinib/gefitinib combination therapy Corresponding endpoints • Progression-free survival (PFS) • Overall survival (OS) b) To evaluate the safety and tolerability of lenvatinib/gefitinib combination therapy Corresponding endpoint • Assessment of safety of the treatment as determined by the incidence, nature, causality, seriousness, and severity of adverse events using NCI CTCAE 5.0 c) To assess quality of life (QoL) data from patients **Exploratory biomarker objective** Determination of molecular biomarkers and their correlation with ORR as well as analysis of genetic alteration spectra in HCC patients with overexpressed EGFR.
Trial design	The trial is designed as single arm, multicenter, open-label, phase II study, which aims to show therapeutic efficacy of lenvatinib with gefitinib in patients with advanced stage hepatocellular carcinoma with positive EGFR immunohistochemistry. It is planned to enroll 30 patients which will be allocated to the following trial treatment:

	Intervention: • Gefitinib – 250 mg per oral (p.o.), once daily (QD) PLUS • Lenvatinib – 8 mg (body weight < 60 kg)/ 12 mg (BW ≥ 60 kg) p.o., QD **Duration of intervention per patient:** All patients will receive lenvatinib in combination with gefitinib for up to 12 months or until intolerable toxicity, disease progression or patients' request, whatever occurs first. **Follow-up per patient:** All patients will be followed up for 12 months after last patient last treatment or until death, withdrawal of consent, or loss to follow-up (whichever occurs first). Potential trial participants will be assessed for eligibility during a 28-day screening period. First treatment should be performed as soon as possible but not later than 14 working days after allocation to the trial. Tumor biopsies (FFPE samples) will be collected for EGFR overexpression analysis via immunohistochemistry (IHC) by the local pathologies prior to enrollment of the participant. Since EGFR overexpression analysis is not a clinical standard, the IHC analysis conditions and cut-off ranges, which will be used at the local pathologies, will be established and local pathologies will be trained for the EGFR analysis prior the trial start. Tumor baseline assessment including imaging will be conducted as part of the screening procedure and tumor response assessment according to mRECIST will be performed every 8 weeks (Q8W) ± 7 days during the treatment and Q12W ± 7 days in the follow-up phase until disease progression, death, loss to follow-up or end of trial, whichever occurs first.
Rationale	Liver cancer is the sixth most common cancer worldwide, with 841,080 new liver cancer cases in 2018, and the fourth leading cause of cancer-related death globally [1]. In Germany, around 9,000 people are diagnosed with liver cancer every year, while the number of deaths is only slightly lower at around 7,500 [2]. The treatment of HCC is assigned to the tumor stages and the expected benefits of major inventions, following the Barcelona Clinic Liver Cancer (BCLC) staging system. Preferred treatment options for patients diagnosed with early-stage HCC include resection or transplantation or radiofrequency ablation. Patients with intermediate stages are first candidates for transarterial chemoembolization (TACE) and those with more advanced disease will first receive systemic therapies [3]. The REFLECT trial showed that the treatment with lenvatinib, a multi-targeted receptor tyrosine kinase inhibitor, was non-inferior to sorafenib in terms of OS (lenvatinib vs. sorafenib: median OS 13.6 vs. 12.3 months (HR 0.92, 95% CI: 0.79, 1.06). Furthermore, lenvatinib significantly improves progression-free survival (7.4 vs. 3.7 months HR 0.66, 95% CI 0.57–0.77; P < 0.001) and overall response rate (24.1 vs. 9.2%; OR 3.13, 95% CI 2.15–4.56; P < 0.0001) compared to sorafenib according to mRECIST [4]. In addition to its ability to inhibit the vascular endothelial growth factor receptor (VEGFR) pathway, lenvatinib is a potent inhibitor of the fibroblast growth factor receptor (FGFR) pathway [5]. In this context a retrospective analysis with 40 patients suggested that tumor FGFR4/FGF19 levels are an independent predictor of response to lenvatinib [6].

	A recent study, aiming to identify combination therapies to improve the clinical benefits of lenvatinib-based treatment for HCC, revealed that inhibition of FGFR by lenvatinib leads to a feedback activation of the EGFR–PAK2–ERK5 cascade that limits sensitivity of liver cancer cells to lenvatinib [7]. Of note, the combination of the epidermal growth factor receptor (EGFR) inhibitor gefitinib and lenvatinib efficiently prevented feedback activation and displayed potent anti-proliferative effects *in vitro* in liver cancer cell lines that express EGFR and *in vivo* in xenografted liver cancer cell lines, immunocompetent mouse models and patient-derived HCC tumors in mice. In contrast to cell lines expressing high levels of EGFR, those expressing low levels were resistant to lenvatinib treatment and the co-treatment with gefitinib [7]. Tissue analysis revealed that ~60% of HCCs express high levels of EGFR, which might be a negative prognostic marker in this disease [7,8]. In addition, EGFR was identified as determinant for sorafenib resistance in HCC *in vitro* before [9,10]. Nevertheless, in contrast to lenvatinib, EGFR inhibitors such as gefitinib or erlotinib did not enhance the activity of sorafenib [7,11] suggesting that lenvatinib synergizes with the EGFR inhibitor mainly by abrogating FGFR signaling. To confirm the promising preclinical data of the lenvatinib-gefitinib co-treatment, a clinical trial to evaluate the safety and anti-tumor activity of lenvatinib plus gefitinib in patients with unresectable HCC, whose tumors had progressed on lenvatinib treatment was conducted in China. Preliminary results of 12 patients of this trial showed 33% partial response, 33% stable disease and 33% disease progression after 4-8 weeks of treatment, indicating the combination as promising strategy for patients with advanced HCC expressing high levels of EGFR in their tumors [7]. Gefitinib is approved as targeted-therapy in non-small cell lung cancer (NSCLC) with a EGFR positive mutations status for several years [12]. Whereat it has not shown promising anti-tumor activity in advanced HCC as monotherapy [13]. Given the recent *in vitro*/ *in vivo* data and preliminary clinical data [7], we hypothesize that addition of gefitinib to lenvatinib will improve the clinical benefit of lenvatinib therapy in EGFR high expressing HCC in second and further line treatment.
Inclusion criteria	Patients must meet the following criteria to be eligible for the trial: 1. Histologically confirmed diagnosis of HCC 2. Confirmed EGFR overexpression via IHC 3. Have a tumor, not eligible for resection or local ablation 4. Have experienced disease progression under previous systemic therapy 5. Have a Child-Pugh Classification score ≤ 6 for assessed liver function within 7 days before allocation 6. Have at least one measurable site of disease based on RECIST v1.1 with spiral CT scan or MRI. Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions 7. Participants* who are at least 18 years of age on the day of signing informed consent 8. Have provided archival tumor tissue sample or newly obtained biopsy of a tumor lesion not previously irradiated for mandatory pre-treatment evaluation (baseline) 9. ECOG ≤ 1 10. Have a life expectancy of ≥ 12 weeks 11. Have adequate organ function

	a. Hemoglobin ≥ 10.0 g/dL b. Absolute neutrophil count ≥ 1,500/µL, platelets ≥ 50,000/µL c. Total bilirubin ≤ 3.0 x upper normal limit (ULN) d. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) ≤ 5x ULN e. International normalized ratio (INR) ≤ 1.25 OR prothrombin time (PT) ≤ 1.5x ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants f. Albumin ≥ 3 g/dL g. Creatinine ≤ 1.5x ULN or calculated creatinine clearance (CrCl) (according to Cockcroft Gault or institutional standard) ≥ 30 mL/min for participant with creatinine > 1.5 x institutional ULN (glomerular filtration rate [GFR] can also be used in place of creatinine or CrCL) h. Urine dipstick for proteinuria ≤ 1+ (within 7 days prior IMP initiation), for participants with proteinuria > 1+: 24-hour urine collection should be performed and must demonstrate < 1 g/ 24 h 12. If participations have concurrent hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, meet the following criteria: • HBV DNA ≤500 IU/mL obtained within 28 days prior to initiation of trial treatment, AND • Anti-HBV treatment (per local standard of care, e.g., entecavir) for a minimum of 14 days prior to the entry of and willingness to continue treatment for the length of the trial • Patients positive for hepatitis C virus (HCV) antibody are eligible, also if PCR testing is positive for HCV ribonucleic acid (RNA) • However, anti-viral therapy against HCV is only allowed prior to but not during the trial 13. Women of childbearing potential as well as male participants with female partners of childbearing potential agree to use contraceptive during the treatment period and for at least 30 days after the last dose of lenvatinib. 14. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial. * There are no data that indicate special gender distribution. Therefore, patients will be enrolled in the trial gender-independently.
Exclusion criteria	Patients who meet at least one of the following criteria are not eligible for trial participation: 1. Known fibrolamellar HCC, sarcomatoid HCC, or mixed cholangiocarcinoma/HCC. 2. Are currently participating in or has participated in a trial with an investigational agent or has used an investigational device within 4 weeks or for a period of at least 5 half-lives of the respective drug/IMP (whichever is longer) prior to or during screening for this trial. Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks or at least 5 half-lives of the respective drug/IMP (whichever is longer) after the last dose of the previous investigational agent. 3. Have a diagnosis of immunodeficiency or are receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or

	any other form of immunosuppressive therapy within 7 days prior to the first dose of IMP. 4. Have a known additional malignancy that is progressing or has required active treatment within the past 2 years. Note: Participants with basal cell or squamous cell carcinoma of the skin or carcinoma in situ (e.g., breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded. 5. Have known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate if they are radiologically stable, i.e., without evidence of progression re-imaging for at least 4 weeks (note that the re-imaging should be performed during screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of IMP. 6. Have severe hypersensitivity (≥ grade 3) to lenvatinib, gefitinib and/or any of its excipients. 7. Have a history of congestive heart failure NYHA > Class II, unstable angina, myocardial infarction or stroke within 6 months of the first dose of IMP, or cardiac arrhythmia requiring medical treatment at screening. 8. Have bleeding or thrombotic disorders or subjects at risk for severe hemorrhage. Note: The degree of tumor invasion/infiltration of major blood vessels (e.g. carotid artery) should be considered because of the potential risk of severe hemorrhage associated with tumor shrinkage/necrosis following lenvatinib therapy. 9. Have uncontrolled blood pressure (systolic > 150 mmHG or diastolic >90 mmHg) despite of an optimized regimen of antihypertensive medication. Participants with initial blood pressure elevations are eligible if initiation or adjustment of antihypertensive medication lowers pressure to meet entry criteria 10. Proteinuria (dipstick ≥ 2+ or ≥ 1 g/24 h) 11. Have active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment and is allowed. 12. Have a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease. 13. Have received a live vaccine or live-attenuated vaccine within 30 days prior to the first dose of IMP. Administration of killed vaccines is allowed. 14. Have an active infection requiring systemic therapy (exception: HBV infection – see inclusion criteria). 15. Have a history of human immunodeficiency virus (HIV) (mandatory testing for HIV during screening is required). 16. Have a history or current evidence of any condition, therapy, or laboratory abnormality that, in the opinion of the investigator, may confound the results

	of the trial, may interfere with the participation throughout the duration of the trial, or is not in the best interest of the participant 17. Have known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. 18. Are pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit and continuing until 30 days after the last dose of IMP. 19. Are unable to swallow orally administered medication or have gastrointestinal disorders likely to interfere with absorption of the IMP. 20. Legal incapacity or limited legal capacity 21. Have received prior therapy with lenvatinib or gefitinib.
Sample size	Screening: 50 patients Enrollment: 30 patients The efficacy assumption is based on the results of the phase III REFLECT trial showing an ORR of 24% and 9% according to mRECIST for patients treated with lenvatinib and sorafenib, respectively, as first line standard of care [4]. We assume an increase of the ORR to 30% for the combination of lenvatinib and gefitinib. Hence, with an ORR ≥ 30% the combination of lenvatinib and gefitinib is considered as desirable candidate for further development. In contrast, with an ORR ≤ 10% the combination would be insufficient for further development. Formally, the hypothesis testing can be written as: H0: P≤0.10 versus H1: P≥0.30. Based on this, the Fleming's single stage testing procedure for the sample size calculation, with the following parameters: P_0=0.10 and P_1=0.30, a one-sided type I error of 0.05 and a power of 90%, showed that a sample size of 30 patients is required. If the number of responses is 6 or more, the null hypothesis that P ≤ 0.10 is rejected with an actual type I error rate of 0.05 (actual type I error of 0.07). If the number of responses is 5 or less, the alternative hypothesis that P ≥ 0.30 is rejected with a target error rate of 0.1 and an actual error rate of 0.08.
Efficacy evaluations/ criteria	On treatment, imaging assessments will be performed Q8W ± 7 days calculated from the date of treatment initiation independent of further treatment time points according to standard of care. Modified RECIST will be used by the site for treatment decisions until first radiologic evidence of disease progression (PD). Subjects who discontinue trial intervention for reasons other than PD will have post-treatment follow-up for disease status performed Q12W ± 7 days until progression, initiation of another anti-cancer treatment, withdrawing consent, lost to follow-up, death or end of the trial, whichever occurs first. Once disease progression is noted, no further tumor assessments are needed per protocol. All subjects will be followed for OS for maximum of 12 months after last patient last treatment, until death, withdrawing consent or lost to follow-up, whichever occurs first.
Translation Research	Tumor biopsy material (FFPE samples) will be collected during screening for EGFR overexpression analysis. Remaining material will be used for the accompanying translational research program. In addition, blood samples (whole blood, plasma) will be collected at the following timepoints: before treatment, 12 weeks after treatment initiation and at time of disease progression or at EOT if EOT due to other

	reasons than disease progression . Tissue samples may be used for the following analyses: • FGFR4/FGF19 expression analysis Blood samples may be used for the following analyses: • ctDNA analysis used to determine the frequency of genetic mutations and to determine mutational load* • Analyses for potential FGFR4/FGF19 pathway biomarkers * Note that NGS analysis is not within the scope of this trial The specified translational research may be adapted taking into account new research data.
Safety evaluation	Since both drugs have established and approved doses, severe overlapping toxicities are not expected and therefore a safety run in phase is not conducted. Additionally, an interim analysis of a currently running trial analyzing the lenvatinib/gefitinib combination in HCC patients showed no severe adverse events until the date of the analysis [7]. However, since there is only a limited amount of safety data available for the combination, an interim safety evaluation will be performed after the 5th patient has completed the first month of therapy. All available safety data will be evaluated by the Medical Scientific Lead and an independent data safety monitoring board (DSMB), respectively. During the interim safety analysis enrollment and therapies will be continued. Safety assessments will include physical examinations, performance status (ECOG), clinical laboratory profile and continuous assessments of adverse events. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI CTCAE v5.0. All AEs will be monitored starting from first administration of trial medication until 30 days after the last dose of the IMP treatment or until initiation of another anti-cancer therapy, whichever occurs first. SAEs and AESIs will continue to be reported until 90 days after the last dose of the IMP treatment or until initiation of new anti-cancer therapy, whichever occurs first. After informed consent has been obtained but prior to initiation of the IMP treatment, only SAEs caused by a protocol-mandated intervention should be reported.
Statistical Analysis	The primary endpoint objective response rate according to mRECIST, defined as the proportion of subjects with best response of complete or partial response, will be evaluated based on Fleming' design decision rules. The ORR together with 95% Clopper-Pearson CIs will also be provided. Missing data for the primary endpoint will be considered as a treatment failure. The primary endpoint will be analyzed as soon as the necessary number of events is available. PFS is defined as time from enrolment to the date of the first objectively documented tumor progression, as determined by investigators (per mRECIST), or death due to any cause. Subjects who die without a reported prior progression will be considered to have progressed on the date of their death. Subjects who did not progress or die will be censored on the date of their last tumor assessment. Subjects who did not have any on-trial tumor assessments and did not die will be censored on the first dosing date of IMP. Subjects who started any subsequent anti-cancer therapy without a prior reported progression will be censored at the last tumor assessment prior to initiation of the subsequent anti-cancer therapy. OS is

	defined as time from date of subjects' enrollment until the date of death from any cause; if no event is observed OS is censored at the date of last known alive. PFS and OS will be estimated by the Kaplan-Meier method with the number of events, median and 25% and 75% quantile of the survival, and the survival rates at certain times with 95% confidence interval. Safety: Frequency tables will be compiled with the number and percentage of patients experienced the AE by treatment and total. Additionally, AEs will be summarized by seriousness of AE, relationship to the IMP, and grading for all NCI CTCAE categories. QoL will be summarized using descriptive statistics and change in EORTC QLQ-C30/ EORTC QLQ-HCC18 before and after treatment at respective time points by treatment and total. Analysis population: The intention-to-treat (ITT) population includes all patients who were allocated to the treatment. The ITT population is the primary population for the description of the patient and treatment characteristics and is used for the primary efficacy analysis. The per protocol (PP) population includes all allocated patients who fulfill the inclusion and exclusion criteria and received at least one dose of lenvatinib and gefitinib. Analyses based on the PP population will serve as sensitivity analyses in order to assess the robustness of the results obtained from the ITT population. The safety population is the primary population for the evaluation of treatment administrations/compliance and all safety endpoints and will comprise all patients enrolled who received at least on dose of lenvatinib and gefitinib.
Duration of the trial (planned)	The estimated trial duration at the trial sites is 36 months (12 months recruitment, 12 months maximum treatment duration, and 12 months maximum follow-up after last patient last treatment). The end of this trial is defined as the date of database closure to ensure the collection of survival data of patients and the active involvement of sites in the data cleaning process (e.g., addition source data may be requested, or an additional monitoring visit may be necessary). The Sponsor may decide to terminate the trial at any time (refer to protocol section 5.5.2 for criteria for early trial termination).
Total number of study sites	This trial will be conducted at approximately 10 centers.