Official Title: A Phase IIIB, Single Arm, Multicenter Study of Atezolizumab in Combination with Bevacizumab to Investigate Safety and Efficacy in Spanish Patients with Unresectable or Unsuitable for Locoregional Treatments Hepatocellular Carcinoma Not Previously Treated with Systemic Therapy

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STATISTICAL ANALYSIS PLAN

TITLE:	**A PHASE IIIB, SINGLE ARM, MULTICENTER STUDY OF ATEZOLIZUMAB IN COMBINATION WITH BEVACIZUMAB TO INVESTIGATE SAFETY AND EFFICACY IN SPANISH PATIENTS WITH UNRESECTABLE OR UNSUITABLE FOR LOCOREGIONAL TREATMENTS HEPATOCELLULAR CARCINOMA NOT PREVIOUSLY TREATED WITH SYSTEMIC THERAPY**
PROTOCOL NUMBER:	ML42600
STUDY DRUG:	Atezolizumab (RO5541267) Bevacizumab (RO4876646)
VERSION NUMBER:	3.0
IND NUMBER:	Not applicable
EUDRACT NUMBER:	2020-005268-71
SPONSOR:	F. Hoffmann-La Roche Ltd
PLAN PREPARED BY:	Prepared by [redacted] Reviewed by [redacted]
DATE FINAL:	See electronic date stamp below
DATE(S) AMENDED:	See electronic date stamp below

STATISTICAL ANALYSIS PLAN AMENDMENT APPROVAL

STATISTICAL ANALYSIS PLAN AMENDMENT RATIONALE

This SAP is amended for the following reasons:

- Language regarding Exploratory Efficacy Endpoints (section 4.4.3), Exploratory Biomarker Analysis for Tissue and Blood biomarker Plan and Exploratory Radiomic Analysis Plan have been updated (Sections 2.2.4 and 4.4.3.7), according to the updates of the protocol ML42600, version 2.

- Immunosuppressive medications have been removed from the prohibited therapy section (4.4.3) and added to the cautionary therapy section (4.3), according to the updates of the protocol ML42600, version 2.

- To expand section of analysis of treatment group comparability (section 4.3) including new patients characteristics and variables related to varices considered of scientific interest for the study.

- To clarify the definition of “intrahepatic lesions”, “extrahepatic lesions”, “new lesions”, and “growth” (section 4.4.3.2).

- To clarify the definition of “Depth of response” and expand and clarify the analyses to be performed (section 4.4.3.6)

- To expand the exploratory efficacy analyses to be performed, including subgroups analyses (section 4.4.5) considered of scientific interest for the study.

- To amend the definition of treatment-emergent adverse events (TEAEs, section 4.6) to extend the follow-up period for those adverse events considered AESIs.

- To expand the safety analyses to be performed, including hepatic adverse events (section 4.6.1), laboratory analyses performed for values outside the normal ranges (section 4.6.5), and vital signs analyses performed for values outside the normal ranges (section 4.6.3)

- To clarify the definition of immune-mediated adverse events (section 4.6)

- To clarify the definition of post progression survival (section 4.4.3.2).

- To remove exploratory efficacy endpoints regarding iRECIST and the modified HCC mRECIST, as it will be not possible to receive the necessary data to perform the analyses (section 4.4.3)

- To expand the exploratory analyses of biomarkers and radiomic (section 4.4.3.7), as the Biomarker and Radiomic plan will not be generated.

Additional minor changes have been made to improve clarity and consistency.

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1. BACKGROUND

BACKGROUND ON HEPATOCELLULAR CARCINOMA

Liver cancer is the fifth most common cancer, accounting for 7% of all cancers, and the second most frequent cause of cancer-related death globally, with 854,000 new cases and 810,000 deaths per year. Hepatocellular carcinoma (HCC) represents approximately 90% of primary liver cancers and thus represents a significant global public health issue. On the basis of annual projections, the World Health Organization estimates that in excess of 1 million people will die from liver cancer in 2030 (Villanueva 2019).

The majority of HCCs occur in patients with underlying liver disease, mostly due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection or alcohol abuse. HBV infection accounts for the majority of HCC cases worldwide; however, in Western countries and Japan, HCV is the main cause of HCC (Villanueva 2019). Universal HBV vaccination and wide implementation of direct-acting antiviral agents against HCV are likely to change the etiologic landscape of HCC. However, the incidence of non-alcoholic fatty liver disease (NAFLD), which is a risk factor for HCC, is increasing worldwide and NAFLD will soon become a leading cause of liver cancer in Western countries (Villanueva 2019).

BACKGROUND ON ATEZOLIZUMAB

Atezolizumab is a humanized immunoglobulin G1 (IgG1) monoclonal antibody that targets programmed death-ligand 1 (PD-L1) and inhibits the interaction between PD-L1 and its receptors, PD-1 and B7-1 (also known as cluster of differentiation 80 [CD80]), both of which function as inhibitory receptors expressed on T cells. Therapeutic blockade of PD-L1 binding by Atezolizumab enhances the magnitude and quality of tumour-specific T-cell responses, resulting in improved anti-tumour activity (Fehrenbacher et al. 2016; Rosenberg et al. 2016). Atezolizumab has minimal binding to Fc receptors, thus eliminating detectable Fc-effector function and associated antibody-mediated clearance of activated effector T cells.

Atezolizumab shows anti-tumour activity in nonclinical models. In the clinical setting, Atezolizumab is being studied as a single agent in the advanced cancer and adjuvant therapy settings, as well as in combination with chemotherapy, targeted therapy, and cancer immunotherapy. Targeting the PD-L1 pathway with Atezolizumab has demonstrated activity in patients with advanced malignancies who have failed standard-of-care therapies. Objective responses have been observed across a broad range of malignancies, including non-small-cell lung carcinoma (NSCLC), urothelial carcinoma, RCC, melanoma, colorectal cancer, head and neck cancer, gastric cancer, breast cancer, and sarcoma. Atezolizumab is currently approved for the treatment of urothelial carcinoma, NSCLC, small-cell lung cancer, hepatocellular carcinoma, and triple-negative breast cancer.

Refer to the Atezolizumab Investigator's Brochure for details on nonclinical and clinical studies.

BACKGROUND ON BEVACIZUMAB

Avastin (Bevacizumab) is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) in in-vitro and in-vivo assay systems. Bevacizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF, and has an approximate molecular weight of 149 kD. Bevacizumab is produced in a mammalian Chinese hamster ovary cell line.

Bevacizumab was first granted marketing approval in the United States on 26 February 2004 (international birth date) in combination with intravenous (IV) 5-fluorouracil (5-FU)-based chemotherapy for the first-line treatment of patients with metastatic colorectal cancer (CRC). As of November 2016, Bevacizumab has been approved for use in over a 100 countries worldwide in a variety of indications, including locally recurrent or metastatic breast cancer; advanced, metastatic, or recurrent NSCLC; advanced and/or metastatic renal cell cancer (RCC); newly diagnosed glioblastoma multiforme (GBM) and GBM after relapse or disease progression; persistent, recurrent, or metastatic cervical cancer; front-line treatment of epithelial ovarian cancer (EOC), primary peritoneal cancer (PPC), or fallopian tube cancer (FTC); and treatment of platinum-sensitive and platinum-resistant recurrent EOC, PPC, or FTC.

2. STUDY DESIGN

2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis included in Appendix 1 referred to protocol ML42600 v2.0 (21-Feb-2023). For additional details, see the Schedule of Assessments in Appendix 2.

2.2 ENDPOINTS

The endpoints of the study described in the Protocol Synopsis (Appendix 1) are summarized below according to efficacy or safety purpose.

2.2.1 Primary Efficacy Endpoint

No primary efficacy endpoint is defined in this study. Primary objective is described as a safety endpoint (see section 2.2.52.2.5).

2.2.2 Secondary Efficacy Endpoints

- OS, defined as the time from initiation of study treatment to death from any cause.
- PFS, defined as the time from initiation of study treatment to the first occurrence of disease progression or death from any cause (whichever occurs first), as determined by the investigator according to RECIST v1.1.
- Time to progression (TTP), defined as the time from initiation of study treatment to the first occurrence of disease progression, as determined by the investigator according to RECIST v1.1 criteria.
- Duration of Response (DOR), defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause (whichever occurs first), as determined by the investigator according to RECIST v1.1.
- Objective response rate (ORR), defined as a complete or partial response, on two consecutive occasions ≥ 4 weeks apart, as determined by the investigator according to RECIST v1.1.
- Number/Rate of patients starting second line treatment.

2.2.3 Exploratory Efficacy Endpoints

- To further, evaluate whether the patterns of tumour responses to Atezolizumab + Bevacizumab treatment using different criteria have a different impact on OS and PFS. The different criteria for evaluation of tumour responses that will be compared are as follows: RECIST 1.1, HCC mRECIST, and EASL criteria.
- To evaluate whether the patterns of tumour progression (growth versus new lesion, intrahepatic versus extrahepatic) have a different impact on OS and PPS, assessed by RECIST 1.1. Additionally, a secondary analysis for the registration of tumour progression will include HCC mRECIST, taking

into consideration the RECIST modifications described for SHARP (Reig 2014).

- To explore whether post-study treatments have an impact on OS. OS will be analyzed according to type and duration of each post-study treatments.
- To evaluate whether reasons for treatment withdrawal (progressive disease,AE, deterorating liver function/clinical condition or radical treatment) have an impact on OS.
- To analyze the organ-specific response rate (OS-RR) using RECIST 1.1 and the cumulative incidence probability of organ-specific progression RR including target lesions from the liver, lungs, lymph-nodes and non-target lesions in bones.
- To determine the applicability of depth of response (decrease in tumour burden) as a surrogate for OS when compared to baseline measurement according to RECIST 1.1, EASL, and mRECIST criteria.

2.2.4 Biomarker and Radiomic Efficacy Endpoints

In the Tissue & Blood Biomarker Plan, patients will have samples taken to identify Tissue and Blood based Biomarkers that might be associated with response patterns to Atezolizumab + Bevacizumab and patient outcomes under Atezolizumab+ Bevacizumab treatment. These samples will be obtained at three different study time points: Screening, Cycle 2 (week 3), Cycle 3 (week 6).
The following analyses will be conducted on blood, plasma, serum, and tumour biopsy samples taken from patients included in the project:

i. Multiplex Immunofluorescence T/B series & PDL1 (6-Plex+DAPI): S5 Oncomine™ TCR Beta-SR Assay Sequencing and RNA sequencing, will be performed on tumour biopsy samples to characterize:
   a. the tumour immune cell infiltrate
   b. the specificities of T lymphocyte receptors against tumour-specific antigens
   c. Specific expression patterns that may constitute gene signatures with prognostic and/or predictive power of response to Atezolizumab + Bevacizumab

ii. ELISA Luminex HCYTA-60K-26 Human Cyto Panel A (48 cytokines), cfTNA Whole Exome Sequencing, Euroflow Panel, Whole Blood RNA sequencing andS5 Oncomine™ TCR Beta-SR Assay Sequencing to evaluate potential non-invasive biomarkers on blood, plasma or serum samples, such us:
   a. Cytokines
   b. cfDNA exome sequencing to capture mutations present in circulating DNA
   c. sequencing the TCRs of circulating T cells to detect specific antigenicity of tumour antigens characterization of leukocyte subpopulations that could predict response

In addition, an exploratory Radiomic Analysis Plan will be performed and will aim to gain a more precise evaluation of response to Atezolizumab and Bevacizumab in patients with unresectable hepatocellular carcinoma. This Radiomic Analysis will be performed over the following CT images: Basal, week 6, week 12 and progression scan. The Radiomic Analysis Plan will also evaluate the use of radiomics features as a means of noninvasive prediction of immuno-oncologic characteristics based on biopsied lesions. To this end, the following analyses will be performed:

i. CT-radiomics signatures (including shape, first-order and higher-order texture

features) at baseline will be correlated with the response to study treatment.
ii. Any early changes in CT-radiomics signatures during the study will be correlated with the response to study treatment.
iii. Any changes in inter- and intra- tumour CT-radiomics will be explored in relation to any differences in responder/non-responder lesions and to the patient clinical outcome.
iv. Any CT-radiomics features will be correlated with tumour mutational status in order to develop radiogenomic phenotypes.
v. Any CT-radiomics features will also be correlated with the tumour microenvironment on biopsied lesions including the tumour immunophenotype, RNA sequences, and vascularization.

2.2.5 Primary Safety Endpoint

- Incidence and severity of adverse events of grade ≥ 3 that lead to discontinuation of Atezolizumab and/ or Bevacizumab.

2.2.6 Other Safety Endpoints

- Incidence of Adverse Event (which severity will be determined according to NCI CTCAE v5.0) during patient's treatment including any AE, AE according to severity, relationship with study treatments, seriousness, immune relationship, leading to treatment withdrawal, AESIs and leading to temporary treatment interruption.
- Change from baseline in targeted Vital signs by visit (treatment cycle)
- Change from baseline in targeted Clinical laboratory test results by visit (treatment cycle)
- Hepatic function assessed according to the following parameters:

- International normalized ratio (INR)
- Presence or absence of Ascites and/or Hepatic Encephalopathy
- Albumin-Bilirubin (ALBI) assessment grades of 1 to 3 (Johnson et al 2015) based on ALBI score calculation ($\log_{10}$ bilirubin [µmol/L] x 0.66) + (albumin [g/L] x -0.0852). ALBI score ≤-2.60 = ALBI grade 1; -2.60<ALBI score ≤-1.39= ALBI grade 2 and -1.39< ALBI score = ALBI grade 3.

2.3 DETERMINATION OF SAMPLE SIZE

This study will enroll approximately a sample of convenience (Lohr 2010) of 100 patients across approximately 20 Spanish sites, possibly according to a competitive enrolment scheme (Kim et al. 2017). Approximately 100 patients will be recruited in 12 months and estimated 25 sites will participate in the study.

With this sample size, estimates of the observed frequencies exceeding 10-20% will have an acceptable precision (e.g. 95% confidence limit ± 5-6%), while for rare AE's, rather imprecise estimates will be obtained. For example, with an expected frequency of 1-2% (2-3 events in the 100 patients) the 95% CI of the observed proportion will cover a range of values

compatible with the expected results with a 12-fold or greater of variation. For the same reason, a 2 to 3-fold increase over the expected frequencies might easily be observed by chance. As a consequence, the results for rare AEs in this study will have to be interpreted with caution.

The table below displays the confidence level and the width at observed frequencies of 10%, 15% and 20%. As shown, a sample size of 100 produces a two-sided 87% confidence interval with a width equal to 0.1 when the sample proportion is 10%, an 80% confidence interval with a width equal to 0.1 when the sample proportion is 15% and a 74% confidence interval with a width equal to 0.1 when the sample proportion is 20%.

Confidence Level	Sample size (N)	Target Width	Actual width	Proportion (P)	Lower limit	Upper limit	Width if p=0.5
0.866	100	0.1	0.1	0.1	0.05	0.15	0.16
0.792	100	0.1	0.1	0.15	0.1	0.2	0.136
0.739	100	0.1	0.1	0.2	0.15	0.25	0.123

2.4 ANALYSIS TIMING

Not applicable.

3. STUDY CONDUCT

3.1 RANDOMIZATION

Not applicable.

3.2 INDEPENDENT REVIEW FACILITY

Not applicable.

3.3 CDATA MONITORING

Not applicable.

4. STATISTICAL METHODS

This is not a hypothesis testing study but an exploratory study with predefined precision of estimates for key safety parameters for sample size determination; there are no formal statistical hypotheses tests to be tested, and there will be no adjustments for multiplicity of endpoints or within-subgroups comparisons.

Continuous data will be summarized using the number of available data (number), mean, SD, median, minimum, and maximum. When applicable, the interquartile range (Q_1 and Q_3) and 95% CI will be also provided. In general, mean, SD and median will have 1 more decimal than the decimals of the evaluated variable.

Categorical and ordinal data will be summarized using the number of available data (number), the absolute and relative frequency (by the percentage). Percentage will be rounded to 1 decimal. When applicable 95% CI will be also provided.

In general, descriptive statistics of quantitative efficacy and safety parameters (result and change from baseline) by scheduled post-baseline visits will be provided on observed cases.

Missing will be also displayed but not included in the percentage calculation.

When specified, missing values will be evaluated and classified according to their underlying missing mechanism (missing completely at random; missing at random; missing not at random) and pattern (monotonic; generalized; univariate) (van Buuren et al. 1999; Little and Rubin 2002; van Buuren 2018). If necessary, missing data will be dealt with via one or more multiple imputation regression models and/or other statistical methods consistent with their underlying missing mechanism (Van Buuren et al., 1999; Little & Rubin 2002; Van Buuren 2018).

All analyses will be performed in SAS, version 9.4 (or update, if applicable).

Baseline value is defined as the last available value prior to the first study treatment intake.

4.1 ANALYSIS POPULATIONS

4.1.1 Randomized Population

Not applicable.

4.1.2 Intent-To-Treat Population

Intent-to treat population (ITT) is defined as all screened patients (patients who have signed the informed consent) and were eligible for the study (all inclusion and exclusion criteria met).

4.1.3 Per Protocol Population

Not Per Protocol population defined in this study.

4.1.4 Pharmacokinetic-Evaluable Population

Not applicable.

4.1.5 Safety Population

Safety population is defined as all screened patients (patients who have signed the informed consent) and received at least one full or partial dose of study treatment.

4.2 ANALYSIS OF STUDY CONDUCT

This section describes the patient disposition for patient study status and the patient analysis populations.

Screened patients are defined as patients who signed the informed consent.

Eligible patients are defined as screened patients who meet all I/E criteria (ITT population).

For patient study status, the total number of patients in each of the following categories will be presented:

- Screened patients
- Screen failure patients and reasons
- Non-eligible patients but treated (patients belonging to Safety but not to ITT populations)
- Patients eligible but not treated (patients belonging to ITT but not to Safety populations)
- Eligible and treated (patients belonging to ITT and Safety populations).
- Patients who completed the study
- Patients who discontinued the study and reasons (AE, Pregnancy, Death, Lost to follow-up, Protocol deviation, Non-compliance with study drug, Withdrawal by subject, Study terminated by sponsor, Physician decision, Progressive disease, Symptomatic deterioration or Other).
- Patients ongoing in the study
- Patients who discontinued the study treatment (either Atezolizumab or Bevacizumab).
- Patients who discontinued Atezolizumab and Bevacizumab.
- Patients who discontinued Atezolizumab treatment and reasons (AE, Pregnancy, Death, Lost to follow-up, Protocol deviation, Non-compliance with study drug, Withdrawal by subject, Study terminated by sponsor, Physician decision, Progressive disease, Symptomatic deterioration, Deteriorating liver function/clinical conditions, Radical treatment or Other).
- Patients who discontinued Bevacizumab treatment and reasons (same reason as displayed above).

As a general rule, for all categories of patients (except for the screened and screen failures), percentages will be calculated using the number of patients in ITT population as the denominator. As an exception to this rule, for all information regarding end of treatment ("Patients who discontinued the study treatment (either Atezolizumab or Bevacizumab)", "Patients who discontinued Atezolizumab and Bevacizumab", "Patients who discontinued Atezolizumab treatment and reasons", "Patients who discontinued Bevacizumab treatment and reasons") percentages will be calculated using the number of patients in safety population as the denominator.

Patients who discontinued any of the study treatments will be listed including the reason and the specifications of the reasons: Physician decision and Other. Same listing will be performed for patients who discontinued the study on the screened patients.

Analysis populations for safety and efficacy will be summarized in a table on screened patients:

- ITT population
- Safety population

Additionally, all major or critical protocol deviations potentially impacting efficacy analyses as well as other major or critical deviations will be summarized in tables (and listed) giving numbers and percentages of patients for each deviation based on ITT population.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

The following parameters will be summarized on the safety population according to the statistical methods defined in section 4:

1. Demographics and baseline data:

- Age (years);
- Age categories (years): <65; ⩾65 and <75; ⩾75/ 18 to 64; 65 to 84; 85 and over;
- Gender (male/ female)
- Female reproductive status (Childbearing potential with contraceptive protection/ Non childbearing potential);
- Ethnicity (Hispanic or Latino/ not Hispanic or Latino/ Not reported/Unknown)
- Race (American Indian or Alaska Native/ Asian/ Black or African American/ Native Hawaiian or Pacific Islander/ White/ Unknown)
- Height (cm)
- Weight (kg)
- Body mass index (BMI) (kg/m^2), calculated as:

$$BMI\ (kg/m^2) = weight\ (kg)/(height\ (m))^2$$

- BMI categories (kg/m^2) :<25; ≥25 and <30; ≥30;
- Systolic Blood Pressure (mmHg);
- Diastolic Blood Pressure (mmHg);
- Pulse Rate (bpm);
- Respiratory Rate (Breaths/min);
- Body temperature (ºC).

2. Smoking, alcohol, and drug abuse histories

- Smoking status (Never/current/previous/Unknown);

If previous selected:

- Time to quitting smoking (years), calculated as:

$$Time\ to\ quitting\ smoking\ (years) = year\ of\ IC - year\ of end\ of\ smoking + 1$$

If previous or current selected:

- Average of cigarettes per day (cigarettes/day);
- Time smoking (years);
- Alcohol status (Never/current/previous/Unknown);

If previous selected:

- Time to alcohol cessation (years), calculated as:

$$Time\ to\ \text{alcohol cessation}\ (years) = year\ of\ IC - year\ of end\ of\ alcohol\ use + 1$$

If previous or current selected:

- Average grams intake per day (g/day);
- Time of alcohol use (years);
- Drug abuse status (Never/current/previous/Unknown);

If previous selected:

- Time to drug abuse cessation (years), calculated as:

$$Time\ to\ \text{drug abuse cessation}\ (years) = year\ of\ IC - year\ of end\ of\ drug\ abuse + 1$$

If previous or current selected:

- Time of drug abuse (years);

3. Other patients characteristics

- Diabetes (Yes/ No);
- Hypertension (Yes/ No);

- Dyslipidemia (Yes/No): Yes if the medical history of the subject includes "Dyslipidaemia" or "Hypercholesterolaemia";
- Varices at screening (Yes/ No). If yes, specify if the varices are treated;
- Alpha-fetoprotein (<400 ng/ml/ ⩾400 ng/ml);
- ECOG (0/ 1),
- Possible cause of hepatocellular carcinoma, defined as:
  - Hepatitis B: If Hepatitis B Surface Antigen = "Positive".
  - Hepatitis C: If Hepatitis C Antibody = "Positive".
  - Non-viral: all patients not included in the previous categories
- Cardiovascular disease (Yes/ No). Yes if the medical history of the subject includes any of the following coded terms:

  MedDRA HLGT Term "Cardiac Arrhythmias", HLGT code 10007521

  MedDRA HLGT Term "Cardiac Valve Disorders", HLGT code 10046973

  MedDRA HLT Term "Ischaemic Coronary Artery Disorders", HLT code 10011085

4. Cancer characteristic and previous disease therapies

- Stage of cancer (BCLC 0/ BCLC A/ BCLC B/ BCLC C/ BCLC D);
- Time to cancer diagnosis (months), calculated as:

$$Time\ to \text{ cancer diagnosis } (months) = (date\ of\ IC - date\ of cancer\ diagnosis + 1)/30.25$$

Prior cancer therapy regimen:

- Therapy setting (Locoregional/ other);
- Line of therapy (1st/ 2nd/ 3rd/ 4th);
- Liver directed therapy type (Radiofrequency ablation/ Percutaneous ethanol or acetic acid injection/ Cryoablation/ High-intensity focused ultrasound/ Transarterial chemoembolization/ Transarterial embolization/ Transarterial radioembolization/ Other)

For each type of therapy, it will be also summarized:

- Number of cycles received;
- Time since finished the therapy (months), calculated as:

$$Time \text{ since finished the therapy } (months) = (\text{date } of\ IC - date\ of ending\ therapy + 1)/30.25$$

Prior cancer radiotherapy:

- Modality (External beam radiotherapy/ Stereotactic radiosurgery/ Transarterial radioembolization);
- Site (Bone/ brain/ breast/ bronchus/ cervix uteri/ colon/ duodenum/ esophagus/ gallbladder/ large intestine/ kidney/ liver/ lung/ lymph node/ abdominal cavity/ mediastinum/ central nervous system/ ovary/ pancreas/

pelvis/ prostate gland/ rectum/ fallopian tube/ skin/ small intestine/ soft tissue/ stomach/ trachea/ bladder/ uterus/other);

- Therapy setting (Limited-stage/ extensive stage/ other);
- Total dose received (Gy);
- Number of fractions;
- Time since finished the radiotherapy (months), calculated as defined for previous therapy;
- Duration of radiotherapy (months), calculated as:

$$\text{Radiotherapy duration } (months) = \frac{date\ of\ ending\ radiotherapy - date\ of starting\ therapy + 1}{30.25}$$

Prior cancer surgery:

- Site (Adrenal gland/ bone/ bone narrow/ brain/ liver/ lung/ lymph node/ mediastinum/ pleura/ pleural effusion/ skin/ chest wall/ abdominal cavity/ abdominal wall/ Other);
- Surgical procedure (liver resection/ liver transplantation/ Metastasectomy/ other);
- Time since surgery (months), calculated as:

$$Time \text{ since surgery } (months) = (\text{date } of\ IC - date\ of\ the\ surgery + 1)/30.25$$

5. Medical history

Medical/surgical history will be coded using the version of Medical Dictionary for Regulatory Activities (MedDRA) currently in effect at the time of database lock.

This information will be summarized by primary SOCs and PT using MedDRA. Table will be sorted by SOC internationally agreed order and in decreasing frequency of PT based on the overall incidence.

6. Varices at screening

- Varices (Yes/ No);
- Varices size (Small/ Large);
- Varices treated (Yes/ No);
- Treatment type.

7. Prior and concomitant therapy

Concomitant therapy consists of any medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated treatment from 7 days prior to initiation of study drug to the

treatment discontinuation visit. Those medications will be coded using the WHO-DD version currently in effect at the time of database lock.

Details related to computation, dates, imputation for missing dates are described in section 4.7.

All medications will be summarized according to the WHO-DD dictionary, considering the first digit of the Anatomical Therapeutic Chemical Classification (ATC) corresponding to the anatomical category (ATC1) and the first 3 digits of the ATC class (therapeutic category) (ATC3). All ATC codes corresponding to a medication will be summarized, and patients will be counted once in each ATC category (anatomic or therapeutic) linked to the medication. Therefore, patients may be counted several times for the same medication.

The tables for all medications will be sorted by decreasing frequency of ATC followed by all other therapeutic classes. In case of equal frequency regarding ATCs (anatomic or therapeutic categories), alphabetical order will be used.

Prohibited medications will be classified as prior and concomitant as well and will be summarized following the same approach as all medications defined above.

4.4 EFFICACY ANALYSIS

This study is not designed to primarily evaluate efficacy. All baseline summaries and efficacy analyses will be based on the ITT analysis set defined as all screened patients.

The analysis of continuous, count and categorical variables will be performed according to the general statistical analysis (see section 4).

Details related to computation, dates, imputation for missing dates are described in section 4.7

4.4.1 Primary Efficacy Endpoint

Not applicable.

4.4.2 Secondary Efficacy Endpoints

4.4.2.1 Secondary Efficacy Endpoint 1: Overall survival

Efficacy variables

This efficacy endpoint will assess the overall survival (OS), defined as time-to-event from initiation of study treatment to OS event (death from any cause), calculated as:

OS (months) = (Date of Death – Treatment Start Date + 1) / 30.25.

Event and censor

Event is defined as any patient who died during the study from any cause. Censor will be defined as any patient who has not died during the study and will be censored at the last known date to be alive.

Analysis
OS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included.

4.4.2.2 Secondary Efficacy Endpoint 2: Progression-free survival

Efficacy variables
This efficacy endpoint will assess the progression-free survival (PFS), defined as time-to-event from initiation of study treatment to PFS event (first occurrence of disease progression or death from any cause, whichever occurs first), as determined by the investigator according to RECIST v1.1, calculated as:

PFS (months) = (Date of Disease Progression/Death − Treatment Start Date +1) / 30.25.

Event and censor
Event is defined as any of the first occurrences between disease progression and death from any cause. Censor is defined as any patient who has not experienced disease progression or death during the study and will be censored at the last known date to be alive or without disease progression.

Analysis
PFS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included.

4.4.2.3 Secondary Efficacy Endpoint 3: Time to progression

Efficacy variables
This efficacy endpoint will assess the time to progression (TTP), defined as time-to-event from initiation of study treatment to TTP event (the first occurrence of disease progression, as determined by the investigator according to RECIST v1.1), calculated as:

TTP (months) = (Date of Treatment Discontinuation – Enrolment Date + 1) / 30.25.

Event and censor
Event is defined as the first occurrence of disease progression. Censor is defined as any patient who has no disease progression and will be censored at the last known date without disease progression.

Analysis:
TTP will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included.

4.4.2.4 Secondary Efficacy Endpoint 4: Duration of response

Efficacy variables

This efficacy endpoint will assess the duration of response (DOR), defined as the time-to-event from the first occurrence of a documented objective response, determined on two consecutive investigator assessments $\geqslant$ 4 weeks apart in patients with measurable disease at baseline, to the time of disease progression as determined by the investigator using RECIST v1.1 or death from any cause, whichever comes first, calculated as:

DOR (months) = (Date of Disease Progression or Death – Date of Documented Objective Response + 1) / 30.25.

Event and censor

Event is any of the first occurrences between disease progression, determined on two consecutive investigator assessments $\geqslant$ 4 weeks apart in patients with measurable disease at baseline, and death from any cause. Censor is defined as any patient who has not experienced disease progression, determined on two consecutive investigator assessments $\geqslant$ 4 weeks apart in patients with measurable disease at baseline, or death during the study and will be censored at the last known date to be alive or without disease progression.

Analysis:

DOR will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included.

4.4.2.5 Secondary Efficacy Endpoint 5: Objective response rate

Efficacy variables

This efficacy endpoint will assess the objective response rate (ORR), defined as a complete or partial response, on two consecutive occasions, 4 weeks apart, before any evidence of progression, as determined by the investigator according to RECIST v1.1.

Analysis:

The ORR will be summarized with appropriate descriptive statistics. It will be calculated as the percentage of patients who have a CR or PR before any evidence of progression. A 95% CI will be derived for the ORR using Wilson score intervals (CIs for a single proportion).

4.4.2.6 Secondary Efficacy Endpoint 6: Second line treatment

Efficacy variables

This efficacy endpoint will assess the number and rate of patients starting second line treatment. With this purpose, duration of second line treatment (months) will be assessed, calculated as:

Duration of 2nd line treatment (months) = (2nd line treatment end date – 2nd line treatment start date)/30.25.

If no end date is available, the date of the last transfer will be used instead.

Analysis:

The patients starting second line treatment will be summarized with appropriate descriptive statistics, including number and percentage of patients, and time and duration of each post-study treatment.

4.4.3 Exploratory Efficacy Endpoints

4.4.3.1 Exploratory Efficacy Endpoint 1: Tumor responses

Efficacy variables

This efficacy endpoint will evaluate whether the patterns of tumor responses to Atezolizumab + Bevacizumab treatment using different criteria have a different impact on PFS. Progression-free survival (PFS) is defined as time-to-event from initiation of study treatment to PFS event (first occurrence of disease progression or death from any cause, whichever occurs first), as determined according to HCC mRECIST, and EASL criteria, calculated as:

PFS (months) = (Date of Disease Progression/Death − Treatment Start Date +1) / 30.25.

Event and censor

Event is defined as any of the first occurrences between disease progression and death from any cause. Censor is defined as any patient who has not experienced disease progression or death during the study and will be censored at the last known date to be alive or without disease progression.

Analysis

PFS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included. Each of these analyses will be performed using data of each of the criteria considered for disease progression (HCC mRECIST, and EASL).

All the information of HCC mRECIST, and EASL will be evaluated externally (see Appendix 3).

4.4.3.2 Exploratory Efficacy Endpoint 2: Patterns of tumor progression

Efficacy variables

This efficacy endpoint will evaluate whether the patterns of tumor progression (growth versus new lesion, intrahepatic versus extrahepatic) have a different impact on OS and PPS, assessed by RECIST 1.1.

A patient will be classified in the group of "growth" or "new lesion" if progressive disease is observed, and according to the reason for the progressive disease (if there is no new lesion by the time of the evaluation, the progression will be considered due to growth, according to the investigator criterion).

Intrahepatic/ extrahepatic lesions will be only evaluated on those patients with progressive disease. Intrahepatic lesions will be defined as those lesions that occur in the liver. If a patient has any lesion outside the liver, that patient will be considered in the group of patients with extrahepatic lesions.

Overall survival (OS) is defined as time-to-event from initiation of study treatment to OS event (death from any cause), calculated as:

OS (months) = (Date of Death – Treatment Start Date + 1) / 30.25.

Post progression survival (PPS), defined as time-to-event from the first occurrence of disease progression to death from any cause, as determined according to RECIST 1.1 and HCC mRECIST criteria, calculated as:

PPS (months) = (Date of Death – Date of Disease Progression +1) / 30.25.

Event and censor
OS and PPS event is defined as any patient who died during the study from any cause. OS and PPS censor will be defined as any patient who has not died during the study and will be censored at the last known date to be alive.
Analysis
OS and PPS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included. All analyses will be performed considering the patterns of tumor progression: *growth* vs *new lesion*, *intrahepatic* vs *extrahepatic and growth intrahepatic vs growth extrahepatic vs new intrahepatic lesion vs new extrahepatic lesion*.

4.4.3.3 Exploratory Efficacy Endpoint 3: Impact of post-study treatments on OS

Efficacy variables
This efficacy endpoint will assess the impact of post-study treatments on OS.
With this purpose, duration of second or further lines of treatment (months) will be assessed, calculated as:

Duration of post-study treatment (months) = (Post-study treatment end date – Post-study treatment start date)/30.25.

If no end date is available, the date of the last transfer will be used instead.

OS will be analyzed according to type and duration of each post-study treatments and is defined as time-to-event from initiation of study treatment to OS event (death from any cause), calculated as:

OS (months) = (Date of Death – Treatment Start Date + 1) / 30.25.

Event and censor
Event is defined as any patient who died during the study from any cause. Censor will be defined as any patient who has not died during the study and will be censored at the last known date to be alive.

Analysis
The patients starting post-study treatments will be summarized with appropriate descriptive statistics, including number and percentage of patients, and duration of each post-study treatment.

OS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included. OS will be described based on the type of post-study treatment (*systemic* compared to *other*). And based on the duration. Quartiles will be used to categorize the duration of therapies.

4.4.3.4 Exploratory Efficacy Endpoint 4: Impact of reasons for treatment withdrawal on OS

Efficacy variables
This efficacy endpoint will assess the impact of reasons for treatment withdrawal on OS. OS is defined as time-to-event from initiation of study treatment to OS event (death from any cause), calculated as:

OS (months) = (Date of Death – Treatment Start Date + 1) / 30.25.

OS based on the following reasons for treatment withdrawal will be described: progressive disease, adverse event, and deteriorating liver function/clinical conditions (based on: INR assessments, presence or increase in ascites and/or hepatic encephalopathy, increase in ALBI assessment scores grading).

ALBI assessment grades of 1 to 3 (Johnson et al 2015) are based on calculated ALBI score (log10 bilirubin [µmol/L] × 0.66) + (albumin [g/L] × −0.0852) values as follows:

- ALBI score $\leqslant$ −2.60 = ALBI grade 1
- > −2.60 to $\leqslant$ −1.39 =ALBI grade 2
- > −1.39 = ALBI grade 3

Event and censor
Event is defined as any patient who died during the study from any cause. Censor will be defined as any patient who has not died during the study and will be censored at the last known date to be alive.

Analysis
OS will be analyzed using Kaplan-Meier (K-M) methods (Kaplan and Meier 1958) and Greenwood's formula (Greenwood 1926). Medians and quartiles with 95% confidence interval (CI) will be derived from the K-M curves. K-M plots will also be included. OS based on the

following reasons for treatment withdrawal will be described: *progressive disease* compared to *adverse event*, and *deteriorating liver function/clinical conditions.* Patients without treatment withdrawal will also be evaluated.

4.4.3.5 Exploratory Efficacy Endpoint 5: Organ-specific response rate

Efficacy variables

This efficacy endpoint will assess organ-specific response rate (OS-RR), defined as a complete or partial response, considering target lesions from the liver, lungs, lymph-nodes and non-target lesions in bones, using RECIST 1.1

Analysis:

The OS-RR will be summarized with appropriate descriptive statistics. It will be calculated as the percentage of patients who have a CR or PR before any evidence of progression for target lesions including in the liver, lungs, and lymph-nodes as well as for non-target lesions in bones. A 95% CI will be derived for the OS-RR using Wilson score intervals (Cis for a single proportion). The cumulative incidence probability of organ-specific progression will be measured.

4.4.3.6 Exploratory Efficacy Endpoint 6: Depth of response

The final exploratory efficacy objective of the study will be to determine the applicability of depth of response (decrease in tumor burden) as a surrogate for OS. Tumor response according to RECIST 1.1, EASL, and mRECIST criteria will be compared to baseline evaluations.

Efficacy variables

This efficacy endpoint will assess the depth of response (decrease in tumor burden), defined as the maximum tumors shrinkage from baseline, based on the sum of diameters (longest diameter for non−lymph node lesions, short axis for lymph node lesions), considering all target lesions at baseline and at each subsequent tumor assessment as a measure of tumor burden. The depth of response (DpR) will be assessed using the evaluations of the investigators (RECIST 1.1), and the external evaluations (RECIST 1.1, HCC mRECIST, and EASL).

The value of the absolute change in tumor burden will be derived as the value in each evaluation minus the baseline value. Percent changes will also be calculated.

Analysis:

For the evaluations of the investigators (RECIST 1.1), the following analyses will be performed:

The summary statistics of the minimum value in the change of tumor burden will be summarized according to the general approach (see section 4). A waterfall plot with the evaluations of each patient will be included (the lowest evaluation per patient).

For the evaluations of the investigators (RECIST 1.1), and the external evaluations (RECIST 1.1, mRECIST, and EASL), the following analyses will be performed:

OS and TTP will be presented, categorizing the patients in tumour shrinkage population (DpR <0%) and non‐tumour shrinkage population (DpR ⩾0%). The OS will be analyzed by means of a Cox proportional hazards regression comparing both groups (>0%, ⩽0%). The Hazard Ratio will be presented, with its 95% confidence interval and associated p-value.Cox proportional hazards regressions will be implemented to assess the effect of depth of response at week 6 and week 12 on the OS. The predictive value of depth of response at week 6 and 12 over the OS at 6, 12, 18, and 24 months will be evaluated by means of ROC curves.

Spider plots will be included for the evaluations of different timepoints (baseline, week 6, week 12, PD/EOS).

4.4.3.7 Biomarker and Radiomic Efficacy Endpoints

Biomarker and Radiomic Efficacy analysis will be performed by external laboratories (see appendix 3).

Radiomic analysis

CT image standardization pipelines will be applied, including image resampling and batch effect correction methods to ensure CT image harmonization (Ligero et al, 2021). Target lesions corresponding to the primary tumour or metastases will be selected for radiomics features extraction. Target lesions will be selected based on size (1 cm in large diameter for solid lesions and 1.5 cm in short diameter for nodes). Semiautomatic segmentation tools from 3D Slicer (Fedorov et al, 2012) will be used for delineating the target lesions (both the primary tumour or the metastatic disease). Radiomics features from target lesions will be extracted using an in-house program based on the Pyradiomics package for Python (van Griethuysen, 2017), including first order features (minimum, maximum, mean, skewness, kurtosis, among others), shape features (volume, surface area, sphericity, among others), and different filters for texture analysis will be applied for higher order statistics texture analysis.

All patients included in the trial with CT scan available at baseline will be included in the radiomic analysis.

State-of-the-art machine learning methods such as Elastic Net and SVM, among others, will be implemented to develop predictive models of response to the combined treatment of bevacizumab and atezolizumab. Response as binary outcome will be defined as the output for the predictive model development. Response will be defined as the presence of CR, PR as best response or SD for more than 6 months by RECIST1.1 (Eisenhauer et al, 2009).

The accuracy of the radiomics-based predictive model will be assessed by the area under the curve (AUC) and confidence interval from the receiver operator characteristic (ROC) and compared by the DeLong method and Mann-Whitney U test. The Kaplan-Meier method will be used to estimate the probability of response and survival over time. Summary statistics will be used to summarize and present data. Where applicable, two-sided tests will be used and p-value < 0.05 will be considered statistically significant.

Biomarker analysis

T lymphocyte repertoire study

Genomic DNA was extracted from circulating blood mononuclear cells using the Genomic DNA purification kit (Promega). The Ion Torrent™ Oncomine™ TCR-Beta assay kit (Thermo) was used to prepare the library from 20 ng of genomic DNA. Libraries were sequenced with a Ion GeneStudio S5 System Series sequencer in the Solid Tumor Genomics Lab of CIMALabs Diagnostics (University of Navarra). The pipeline use for primary analysis of CDR1, CDR2 and CDR3 gene sequence was Ion Reporter Software 5.10 (Thermo). Data required preprocessing: trimming of adapters and low-quality bases from raw sequencing reads, filtering out sequencing errors and PCR artifacts, assigning TCR sequences to individual samples based on unique molecular identifiers (UMIs) if available and collapsing identical TCR sequences to reduce redundancy. Clonotypes were defined as unique TCR sequences or groups of TCR sequences sharing the same V(D)J rearrangements and CDR3 sequences. Then, clonotype frequencies were assigned to each sample. The abundance of clonotypes changes in clonotype frequencies were calculated over time, globally and separating large, medium and low frequency clonotypes. Emerging or disappearing clonotypes at any time point (baseline, 3 and 6 w) were identified. Clonotype turnover rate and clonotype persistence were quantified. When available, clonotypes in periphery were compared to FFPE-extracted tumor TCRb sequencing-detected clonotypes. The large-clone abundance, clonality, diversity and evenness baseline and changes along the time points, for each patient was calculated and used to compare responders vs non responders taking into account different types of response (logistic regression) and survival (LogRank test and Kaplan Meyer curves. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Flow Cytometry of circulating Blood Cells

Samples were shipped at room temperature overnight for centralized processing. PBMC were separated by using a Ficoll gradient and resulting cells were frozen in aliquots for subsequent analyses. Upon completion of sample collection and storage, they were thawed for simultaneous use in flow cytometry experiments. After vial thawing, cells were incubated with DNAse I for 20 min at 37 ºC, washed and resuspended in FACS buffer for subsequent staining. Then, after a 15 minute incubation with Fc Block, cells were washed and incubated with two panels of antibodies to determine the proportion of lymphocytes and myeloid cells. In addition to cell frequencies determined using lineage markers, expression of CTLA-4, PD-1, TIM-3, LAG-3 and CD39 was determined in lymphocytes, and CD86 and PD-L1 in myeloid cells. In the lymphocyte panel, after surface staining, cells were treated with Fix/Perm buffer (Invitrogen) before intracellular staining with antibodies against FOXP3 and CTLA-4. Statistical analyses: after checking for normality using the Shapiro-Wilk test, results were analyzed using unpaired T-test for comparisons at each time point, and a mixed-effect analysis for multiple comparisons when analyzing changes between time points. Log-rank test was used for comparisons of overall survival, progression-free survival and time to progression. In all cases, $P<0.05$ was considered statistically significant. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Bulk RNA sequencing of circulating blood cells

RNA was extracted from whole blood collected with Streck tubes (29mmune29zi). Illumine TruSeq double stranded RNA sequencing library was built using customer recommendation and adapted and indexed libraries were sequenced in a NextSeq2000 sequencer, at a depth of 20 million 50 x 2 bp reads. Sequences were then aligned to Homo Sapiens transcriptome using STAR and counts were obtained using RSEM. Counts were then normalized using EdgeR and cpm and logcpm were obtained. Principal Component analysis was used to see differences between time points and between responders and not responders. CIBERSORT and GSVA was the use to infer the enrichment of immune populations. The data obtained in % of cell types (CiberSort) and in normalized scores (GSVA) has the used in downstream analysis. Overall survival (OS) and progression-free survival (PFS) were estimated employing the Kaplan–Meier method, with the association between OS and circulating signatures assessed via the log-rank test. Cox proportional hazards models were employed to evaluate the association of ctDNA with other prognostic factors. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Analysis of Somatic Variants using plasma cell-free DNA

Since there is no NGS panel currently in place to analyze most common variants found in HCC, in collaboration with CIMALabs diagnostics and Thermo, we have designed a custom panel which includes the variants of 14 genes encountered at least in 5 patients in a merged WES/WGS database of around 800 patients. If successful, data on TP53, CTNNB1, TERT mutation status (YES/NO) plus other less frequent variants will be obtained for each patient. Overall survival (OS) and progression-free survival (PFS) were estimated employing the Kaplan–Meier method, with the association between OS and driver gene mutation status assessed via the log-rank test. Cox proportional hazards models were employed to evaluate the association of ctDNA with other prognostic factors. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Analysis of large structural variations from plasma cell-free DNA using low pass Whole Genome Sequencing

cfDNA extraction was performed employing the QIAamp Circulating Nucleic Acid kit. The concentration of extracted cfDNA was quantified using the Qubit dsDNA High-Sensitivity assay, following which it was preserved in LoBind Eppendorf tubes at -80°C until further examination. Library construction of cfDNA was executed using the NEBNext Ultra II DNA Library Prep Kit, with 2.5 ng of cfDNA input utilized for ULP-WGS. The sequencing libraries generated were pooled and subjected to sequencing on a NextSeq2000 platform (Illumina) utilizing 100 bp paired-end runs, achieving an average coverage of 0.3×. Fastq files underwent quality filtration employing TrimGalore, with sequences shorter than 50 bp discarded. Alignment of sequences to the hg19 reference genome was accomplished using Bowtie2, and resulting bam files were sorted and indexed utilizing Samtools. Duplicates were identified and tagged using the MarkDuplicates feature from Picard tools. ichorCNA package was utilized,

following the workflow delineated by its developers. This encompassed read coverage computation, data normalization, and can prediction employing a Hidden Markov Model. Statistical analysis involved categorization of patients based on ctDNA positivity, with subsequent assessment of associations with clinical and demographic features utilizing Fisher's exact or chi-square tests and t-tests as applicable. Overall survival (OS) and progression-free survival (PFS) were estimated employing the Kaplan–Meier method, with the association between OS and ctDNA positivity assessed via the log-rank test. Cox proportional hazards models were employed to evaluate the association of ctDNA with other prognostic factors. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Olink analysis of circulating cytokines

Proteomic profiling on blood plasma samples was performed using the Primer Extension Assay (Olink Explore). A total of 1,300 distinct proteins were measured. After quality control, Normalized Protein eXpression (NPX) values were calculated for each protein. We tested the association between Response and Survival and protein levels in three time points (baseline, 3 and 6 w) by linear regression analysis adjusting for age, gender, macrovascular invasion, extrahepatic spread and AFP><400. All plasma protein values were rank-based inverse normal transformed before the analysis. We corrected the marginal associations for multiple testing using Benjamini-Hochberg method, and plasma proteins with a corrected p-value <0.05 were deemed as significant. R Bioconductor packages for graphics and STATA 18 for statistics was used.

Inmunofluorescence (Multiplex Immunofluorescence T/B series & PDL1 (6-Plex+DAP) vs WES vs Variant analysis

Currently in planning process. Only a fraction of biopsies are currently available. Final decision to be made based on results from NGS panel in circulating DNA.

Integration of multiparametric data

All pertinent biological data concerning each immune and tumor component was meticulously chosen for comprehensive analysis. This selection aimed at facilitating a comprehensive examination of biomarkers associated with response and survival outcomes. Determining the appropriate statistical tests necessitates an initial investigation focusing on individual biomarkers and distinct data types.These analyses of biomarkers and radiomics could be performed after the final analysis, depending on the availability of the data. If it is considered necessary, further analyses could be included in this section.

4.4.4 Sensitivity Analyses

A sensitivity analysis will be performed for the analysis corresponding to the secondary endpoint 2 (PFS) for the external evaluations (RECIST1.1), replicating the corresponding tables using these evaluations.

4.4.5 Subgroup Analyses

Subgroup analyses will be performed for the analyses corresponding to secondary endpoint 1 (OS) and secondary endpoint 2 (PFS), for the following subgroups:

- Alpha-fetoprotein at baseline: <400 ng/ml/ ≥400 ng/ml;
- Baseline BCLC category: BCLC C / BCLC B;

Cox proportional hazards regression could be implemented, comparing both groups of each subgroup, presenting the Hazard Ratio with its 95% confidence interval and associated p-value.

Subgroup analyses will be performed for the analyses corresponding to secondary endpoint 1 (OS) for the subgroup of patients treated beyond progression (TBP), which includes those patients who have a radiological response of PD and continued the treatment of Atezolizumab, Bevacizumab, or both.

4.5 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

Not applicable.

4.6 SAFETY ANALYSES

All safety analyses will be based on the Safety population, and during the treatment emergent study period, defined from day of the first study treatment intake up to the day of the last study treatment intake + 30 (either Atezolizumab or Bevacizumab).

Serious adverse events (SAEs) and AESIs will be considered treatment emergent from day of the first study treatment intake up to the day of the last study treatment intake + 90 (either Atezolizumab or Bevacizumab) or until the start of a new anticancer therapy.

Hepatic AEs (HAEs) are defined as those AE that have the liver as the target organ or represent usual complications of cirrhosis, including hepatobiliary events, liver-related investigations, thrombocytopenia, ascites, encephalopathy, spontaneous bacterial peritonitis and gastrointestinal hemorrhage. The ATCs for the hepatic AEs are included in appendix 4.

Immune-mediated AEs are defined as those treatment-emergent AESIs that required the use of systemic corticosteroids.

4.6.1 Adverse Events

All adverse events will be coded to a lower-level term (LLT), preferred term (PT), high-level term (HLT), high-level group term (HLGT), and associated primary system organ class (SOC) using the version of MedDRA currently in effect at the time of database lock.

Only treatment emergent AEs will be analyzed.

Details related to computation, dates, imputation for missing dates are described in section 4.7

Adverse event incidence tables will present by SOC (sorted by internationally agreed order) and PT (sorted by decreasing frequency of PTs within SOCs based on overall frequency), the number (n) and percentage (%) of patients experiencing at least one adverse event. Multiple occurrences of the same event in the same patient will be counted only once at the maximum severity in the tables. The denominator for computation of percentages is the number of patients in safety population.

Severity of the AEs is determined according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0 [NCI CTCAE v5.0].

4.6.1.1 Primary Safety Endpoint

For the primary safety endpoint, treatment emergent adverse events with severity grade ≥ 3 leading to treatment discontinuation of Atezolizumab and/ or Bevacizumab will be summarized in a table.

In the table, 4 different columns will be displayed:

- Number of events, and number and (%) of patients with at least one TEAEs by SOC and PT with severity grade ≥ 3 leading to Atezolizumab discontinuation

- Number of events, and number and (%) of patients with at least one TEAEs by SOC and PT with severity grade ≥ 3 leading to Bevacizumab discontinuation

- Number of events, and number and (%) of patients with at least one TEAEs by SOC and PT with severity grade ≥ 3 leading to Atezolizumab and Bevacizumab discontinuation

- Number of events, and number and (%) of patients with at least one TEAEs by SOC and PT with severity grade ≥ 3 leading to Atezolizumab and/or Bevacizumab discontinuation.

For the overall percentages of patients with at least one TEAE in each of the above categories, 95% CI will be calculated by Clopper-Pearson test.

4.6.1.2 Other AE analyses

The following treatment-emergent adverse event summaries will be generated:

1 Overview of treatment-emergent adverse events, summarizing the number of events, and number and (%) of patients with any:
    - TEAE;
    - Serious TEAE;
    - TEAE severity grade 3-4;
    - Related TEAE (either Atezolizumab or Bevacizumab);
    - Serious related TEAE (either Atezolizumab or Bevacizumab);
    - TEAE leading to treatment discontinuation (either Atezolizumab or Bevacizumab);

- Severe TEAE leading to treatment discontinuation (either Atezolizumab or Bevacizumab);
- TEAE leading to death;
- Treatment emergent AESI;
- TEAE leading to temporary interruption (either Atezolizumab or Bevacizumab).

2 Number of events, and number and (%) of patients with at least one TEAE by SOC and PT.

3 Number of events, and number and (%) of patients with at least one severe TEAE by SOC and PT (severity grade 3 or 4). TEAEs grade 3, 4 and 3-4 will be summarized in the table.

4 Number of events, and number and (%) of patients with at least one treatment related TEAE by SOC and PT (either Atezolizumab or Bevacizumab). TEAEs will be summarized in the table for each of the components, for both components, and for any component.

5 Number of events, and number and (%) of patients with at least one severe treatment related TEAE by SOC and PT (either Atezolizumab or Bevacizumab, severity grade 3 and 4). TEAEs will be summarized in the table for each of the components, for both components, and for any component, and by grade of severity (grade 3, 4 and 3-4).

6 Number of events, and number and (%) of patients with at least one serious TEAE by SOC and PT.

7 Number and of events, and number (%) of patients with at least one serious treatment related TEAE by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component.

8 Number of events, and number and (%) of patients with at least one treatment emergent related AESI by SOC and PT.

9 Number of events, and number and (%) of patients with at least one TEAE leading to treatment withdrawal by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component.

10 Number of events, and number and (%) of patients with at least one severe TEAE leading to treatment withdrawal (grade 3 or 4) by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component.

11 Number of events, and number and (%) of patients with at least one TEAE leading to death by SOC and PT (severity grade 5).

12 Number of events, and number and (%) of patients with at least one related TEAE leading to death by SOC and PT (severity grade 5).

13 Number of events, and number and (%) of patients with at least one treatment emergent AE of special interest (AESI) by SOC and PT.

14 Number of events, and number and (%) of patients with at least one severe treatment emergent AESI (severity grade 3 or 4) by SOC and PT (either Atezolizumab or Bevacizumab, severity 3 and 4). AESIs will be summarized in the table by grade of severity (grade 3, 4 and 3-4).

15 Number of events, and number and (%) of patients with at least one TEAE leading to temporary interruption by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component.

16 Number of events, and number and (%) of patients with at least one severe TEAE leading to temporary interruption (severity grade 3 or 4) by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component, and also by grade (3-4, 3, and 4).

17 Number of events, and number and (%) of patients with at least one hepatic TEAE by SOC and PT.

18 Number of events, and number and (%) of patients with at least one treatment-emergent AESI with requirement of systemic corticosteroids (immune-mediated) by SOC and PT.

19 Number of events, and number and (%) of patients with at least one TEAE related to Atezolizumab with requirement of systemic corticosteroids by SOC and PT.

20 Number of events, and number and (%) of patients with at least one non-serious TEAE, with an incidence > 5%, by SOC and PT

Number of events, and number and (%) of severe TEAEs leading to temporary interruption (severity grade 3 or 4) by SOC and PT. TEAEs will be summarized in the table for each of the components, for both components, and for any component, and also by grade (3-4, 3, and 4).

4.6.2 Exposure of Study Medication

Drug exposure will be summarized by descriptive statistics following the general approach (see section 4). The following variables will be included by treatment component:

- Treatment duration of Atezolizumab/ Bevacizumab (months), calculated as:

$$Treatment\ duration\ (months) = (Treatment\ end\ date - Treatment\ start\ date) + 1/30.25$$

- Number of doses administered
- Dose at baseline (mg)

4.6.3 Vital Signs

Vital signs assessments will include the following variables:

- Weight (kg)
- Systolic Blood Pressure (mmHg)
- Diastolic Blood Pressure (mmHg)
- Pulse rate (bpm)

- Respiratory Rate (breaths/min)
- Body temperature (ºC)

The summary statistics of all vital signs variables (values and changes from baseline) will be included for each visit or study assessment, and performed according to the general approach (see section 4).

Vital signs outside the normal limits among subjects without abnormality at baseline will be also summarized, considering the following values as the normal limits:

- Systolic blood pressure is considered low if =<90 mmHg, and high if >150 mmHg.
- Diastolic blood pressure is considered low if =<60 mmHg, and high if >=100 mmHg.
- Pulse rate is considered low if <60 bpm, and high if >100 bpm.
- Respiratory rate is considered low if <12 breaths/min, and high if >20 breaths/min.
- Temperature is considered low if <35 ºC, and high if >38 ºC.

If more than one post-dose evaluation is available for a cycle, the worst case will be considered as post-dose value, which is calculated according to:

1. If there are values outside the normal limits (high), the highest value is considered the worst.
2. If there are no high values, but there are values outside the normal limits (low), the lowest value is considered the worst.
3. If no values outside the normal limits (either high or low), the highest value will be considered the worst.

4.6.4 ECOG

The ECOG performance status will be displayed at each cycle. The statistical approach will be the general approach (see section 4), including only descriptive analyses.

4.6.5 Laboratory Data

Laboratory data will be summarized according to the general approach (see section 4), including descriptive analyses by cycles. Descriptive analyses will be included for the following parameters:

- Haematology: white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells);

- Chemistry panel (serum or plasma): bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, magnesium, chloride, glucose, BUN or urea, creatinine, total protein, albumin, phosphate, calcium, total bilirubin, alkaline phosphatase, ALT, AST, and LDH;

- Coagulation: INR, and aPTT;

- Thyroid function testing: thyroid-stimulating hormone (TSH), free triiodothyronine (T3) (or total T3 for sites where free T3 is not performed), and free thyroxine (also known as T4).

- HIV serology: HIV-1 antibody;

- HBV serology: HBsAg, HBsAb, and total HBcAb, for all patients; HBV DNA for patients with negative HBsAg and HBsAb tests and a positive total HBcAb test;

- HCV serology: HCV antibody and (if HCV antibody test is positive) HCV RNA;

- Urinalysis (pH, specific gravity, glucose, protein, ketones, and blood); dipstick permitted.

Other descriptive analyses, considering only those post-baseline values outside the normal ranges (low or high values), will be also summarized by grade for the following parameters:

Parameter	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Bilirrubin (increase)	>ULN - 1.5 x ULN if baseline was normal; > 1.0 - 1.5 x baseline if baseline was abnormal	>1.5 - 3.0 x ULN if baseline was normal; >1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 10.0 x ULN if baseline was normal; >3.0 - 10.0 x baseline if baseline was abnormal	>10.0 x ULN if baseline was normal; >10.0 x baseline if baseline was abnormal
Alanine aminotransferase (increase)	>ULN - 3.0 x ULN if baseline was normal; 1.5	>3.0 - 5.0 x ULN if baseline was normal; >3.0 - 5.0 x baseline if	>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline	>20.0 x ULN if baseline was normal; >20.0 x baseline if

	- 3.0 x baseline if baseline was abnormal	baseline was abnormal	if baseline was abnormal	baseline was abnormal
Alkaline phosphatase (increase)	>ULN - 2.5 x ULN if baseline was normal; 2.0 - 2.5 x baseline if baseline was abnormal	>2.5 - 5.0 x ULN if baseline was normal; >2.5 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal
Aspartate aminotransferase	>ULN - 3.0 x ULN if baseline was normal; 1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 5.0 x ULN if baseline was normal; >3.0 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal

For alpha-fetoprotein, a descriptive analysis of the evolution of those patients who had abnormal values at baseline (>ULN reported in the EDC) will be included.

4.7 MISSING DATA

4.7.1 Missing or partial dates in adverse events

The algorithm for imputing date/time of onset will be conservative and will classify an adverse event as treatment emergent unless there is definitive information to determine it is not treatment emergent. Missing or partial adverse event dates and/or times will be imputed just for categorization purpose but will not appear in the listings.

4.7.2 Missing or partial dates in medications different than study treatment

Similar approach as followed for missing or partial dates will be performed for the missing or partial medications dates. If a medication date or time is missing or partially missing and it cannot be determined whether it was taken prior or concomitantly with the study treatment, it will be considered a prior and concomitant.

4.7.3 Missing or partial dates in time-to-event variables

No imputation of partial dates of start and/or end dates of any date used to define time-to-event variables will be performed.

4.8 INTERIM ANALYSES

One interim analysis of safety will be performed at the time of 50 recruited patients, estimated to occur at approximately 6 months after first patient in.

5. REFERENCES

Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009

Fedorov A, Beichel R, Kalpathy-Cramer J, et al: 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging 30:1323-41, 2012

Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387:1837–46.

Greenwood M. The natural duration of cancer. Reports on Public Health and Medical Subjects 1926;33:1-26.

Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958;53:457–81.Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annual Rev Immunol 2008;26:677–704.

Kim CH, Han JK, Yang HM, Park KW, Lee HY, et al. Study protocol for39mmune39zinsed controlled tria39mmune39zinging optimal strategy for treatment of coronary artery stenos–s - coronary intervention with next-generation drug-eluting stent platforms and abbreviated dual antiplatelet therapy (HOST-IDEA) trial. BMJ Open 2017;7:e016617.

Ligero M, Jordi-Ollero O, Bernatowicz K, et al: Minimizing acquisition-related radiomics variability by image resampling and batch effect correction to allow for large-scale data analysis. Eur Radiol 31:1460-1470, 2021Lohr SL. Sampling: design and analysis. 2nd ed. Boston (MA): Brooks/Cole, 2010.

Reig, Maria & Darnell, Anna & Forner, Alejandro & Rimola, Jordi & Ayuso, Carmen & Bruix, Jordi. (2014). Systemic Therapy for Hepatocellular Carcinoma: The Issue of Treatment Stage Migration and Registration of Progression Using the BCLC-Refined RECIST. Seminars in liver disease. 34. 444-55. 10.1055/s-0034-1394143.

Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909–20.

van Griethuysen JJM, Fedorov A, Parmar C, et al: Computational Radiomics System to Decode the Radiographic Phenotype. Cancer Res 77:e104-e107, 2017

Villanueva A. Hepatocellular carcinoma. N Engl J Med 2019;380:1450–62.

6. APPENDICES

Appendix 1
Protocol Synopsis

TITLE:	**A PHASE IIIB, SINGLE ARM, MULTICENTER STUDY OF ATEZOLIZUMAB IN COMBINATION WITH BEVACIZUMAB TO INVESTIGATE SAFETY AND EFFICACY IN SPANISH PATIENTS WITH UNRESECTABLE OR UNSUITABLE FOR LOCOREGIONAL TREATMENTS HEPATOCELLULAR CARCINOMA NOT PREVIOUSLY TREATED WITH SYSTEMIC THERAPY**
ACRONYM:	ATHECA study
PROTOCOL NUMBER:	ML42600
VERSION NUMBER	2
EUDRACT NUMBER:	2020-005268-71
IND NUMBER:	Not applicable
NCT NUMBER:	NCT04732286
TEST PRODUCT:	Atezolizumab (RO5541267) Bevacizumab (RO4876646)
PHASE:	IIIb
INDICATION:	Hepatocellular carcinoma
SPONSOR:	Roche Farma S.A.

Objectives and Endpoints

This study will evaluate primarily the safety of Atezolizumab in combination with Bevacizumab in patients with unresectable hepatocellular carcinoma who have received no prior systemic treatment and are considered unsuitable for locoregional therapy, by assessing the incidence and severity of adverse events that lead to discontinuation of any study agent. In this protocol, "study treatment" refers to the combination of agents assigned to patients as part of this study (i.e., Atezolizumab and Bevacizumab). Efficacy of Atezolizumab in combination with Bevacizumab in this study, in contrast with IMbrave150 study, is considered within secondary and exploratory objectives.

Primary Objective	**Corresponding Endpoint**
To evaluate the safety of Atezolizumab + Bevacizumab	- Incidence and severity of adverse events of grade ≥ 3. that lead to discontinuation of Atezolizumab and/ or Bevacizumab.
Main Secondary Objective	**Corresponding Endpoint**
To evaluate the efficacy of Atezolizumab + Bevacizumab	**OS**, defined as the time from initiation of study treatment to death from any cause.
Other Secondary Objectives	**Corresponding Endpoints**
To further evaluate the safety of Atezolizumab + Bevacizumab	- Adverse Event severity will be determined according to NCI CTCAE v5.0 during patient's treatment. - Change from baseline in targeted Vital signs. - Change from baseline in targeted Clinical laboratory test results.
To further evaluate the efficacy of Atezolizumab + Bevacizumab	- **PFS**, defined as the time from initiation of study treatment to the first occurrence of disease progression or death from any cause (whichever occurs first), as determined by the investigator according to RECIST v1.1. - **Objective response rate (ORR)**, defined as a complete or partial response, on two consecutive occasions ≥ 4 weeks apart, as determined by the investigator according to RECIST v1.1. - **Time to progression (TTP)**, defined as the time from initiation of study treatment to the first occurrence of disease progression, as determined by the investigator according to RECIST v1.1 criteria. - **Duration of Response (DOR)**, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause (whichever occurs first), as determined by the investigator according to RECIST v1.1. - **Number/Rate of patients** starting second line treatment
To evaluate deterioration of Liver function during the treatment	- **Hepatic function assessed** according to the following parameter: o International normalized ratio (INR). o Presence or absence of Ascites and /or Hepatic Encephalopathy. o Albumin-Bilirubin (ALBI) assessment grades of 1 to 3.*

***** ALBI assessment grades 1 to 3 (Johnson et al. 2015) are based on calculated ALBI score (log10 bilirubin [µmol/L] × 0.66) + (albumin [g/L] × −0.0852) values as follows: ALBI score ≤ −2.60 = ALBI grade 1; > −2.60 to ≤ −1.39 =ALBI grade 2); and > −1.39 = ALBI grade 3.

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NCI CTCAE v5.0 = National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0; OS = overall survival; PFS = progression-free survival; RECIST v1.1 = Response Evaluation Criteria in Solid Tumours, Version 1.1.

Exploratory Response Objectives	Corresponding Endpoint
To further, evaluate whether the Tumour Responses patters to Atezolizumab + Bevacizumab treatment using different criteria have a different impact on OS and PFS	- **Tumour Response** will be primarily assessed by RECIST 1.1. And the results in efficacy on terms of OS and PFS reported accordingly. - Additionally, a secondary analysis will include HCC mRECIST, EASL and iRECIST criteria, based on the following patterns of progression: o **HCC mRECIST,** by measuring for any increase (confirmed in follow up visit) in the sum of longest diameter of viable tumour (unidimensional measurement). o **EASL**, by measuring for any increase (confirmed in follow up visit) in the area of viable tumour (bidimensional measurement). o **iRECIST**, by taking into consideration tumour pseudoprogression.
To further evaluate whether the patterns of tumour progression (growth versus new lesion, intrahepatic versus extrahepatic) have a different impact on OS and PFS	- **Tumour Progression** (and the results in efficacy on terms of OS and PFS) will be primarily assessed by RECIST v1.1 criteria based on the following patterns of progression: o > 20% increase in tumour size against a known baseline lesion (intrahepatic growth [IHG] or extrahepatic growth [EHG]) o new intrahepatic lesion (NIH) o new extrahepatic lesion and/or vascular invasion (NEH). - Additionally, a secondary analysis for the registration of tumour progression will include HCC mRECIST, taking into consideration the RECIST modifications described for SHARP (Reig 2015). - Exploratory assessment on OS and PFS will be performed based on the following patterns of progression: o New intrahepatic nodules are considered progression if: - They exceed 10 mm in diameter and present arterial enhancement at dynamic imaging. - Non-specific non-hypervascular nodules ≥ 10 mm (absence of the above definitions) present a doubling tumour size reaching a diameter > 20 mm since its initial detection or showing hyperenhancement during the follow-up. - Ascites and pleural effusion reflect progression only if malignant cells are pathology (cytology) proven. - Vascular invasion may be classified as progression if expansive and/or displaying arterial enhancement at dynamic imaging. - Hilar lymph nodes will be considered malignant if the smaller diameter exceeds 20 mm. Growth of existing nodes uses the same cut-offs as other lesions. - Lobar or segmental portal invasion with growth of the tumour thrombus reaching the main trunk of the portal vein.
To explore if post-study treatments have an impact on OS	- OS will be analysed according to type and duration of each post-study treatment.

To evaluate if reasons for treatment withdrawal have an impact on OS	- OS based on the following reasons of treatment withdrawal:
	  ○ PD vs AE vs deteriorating liver function based on:
	  - INR assessments;
	  - presence or increase in ascites and/or hepatic encephalopathy;
	  - increase in ALBI assessment scores grading.*

To analyse the organ-specific response rate (OS-RR) using RECIST 1.1 and the cumulative incidence probability of organ-specific progression	- We hypothesized that treatment efficacy varies across different metastatic sites. Comparison of OS-RR including target lesions from the liver, lungs, lymph nodes and non-target lesions in bones.

To determine the applicability of depth of response as surrogate for OS	- Depth of response (decrease in tumour burden) compared to baseline measurement according to:
	  ○ RECIST 1.1
	  ○ EASL
	  ○ mRECIST
	  ○ iRECIST

Exploratory Biomarker Objective	**Corresponding Endpoint**
To identify Tissue & Blood based Biomarkers that might be associated with response patterns to Atezolizumab + Bevacizumab and patient outcomes under Atezolizumab+ Bevacizumab treatment	**Tumour biopsy sample** if available to characterize: (1) Tumour immune-cell infiltrate (2) The specificities of T lymphocyte receptors against tumour-specific antigens. (3) Specific expression patterns that may constitute gene signatures with prognostic and/or predictive power of response to Atezolizumab + Bevacizumab. (4) Specific set of mutations associated with response to Atezolizumab + Bevacizumab **Biomarker (blood, plasma, and serum) samples** to evaluate one or more of the following: (1) Cytokines. (2) cfDNA exome sequencing to capture mutations present in circulating DNA. (3) Sequencing the TCRs of circulating T cells to detect specific antigenicity of tumour antigens. (4) Characterization of circulating immune populations which changes could predict response to Atezolizumab + Bevacizumab

***** ALBI assessment grades 1 to 3 (Johnson et al. 2015) are based on calculated ALBI score (log10 bilirubin [µmol/L] × 0.66) + (albumin [g/L] × −0.0852) values as follows: ALBI score ≤ −2.60 = ALBI grade 1; > −2.60 to ≤ −1.39 =ALBI grade 2); and > −1.39 = ALBI grade 3.

ALBI = Albumin-Bilirubin; EASL = European Association for the Study of the Liver; HCC = hepatocellular carcinoma; iRECIST = modified Response Evaluation Criteria in Solid Tumours for immune therapeutics; mRECIST = modified Response Evaluation Criteria in Solid Tumours; ORR = Objective Response Rate; OS = overall survival; PD = progressive disease; PFS = progression-free survival; RECIST v1.1 = Response Evaluation Criteria in Solid Tumours, Version 1.1.

Exploratory Radiomic Objective	Corresponding Endpoint
CT-radiomics analysis towards a more precise evaluation of response to Atezolizumab and Bevacizumab in patients with unresectable hepatocellular carcinoma included.	- To explore correlation of CT-radiomics signatures (including shape, first-order and higher-order texture features) at baseline with response to treatment. - To explore correlation in early changes in CT-radiomics signatures with response to treatment. - To explore inter- and intra- tumour CT-radiomics changes in relation to differences in responder/non-responder lesions and with the patient clinical outcome. - To explore correlation of radiomics features with tumour mutational status to develop radiogenomics phenotypes. - To explore correlation of CT-radiomics features with the tumour microenvironment including tumour immunophenotype by RNA seq and multiparametric immunofluorescence and vascularisation from tumour samples.

Efficacy Objectives

Secondary Efficacy Objective

Efficacy analysis includes both main and other secondary objectives.

All baseline summaries and efficacy analyses will be based on the ITT (intent-to-treat) analysis set defined as all recruited patients.

Time-dependent variables OS, PFS, TTP and Duration of Response (DOR) will be analysed using Kaplan-Meier (K-M) methods and Greewood's formula. Medians and the quartiles with 95% confidence interval will be derived from the K-M curves. Kaplan-Meier plots with a 95% CI for OS, PFS, PPS and DOR will be prepared.

ORR will simply be summarized. The ORR will be calculated as the percentage of patients who have a CR or PR before any evidence of progression. A 95% CI will be derived for the ORR using Wilson score intervals (CIs for a single proportion).

Following disease progression, patients will be followed for survival to evaluate whether the patterns of tumour progression (growth versus new lesion, intrahepatic versus extrahepatic) have a different impact on OS and PPS. OS and PPS will be described based on the following patterns of progression:

- > 20% increase in tumour size against a known baseline lesion (intrahepatic growth [IHG] or extrahepatic growth [EHG])
- new intrahepatic lesion (NIH)
- new extrahepatic lesion (NEH) and/or vascular invasion

To evaluate if post-study treatments have an impact on OS following disease progression, patients will be followed for anti-cancer therapies and survival with a descriptive analysis. In details, number and rate of patients starting second or further lines of treatment will be described indicating time and duration of each post-study

treatment. OS based on the type and duration of each post-study treatments will be described.

To evaluate if reason of treatment withdrawal has impact on OS, OS based on the following reasons of treatment withdrawal will be described:

- Progressive disease (PD) vs adverse event (AE) vs deteriorating liver function/clinical conditions vs radical treatment.

Missing values will be classified and managed using the methods outlined described above.

Continuous and count variables:

- location measures: mean and median;
- dispersion measures: standard deviation and range;
- categorical variables: absolute and relative frequency.

Study Design

a) Description of the Study

This is a Phase IIIb, one arm, multicentre, open - label study designed to primarily evaluate the safety and efficacy of Atezolizumab + Bevacizumab in patients with locally advanced or metastatic HCC who have received no prior systemic treatment.

The treatment schemed for all patients included in the study will be:

- Atezolizumab 1200 mg IV infusions Q3W (dosed in 3-week cycles) + Bevacizumab 15 mg/kg Q3W (dosed in 3-week cycles).

Patients treated with Atezolizumab + Bevacizumab, who transiently withhold or permanently discontinue either Atezolizumab or Bevacizumab, may continue on single-agent therapy as long as the patients are experiencing clinical benefit in the opinion of the investigator and after discussion with the Medical Monitor (i.e., patients transiently withhold or permanently discontinue Bevacizumab for adverse effects may continue Atezolizumab monotherapy and vice versa).

Patients will receive Atezolizumab and/ or Bevacizumab until unacceptable toxicity or loss of clinical benefit as determined by the investigator after an integrated assessment of radiographic and biochemical data, and clinical status (e.g., symptomatic deterioration such as pain secondary to disease).

The patients who are clinically stable will be allowed to continue on treatment beyond initial RECIST v1.1 defined progression, until next assessment (between 4 to 8 weeks later) to ensure patient's suitability for treatment, as recommended by iRECIST criteria.

In the absence of unacceptable toxicity, patients who meet criteria for disease progression per RECIST v1.1 while receiving Atezolizumab and/or Bevacizumab will be permitted to continue the study treatment if they meet all of the following criteria:

- Evidence of clinical benefit, as determined by the investigator following a review of all available data.

- Absence of symptoms and signs (including laboratory values) indicating unequivocal progression of disease.

- Absence of decline in ECOG Performance Status that can be attributed to disease progression.

- Absence of tumour progression at critical anatomical sites (e.g., leptomeningeal disease or brain metastases) that cannot be managed by protocol-allowed medical interventions.

Safety assessments will include the incidence, nature, and severity of adverse events and laboratory abnormalities graded per the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0 (NCI CTCAE v5.0). Laboratory safety assessments will include the regular monitoring of haematology and blood chemistry.

Tumour assessments will be performed at baseline and at regular intervals during study treatment. Additional scans will be performed as clinically indicated. Tumour assessments will continue until disease progression according to RECIST v1.1 criteria and confirmed in the patient's next follow-up (providing there is a further increase of at least 5 mm of target tumour burden or new target lesion or any increase in non-target disease), regardless of whether treatment has been discontinued (e.g., for toxicity). Patients who meet RECIST v1.1 criteria for progression will undergo tumour assessments until loss of clinical benefit. In the absence of disease progression, tumour assessments should continue until consent is withdrawn, death, or the study is terminated by the Sponsor, whichever occurs first.

Following disease progression, patients will be followed for survival and subsequent anti-cancer therapies until death, loss to follow-up, withdrawal of consent, or study termination by Sponsor, whichever occurs first.

Patients my withdraw their consent for study participation but can continue to participate in the study survival follow up program should they wish to do so.

Patient samples, including serum and plasma, will be collected for the exploratory Tissue and Blood Biomarker Plan assessments.

b) Assessment of response and progression

Registration of Response: Tumour Response will be primarily assessed by RECIST 1.1 as a main assessment. Since activity may be detected by appearance

of necrosis, a secondary **exploratory** analysis will include HCC mRECIST and EASL criteria. HCC mRECIST measures the sum of longest diameter of viable tumour and EASL the area of viable tumour.
Registration of response will be also assessed by applying iRECIST rules and compared with other response criteria in terms of OS and PFS.
Bevacizumab induces vasoconstriction and this reduces splanchnic blood flow and thus, hepatic artery blood flow. This may reduce the intensity of contrast uptake that should not be registered as necrosis. In any case, registration of response or of stable disease will not affect in treatment maintenance.
Registration of progression: Tumour Progression will be primarily assessed by RECIST 1.1 and the efficacy results in in terms of OS and PFS will be registered based on the following patterns of progression:

- > 20% increase in tumour size against a known baseline lesion (intrahepatic growth [IHG] or extrahepatic growth [EHG])

- new intrahepatic lesion (NIH)

- new extrahepatic lesion and/or vascular invasion (NEH)

Secondary exploratory analysis will include HCC mRECIST, taking into consideration the RECIST modifications described for SHARP (Reig 2015) for the capture of tumour progression to prevent over registration of progression and improper treatment interruption. Exploratory assessment on OS and PFS will be performed based on the following patterns of progression:

- New intrahepatic nodules will be considered progression if:

  - They exceed 10 mm in diameter and present arterial enhancement at dynamic imaging.

  - Non-specific non-hypervascular nodules ≥ 10 mm (absence of the above definitions) present a doubling tumour size reaching a diameter > 20 mm since its initial detection or showing hyperenhancement during the follow-up.

  - Ascites and pleural effusion reflect progression only if malignant cells are pathology (cytology) proven.

  - Vascular invasion may be classified as progression if expansive and/or displaying arterial enhancement at dynamic imaging.

  - Hilar lymph nodes are considered malignant if the smaller diameter exceeds 20mm and/or are hypervascular with these characteristics confirmed on the next follow up. Growth of existing nodes uses the same cut-offs as other target lesions.

- Registration of progression will be also assessed by applying iRECIST rules and compared with other response criteria in terms of OS and PFS.

- Additional exploratory assessment of the depth of response by means of changes in TL tumour burden and its association with OS and PFS will be analysed.

- Finally, based on the hypothesis that treatment efficacy varies across different metastatic sites, will carry out a comparison of organ-specific – response rate including target lesions from the liver, lungs, lymph nodes and non-target lesions in bones.

c) Criteria for treatment discontinuation

Safety events related to treatment discontinuation is the primary endpoint of the study; this may be reached through several events.

- **Development of SAEs** ≥ grade 3 clinically significant considered related to any study treatment and irrespective of tumour evolution.

- **Tumour progression outside the liver:** this includes confirmed growth in sequential imaging, and metastasis spread in any location, new lymph nodes, new vascular invasion – note that vascular invasion should never be considered a target lesion.) assessed by RECIST v1.1 and confirmed by iRECIST

- **Tumour progression within the liver:** assessed by RECIST v1.1as confirmed by iRECIST for target lesions. However, in case of new intrahepatic (NIH) tumour sites, treatment should not be interrupted upon detection of new nodules at first time. Further growth during follow-up should be registered to decide treatment interruption.

- **Liver function deterioration:**

    - Associated to progression will also represent a criterion for treatment interruption as it will also fit into SAEs ≥ 3.

    - Associated to appearance of ascites in need of treatment, jaundice or encephalopathy will be considered also a criterion for treatment discontinuation.

According to these definitions, treatment may be maintained beyond progression in very specific circumstances following the iRECIST and the refinements related to HCC as per registration of progression.

Number of patients

This study will enrol approximately 100 patients in one arm of treatment.

a) Target Population Inclusion Criteria

Patients must meet the following criteria for study entry:

- Signed Informed Consent Form.
- Age ≥ 18 years at time of signing Informed Consent Form.
- Ability to comply with the study protocol, in the investigator's judgment.
- Locally advanced or metastatic and/or unresectable HCC with diagnosis confirmed by histology or radiologically, following the AASLD criteria.
- Disease that is not amenable to curative surgical and/or locoregional therapies, or progressive disease after surgical and /or locoregional therapies.
- No prior systemic therapy (including systemic investigational agents) for HCC.
- At least one measurable (per RECIST 1.1) untreated lesion detected by CT scan.
- Patients who received prior local therapy such as radiofrequency ablation, percutaneous ethanol or acetic acid injection, cryoablation, high-intensity focused ultrasound, transarterial chemoembolization, transarterial embolization (excluding transarterial radioembolization.) are eligible provided the target lesion(s) have not been previously treated with local therapy or the target lesion(s) within the field of local therapy have subsequently progressed in accordance with RECIST version 1.1: Those patients.
- For those patients who received external beam radiotherapy as prior locoregional therapy should be necessary to wait at least 3 months before they could be included in this study.
- ECOG Performance Status of 0 or 1 within 7 days prior to recruitment.
- Child-Pugh class A with compensated ascites, within 7 days prior to recruitment.
- Patients should submit a pre-treatment tumour tissue sample. If tumour tissue is not available (e.g., depleted for prior diagnostic testing), it is **recommendable** to take a new biopsy if it´s clinically possible. If tumour tissue is available: A formalin-fixed, paraffin-embedded (FFPE) tumour specimen in a paraffin block

(preferred) or a total of 28 slides (12 slides for histologic studies and 2x8 slides for genomic studies) containing unstained, freshly cut, serial sections should be submitted as detailed in the sample manual.

- Adequate haematologic and end-organ function, defined by the following laboratory test results, obtained within 7 days prior to recruitment, unless otherwise specified:

  – ANC ≥ 1.5 × 10^9/L (1500/μL) without granulocyte colony-stimulating factor support.

  – Lymphocyte count ≥ 0.5 × 10^9/L (500/μL).

  – Platelet count ≥ 75 × 10^9/L (75,000/μL) without transfusion.

  – Haemoglobin ≥ 90 g/L (9 g/dL).

  – AST, ALT, and alkaline phosphatase (ALP) ≤ 5 × upper limit of normal (ULN)

  – Serum bilirubin ≤ 3 × ULN.

  – Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 50 mL/min (calculated using the Cockcroft-Gault formula).

  – Serum albumin ≥ 28 g/L (2.8 g/dL) without albumin infusion.

  – INR ≤ 1.5.

  – Urine dipstick for proteinuria < 2+ (within 7 days prior to initiation of study treatment).

    Patients discovered to have ≥ 2+ proteinuria on dipstick urinalysis at baseline should undergo a 24-hour urine collection and must demonstrate < 1 g of protein in 24 hours.

- Resolution of any acute, clinically significant treatment-related toxicity from prior therapy to Grade ≤ 1 prior to study entry.

- Negative HIV test at screening.

- Documented virology status of hepatitis, as confirmed by screening HBV and HCV serology test.

- For patients with active hepatitis B virus (HBV):

HBV DNA < 500 IU/mL obtained within 28 days prior to initiation of study treatment, and

Anti-HBV treatment (per local standard of care, e.g., entecavir) for a minimum of 14 days prior to study entry and willingness to continue treatment for the length of the study.

- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods with a failure rate of < 1% per year during the treatment period and for at least 5 months after the last dose of Atezolizumab and 6 months after the last dose of Bevacizumab. Women must refrain from donating eggs during this same period.

  A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

  Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.

  The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

- For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:

  With female partners of childbearing potential, men must remain abstinent or use a condom plus an additional contraceptive method that together result in a failure rate of < 1% per year during the treatment period and for 6 months after the last dose of Bevacizumab. Men must refrain from donating sperm during this same period.

  With pregnant female partners, men must remain abstinent or use a condom during the treatment period and for 6 months after the last dose of Bevacizumab to avoid exposing the embryo.

  The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

b) Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

- Active or history of autoimmune disease or immune deficiency, including, but not limited to, myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis, with the **following exceptions**:

    - Patients with a history of autoimmune-related hypothyroidism who are on thyroid-replacement hormone treatment are **eligible for the study.**

    - Patients with controlled Type 1 diabetes mellitus who are on an insulin regimen are **eligible for the study.**

    - Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are **eligible for the study** provided all of following conditions are met:

        - Rash must cover < 10% of body surface area.

        - Disease is well controlled at baseline and requires only low-potency topical corticosteroids.

        - No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high-potency or oral corticosteroids within the previous 12 months.

- History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan.

    - History of radiation pneumonitis in the radiation field (fibrosis) **is permitted**.

- Known active tuberculosis.

- Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident)

within 3 months prior to initiation of study treatment, unstable arrhythmia, or unstable angina.

- History of congenital long QT syndrome or corrected QT interval > 500 ms (calculated with use of the Fridericia method) at screening.

- History of uncorrectable electrolyte disorder affecting serum levels of potassium, calcium, or magnesium within the previous 12 months.

- Major surgical procedure, other than for diagnosis, within 4 weeks prior to initiation of study treatment, or anticipation of need for a major surgical procedure during the study.

- History of malignancy other than HCC within 5 years prior to screening, with the **exception of** malignancies with a negligible risk of metastasis or death (e.g., 5-year OS rate > 90%), such as adequately treated carcinoma in situ of the cervix, non-melanoma skin carcinoma, localized prostate cancer, ductal carcinoma in situ, Stage I uterine cancer or bladder carcinoma in situ.

- Severe infection within 4 weeks prior to initiation of study treatment, including, but not limited to, hospitalization for complications of infection, bacteraemia, or severe pneumonia.

- Treatment with therapeutic oral or IV antibiotics within 2 weeks prior to initiation of study treatment.

    - Patients receiving prophylactic antibiotics (e.g., to prevent a urinary tract infection or chronic obstructive pulmonary disease exacerbation) or patients receiving Rifaximin as prevention of encephalopathy are **eligible for the study**.

- Prior allogeneic stem cell or solid organ transplantation.

- Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that contraindicates the use of an investigational drug, may affect the interpretation of the results, or may render the patient at high risk from treatment complications.

- Treatment with a live, attenuated vaccine within 4 weeks prior to initiation of study treatment, or anticipation of need for such a vaccine during Atezolizumab treatment or within 5 months after the last dose of Atezolizumab.

- History of severe allergic anaphylactic reactions to chimeric or humanized antibodies or fusion proteins.

- Known hypersensitivity to Chinese hamster ovary cell products or to any component of the Atezolizumab or Bevacizumab formulation.

- Pregnancy or breastfeeding, or intention of becoming pregnant during study treatment or within at least 5 months after the last dose of Atezolizumab and 6 months after the last dose of Bevacizumab.

    - Women of childbearing potential must have a negative serum pregnancy test result within 14 days prior to initiation of study treatment.

- Known fibrolamellar HCC, sarcomatoid HCC, or mixed cholangiocarcinoma and HCC.

- Untreated or incompletely treated oesophageal and/or gastric varices with bleeding or high-risk for bleeding.

    - Patients must undergo an esophagogastroduodenoscopy (EGD), and all size of varices (small to large) must be assessed and treated per local standard of care prior to enrolment. Patients who have undergone an EGD within 6 months of prior to initiation of study treatment do not need to repeat the procedure provided they had no active varices or varices at high risk of bleeding.

    - A prior bleeding event due to oesophageal and/or gastric varices within 6 months prior to initiation of study treatment.

- Clinically evident moderate or severe ascites that might require any treatment.

- At least one clinically evident episode of encephalopathy in the past three months.

- Co-infection of HBV and HCV.

    - Patients with a history of HCV infection but who are negative for HCV RNA by PCR will be considered non-infected with HCV.

- Symptomatic, untreated, or actively progressing central nervous system (CNS) metastases.

- Uncontrolled tumour-related pain.

    - Patients requiring pain medication must be on a stable regimen at study entry.

    - Symptomatic lesions (e.g., bone metastases or metastases causing nerve impingement) amenable to palliative radiotherapy should be treated prior to enrolment. Patients should be recovered from the effects of radiation. There is no required minimum recovery period.

    - Asymptomatic metastatic lesions that would likely cause functional deficits or intractable pain with further growth (e.g., epidural metastasis that is not currently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to enrolment.

- Uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures (once monthly or more frequently).

- Uncontrolled or symptomatic hypercalcemia (ionized calcium $>$ 1.5 mmol/L, calcium $>$ 12 mg/dL or corrected serum calcium $>$ ULN).

- Treatment with investigational therapy within 28 days prior to initiation of study treatment.

- Treatment with strong CYP3A4 inducers within 14 days prior to initiation of study treatment, including rifampin (and its analogues) or St. John's wort.

- Prior treatment with CD137 agonists or immune checkpoint blockade therapies, including anti−CTLA-4, anti−PD-1, and anti−PD-L1 therapeutic antibodies.

- Treatment with systemic immunostimulatory agents (including, but not limited to, interferon and interleukin 2 [IL-2]) within 4 weeks or 5 half-lives of the drug (whichever is longer) prior to initiation of study treatment.

- Treatment with systemic immunosuppressive medication (including, but not limited to, corticosteroids, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti−TNF-α agents) within 2 weeks prior to initiation of study treatment, or anticipation of need for systemic immunosuppressive medication during study treatment, with the following exceptions:

    - Patients who received acute, low-dose systemic immunosuppressant medication or a one-time pulse dose of systemic immunosuppressant

medication (e.g., 48 hours of corticosteroids for a contrast allergy) are **eligible for the study** after Medical Monitor approval has been obtained.

  - Patients who received mineralocorticoids (e.g., fludrocortisone), corticosteroids for chronic obstructive pulmonary disease (COPD) or asthma, or low-dose corticosteroids (e.g., prednisone 10mg or equivalent) for orthostatic hypotension or adrenal insufficiency **are eligible for the study**.

- Inadequately controlled arterial hypertension (defined as systolic blood pressure (BP) $\geq$ 150 mmHg and/or diastolic blood pressure $>$ 100 mmHg), based on an average of $\geq$ 3 BP readings on $\geq$ 2 sessions.

  - Anti-hypertensive therapy to achieve these parameters is **allowable.**

- Prior history of hypertensive crisis or hypertensive encephalopathy.

- Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to initiation of study treatment.

- History of haemoptysis ($\geq$ 2.5 mL of bright red blood per episode) within 1 month prior to initiation of study treatment.

- Evidence of bleeding diathesis or significant coagulopathy.

- Current or recent (within 10 days of first dose of study treatment) use of aspirin ($>$ 325 mg/day) or treatment with dipyramidole, ticlopidine, clopidogrel, and cilostazol.

- Current or recent (within 10 days prior to study treatment start) use of full-dose oral or parenteral anticoagulants or thrombolytic agents for therapeutic (as opposed to prophylactic) purpose.

  - Prophylactic anticoagulation for the patency of venous access devices is allowed provided the activity of the agent results in an INR $<$ 1.5 × ULN and aPTT is within normal limits within 14 days prior to initiation of study treatment.

  - For prophylactic use of anticoagulants or thrombolytic therapies, local label approved dose levels may be used.

- Core biopsy or other minor surgical procedure, excluding placement of a vascular access device, within 3 days prior to the first dose of Bevacizumab.

- History of abdominal or tracheoesophageal fistula, gastrointestinal (GI) perforation, or intra-abdominal abscess within 6 months prior to initiation of study treatment.

- History of intestinal obstruction and/or clinical signs or symptoms of GI obstruction including sub-occlusive disease related to the underlying disease or requirement for routine parenteral hydration, parenteral nutrition, or tube feeding prior to initiation of study treatment.

    - Patients with signs/symptoms of sub-/occlusive syndrome/intestinal obstruction at time of initial diagnosis **may be enrolled** if they had received definitive (surgical) treatment for symptom resolution.

- Evidence of abdominal free air that is not explained by paracentesis or recent surgical procedure.

- Serious, non-healing or dehiscing wound, active ulcer, or untreated bone fracture.

- Metastatic disease that involves major airways or blood vessels like vena cava, or centrally located mediastinal tumour masses (< 30 mm from the carina) of large volume.

    - Patients with vascular invasion of the portal or hepatic veins may be enrolled.

- History of intra-abdominal inflammatory process within 6 months prior to initiation of study treatment, including but not limited to complicated active peptic ulcer disease, diverticulitis, or colitis.

- Radiotherapy within 28 days and abdominal/ pelvic radiotherapy within 60 days prior to initiation of study treatment, except palliative radiotherapy to bone lesions within 7 days prior to initiation of study treatment

- Local therapy to liver (e.g., radiofrequency ablation, percutaneous ethanol or acetic acid injection, cryoablation, high-intensity focused ultrasound, transarterial chemoembolization, transarterial embolization, transarterial radioembolization etc.) within 28 days prior to initiation of study treatment or non-recovery from side effects of any such procedure

- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to initiation of study treatment, or abdominal surgery, abdominal interventions or significant abdominal traumatic injury within 60 days prior to initiation of study treatment or anticipation of need for major surgical procedure during the course of the study or non-recovery from side effects of any such procedure

- Chronic daily treatment with a non-steroidal anti-inflammatory drug (NSAID)

    - Occasional use of NSAIDs for the symptomatic relief of medical conditions such as headache or fever **is allowed**.

End of Study and Length of Study

a) End of Study

The end of study will occur when all enrolled patients have either died, withdrawn consent, are lost to follow up, or have been followed for 24 months since the last study patient is enrolled, whichever occurs first.
In addition, the Sponsor may decide to terminate the study at any time.

b) Length of Study

The total length of the study, from screening of the first patient to the end of the study is approximately 3 years (12 months enrolment).

Investigational Medicinal Products

Patients will receive treatment as outlined below until unacceptable toxicity or loss of clinical benefit as determined by the investigator after an integrated assessment of radiographic and biochemical data, and clinical status (e.g., symptomatic deterioration such as pain secondary to disease). For those patients clinically stable to continue on treatment beyond initial RECIST v1.1 defined progression, will be allowed to continue on treatment until next assessment (between 4 to 8 weeks later) to ensure patient's suitability for treatment, as is it recommended by iRECIST criteria.

Treatment Arm	Cycle Length	Dose, Route, and Regimen (drugs listed in order of administration)
A	21 days	Atezolizumab 1200 mg IV on Day 1
		Bevacizumab 15 mg/kg IV on Day 1

Comparator

Not applicable

Non-Investigational Medicinal Products

Not applicable

Statistical Methods

This is not a hypothesis testing study but an exploratory study with predefined precision of estimates for key safety parameters for sample size determination; there are no formal statistical hypotheses tests to be tested, and there will be no adjustments for multiplicity of endpoints or within-subgroups comparisons.

The safety analysis population will consist of all enrolled patients who received at least one full or partial dose of study treatment.

Verbatim adverse event terms will be mapped to Medical Dictionary for Regulatory Activities (MedDRA) thesaurus terms, and adverse event severity will be graded according to NCI CTCAE v5.0.

Drug exposure will be summarized by descriptive statistics to include treatment duration, number of doses, and dose intensity.

The following events occurring during or after the first dose of study treatment will be summarized by NCI CTCAE v5.0:

- All Adverse Events (AEs).
- All severe AEs (Grade 3-4).
- All treatment related AEs.
- All severe treatment related AEs (Grade 3 -4).
- All serious AEs.
- All treatment related serious AEs.
- Immune-related AEs.
- All severe immune-mediated AEs (Grade 3-4).
- All adverse events leading to withdrawal from any component.
- All adverse events leading to withdrawal from Atezolizumab.
- All adverse events leading to withdrawal from Bevacizumab.
- All Grade 5 AEs.
- All treatment related Grade 5 AE.
- All adverse event of special interest (AESIs) of Atezolizumab.
- All severe AESIs of Atezolizumab (Grade 3-4).
- All adverse events leading to temporary interruption of any component.
- All adverse events leading to temporary interruption of Atezolizumab.
- All adverse events leading to temporary interruption of Bevacizumab.

Multiple occurrences of the same event will be counted once at the maximum severity.

Laboratory data with values outside the normal ranges will be identified. In addition, selected laboratory data will be summarized by grade.

Descriptive statistics will be used to summarize changes in vital signs.

Deaths with causes of death reported during the study will be summarized.

Additional analyses may be performed as indicated.

Determination of Sample Size

This is a Phase IIIb, one arm, prospective multicentre, open - label study designed to evaluate the safety and efficacy of Atezolizumab + Bevacizumab in patients with untreated or unsuitable for locoregional treatments hepatocellular carcinoma who have received no prior systemic treatment.

Approximately 100 patients will be recruited in 12 months, and we estimate 25 sites will participate in the study.

There is no formal statistical hypothesis, hence all safety (primary) endpoints results will be presented by 95% confidence intervals and descriptively explained.

For proportions exceeding 10-20%, estimates of the observed frequencies will have an acceptable precision (e.g., 95% confidence limit ± 5-6%) while for rare AE's, rather imprecise estimates will be obtained. For example, with an expected frequency of 1-2% (2-3 events in the 100 patients) the 95% CI of the observed proportion will cover a range of values compatible with the expected results with a 12-fold or greater of variation.

For the same reason, a 2-3-fold increase over the expected frequencies might easily be observed by chance. As a consequence, for rare AEs the results of this study will have to be interpreted with caution.

Exploratory Biomarker Analysis for Tissue and Blood biomarker Plan

Exploratory biomarker analyses will be performed in an effort to understand the association of tissue or blood-based biomarkers with response to Atezolizumab + Bevacizumab and increase the understanding of HCC disease evolution under Atezolizumab + Bevacizumab treatment. This may include appropriate multivariate analyses. Blood based samples will be obtained at several timepoints of the study: Screening, Cycle 2 (Week 3) and Cycle 3 (Week 6).

The exploratory biomarker analyses of the Tissue and Blood Biomarker Plan include the following assessments:

- Multiplex Immunofluorescence T/B series & PDL1 (6-Plex+DAPI): S5 Oncomine™ TCR Beta-SR Assay Sequencing, NGS-based somatic mutation panel, and RNA sequencing, will be performed on tumour biopsy samples to characterize:
  - the tumour immune cell infiltrate
  - the specificities of T lymphocyte receptors against tumour-specific antigens
  - Specific expression patterns that may constitute gene signatures with prognostic and/or predictive power of response to Atezolizumab + Bevacizumab
  - Specific set of mutations associated with response to Atezolizumab + Bevacizumab
- Primer Extension Assay (PEA) identification of plasma cytokines and chemokines (384-plex, Olink), cfTNA Whole Exome Sequencing, Euroflow Panel, Whole Blood RNA sequencing andS5 Oncomine™ TCR Beta-SR Assay Sequencing to evaluate potential non-invasive biomarkers on blood, plasma or serum samples, such us:
  - Cytokines
  - cfDNA exome sequencing to capture mutations present in circulating DNA
  - Flow Cytometry and whole blood cell RNAseq to characterize circulating immune populations which changes could predict response to Atezolizumab + Bevacizumab
  - sequencing the TCRs of circulating T cells to detect specific antigenicity of tumour antigens

Exploratory Radiomic Analysis Plan

An exploratory Radiomic Analysis Plan will be performed to obtain a more precise evaluation of response to Atezolizumab + Bevacizumab in patients with unresectable hepatocellular carcinoma to correlate early changes in CT-radiomics signatures with PFS and OS. The Radiomic Analysis Plan will also evaluate the use of radiomics features for non-invasive prediction of immuno-oncologic characteristics based on biopsied lesions.

Target lesions corresponding to the primary tumour and metastases will be selected for radiomics features extraction. Target lesions will be selected based on size (≥ 1cm in large diameter for solid lesions and ≥ 1.5 cm in short diameter for nodes), preferably well-defined and avoiding for cystic changes and cavitation.

Multiple radiomics features from the target lesions will be extracted, including:

1. First order features (energy, entropy, minimum, percentiles, maximum, mean, median, interquartile range, range, standard deviation, skewness, kurtosis, among others).

2. Shape Features (volume, surface area, sphericity, among others).

3. Different filters for texture analysis will be applied for higher order statistics texture analysis, including: Gray Level Co-occurrence Matrix (GLCM) and Gray Level Run Length Matrix (GLRLM) features among others.

Interim Analyses

One interim analysis of safety is planned at the time of 50 recruited patients. This analysis is estimated to occur at approximately 6 months after the first patient was included in the study. The primary intent of the interim analysis is to allow the study to stop early if signs of safety risks for the patients are detected.

Appendix 2
Schedule of Assessments

Assessment Window (Days) [a]	Screening [b]			Treatment Phase (Q3W)	Treatment Discontinuation [c]	Survival Follow-Up
	–28 to –1	–14 to –1	-7 to -1	Day 1 of Each Cycle [c]	≤ 30 Days after Last Dose	
Signed Informed Consent Form(s) [b]	x					
Review of eligibility criteria	x					
Medical, surgical, and cancer histories, including demographic information [d]	x					
Complete physical examination [e]	x					
Limited physical examination [f]				x [g]	x	
ECOG Performance Status [g]			x	x [g]	x	
Child Pugh			x			
Radiological tumour assessment [h]	x			See footnote [h]	x	x
Vital signs [j]	x			x	x	
Weight	x			x [k]	x	
Height	x					
12-lead ECG [i]	x			Perform as clinically indicated		
Oesophageal and/or gastric varices assessment (EGD [l])				Perform as clinically indicated		
Haematology [m, y]			x	x [g]	x	
Serum chemistry [n, y]			x	x [g]	x	
HIV, HBV, HCV serology [o]	X					
Quantitative HBsAg, HBV DNA, HCV RNA [p]	x			x [p]	x [p]	
Alpha fetoprotein (local laboratory)	x			x	x	

Coagulation panel (aPTT, INR) [y]			**x**	**x [g]**	**x**	
Urinalysis [q, y]			**x**	**x [g]**	**x**	
TSH, free T3, free T4	**x**			**Cycles 5, 9, 13, etc. (every 4 cycles)**	**x**	
Pregnancy test		**x [r]**		**x [s]**	**x**	
Concomitant medications [t]		**x**		**x**	**x**	
Adverse events [u]	**x**			**x**	**x**	
Identification and quantification of health care resources consumed for managing Grade 3 and 4 AEs				**x**	**x**	
Study treatment infusion [v]				**x**		
Survival and anti-cancer therapy follow-up [w]						**x**
Tumour biopsy sample (if available) for biomarker plan	**x**					
Blood samples for biomarker plan	**x**			**Day 1 of Cycle 2 and then Day 1 of Cycle 3**		
CT Scan for Radiomic plan	**x**			**Week 6 (prior cycle 3) & week 12 (prior cycle 5)**	**x[x]**	

CT = computed tomography; EGD = esophagogastroduodenoscopy; HBcAb = hepatitis B core antibody; HBsAb = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; MRI = magnetic resonance imaging; PET = positron emission tomography; Q3W = every 3 weeks; RECIST v1.1 = Response Evaluation Criteria in Solid Tumours, Version 1.1; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone.

Note: Assessments scheduled on the days of study treatment infusions should be performed before the infusion unless otherwise noted. Each cycle is 21 days in length.

[a] All visits and infusions may be administered with a window of ± 3 days.

[b] Written informed consent can be obtained up to 30 days prior to study entry and is required before performing any study-specific tests or procedures. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and per protocol relevant window may be used for screening assessments rather than repeating such tests. Screening local laboratory assessments obtained ≤ 96 hours prior to the initiation of study treatment do not have to be repeated for Cycle 1. Test results should be reviewed prior to administration of study treatment.

[c] Patients will be asked to return to the clinic 30 days after the last dose of study treatment for an end-of-treatment visit. After this visit, serious adverse events and protocol defined adverse events of special interest, regardless of attribution, will be recorded until 90 days after the last dose of study treatment or until initiation of another systemic anti-cancer therapy, whichever occurs first. Ongoing adverse events thought to

be related to study treatment will be followed until the event has resolved to baseline grade or better, the event is assessed by the investigator as stable, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it is determined that the study treatment or participation is not the cause of the adverse event. Scans performed within 6 weeks prior to the treatment discontinuation visit do not need to be repeated.

[d] Cancer history includes stage, date of diagnosis, and prior anti-tumour treatment. Demographic information includes age and self-reported race/ethnicity. Reproductive status and smoking history should also be captured.

[e] A complete physical examination at screening should include the evaluation of head, eye, ear, nose, and throat and cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems. Changes in abnormalities noted at baseline should be recorded at the end of the visit. New or worsened abnormalities should be recorded as adverse events if appropriate.

[f] A limited physical examination will be performed at other visits to assess changes from baseline abnormalities and any new abnormalities and to evaluate patient reported symptoms. New or worsened abnormalities should be recorded as adverse events if appropriate.

[g] ECOG Performance Status at screening must be recorded between day -7 and day -1 before Day 1 of Cycle 1. ECOG Performance Status, limited physical examination and local laboratory assessments may be obtained ≤ 96 hours before Day 1 of each cycle.

[h] All measurable and evaluable lesions should be assessed and documented at the screening visit. Radiologic imaging performed during the screening period should consist of 1) CT and/or MRI of the chest/abdomen/pelvis and brain, 2) bone scan or PET scan as clinically indicated, and 3) any other imaging studies (CT scan of the neck, plain films, etc.) as clinically indicated by the treating physician. The same radiographic procedures and technique must be used throughout the study for each patient (e.g., if the patient had CT chest/abdomen/pelvis performer during screening, then she should subsequently undergo CT performed using the same radiologic protocol throughout the remainder of the study). Results must be reviewed by the investigator before dosing at the next cycle. Tumour assessments will be performed at baseline, every 6 weeks (± 1 week) for the first 54 weeks following the initiation of study treatment, and every 12 weeks (± 1 week) thereafter, with additional scans as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumour evaluation. Tumour response will be evaluated by the investigator using RECIST Version 1.1. In the absence of disease progression, tumour assessments should continue regardless of whether patients discontinue study treatment or start new anti-cancer treatment, unless the patient dies, withdraws consent, or the study is terminated by the Sponsor, whichever occurs first.

[i] Patients should be resting and in a supine position for at least 10 minutes prior to each ECG collection.

[j] Vital signs include heart rate, respiratory rate, blood pressure, and temperature. On days of study treatment administration (Atezolizumab and Bevacizumab), the patient's vital signs should be determined up to 60 minutes before all infusions. Vital signs will be measured at the end of Bevacizumab infusion and 2 (± 1) hours after end of the infusion and will also be collected during and after every infusion of Atezolizumab if clinically indicated.

[k] The dose of Bevacizumab will be based on the patient's weight (in kilograms) measured ≤ 14 days prior to baseline (the initiation of study treatment) and will remain the same throughout the study unless there is a weight change of > 10% from baseline. If re-baseline is needed the latest baseline weight should always be used to calculate percent change in weight for all subsequent doses.

[l] All patients must undergo an EGD within 6 months of starting the study and all size of varices (small to large) must be assessed and treated per local standard of care prior to enrolment.

[m] Haematology consists of CBC, including RBC count, haemoglobin, haematocrit, WBC count with differential (neutrophils, eosinophils, lymphocytes, monocytes, basophils, and other cells), and platelet count. A manual differential can be done if clinically indicated.

[n] Serum chemistry includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, magnesium, chloride, glucose, BUN or urea, creatinine, total protein, albumin, phosphorus, calcium, total bilirubin, alkaline phosphatase, ALT, AST, LDH, amylase and lipase.

[o] All patients will be tested for HIV locally prior to the inclusion into the study and if not in contradiction with local legislation; HIV-positive patients will be excluded from the clinical study. HBsag, HBsAb, and total HBcAb should be collected during screening and tested locally. HBV DNA must be collected prior to Cycle 1, Day 1 in patients who have negative serology for HBsAg and HBsAb tests and a total HBcAb.

[p] Only if patient tests serologically positive for HBsAg, HBcAb, quantitative HBsAg and HBV DNA will be tested during screening; Cycle 5, Day 1; Cycle 9, Day 1; and at treatment discontinuation. Quantitative HBsAg will be tested locally. If a patient tests positive for HCV antibody at screening, quantitative HCV RNA must be tested locally at screening, Cycle 5 Day 1, Cycle 9 Day 1, and at treatment discontinuation.

[q] Urine dipstick includes specific gravity, pH, glucose, protein, ketones, and blood and should be repeated before every cycle during treatment. Urine dipstick for proteinuria must be < 2+ within 7 days prior to initiation of study treatment. Patients discovered to have ≥ 2+ proteinuria on dipstick urinalysis at baseline should undergo a 24-hour urine collection and must demonstrate < 1 g of protein in 24 hours.

[r] Serum pregnancy test within 14 days before Cycle 1, Day 1.

[s] Urine pregnancy test: if a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test.

[t] Concomitant medications include any prescription medications or over-the-counter medications. At screening, any medications the patient has used within the 7 days prior to initiation of study treatment should be documented. At subsequent visits, changes to current medications or medications used since the last documentation of medications will be recorded.

[u] After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until 30 days after the last dose of study treatment or until initiation of another anti-cancer therapy, whichever occurs first. Serious adverse events and adverse events of special interest will continue to be reported until 90 days after the last dose of study treatment or until initiation of new anti-cancer therapy, whichever occurs first. After this period, investigators should report any serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, new *systemic* anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it is determined that the study treatment or participation is not the cause of the adverse event. Every effort should be made to follow all serious adverse events considered to be related to study drug or study-related procedures until a final outcome can be reported.

[v] The initial dose of Atezolizumab will be delivered over 60 (± 15) minutes. If the first infusion is tolerated without infusion-associated adverse events, the second infusion may be delivered over 30 (± 10) minutes. If the 30-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 (± 10) minutes. The initial dose of Bevacizumab will be delivered over 90 (± 15) minutes. If the first infusion is tolerated without infusion-associated adverse events, the second infusion may be delivered over 60 (± 10) minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 (± 10) minutes. Atezolizumab will be administered first followed by Bevacizumab, with a minimum of 5 minutes between dosing. In the absence of unacceptable toxicity, patients may continue the study treatment until there is evidence of disease progression or lack of clinical benefit.

[w] Survival follow-up information will be collected via telephone calls, patient medical records, and/or clinic visits approximately every 3 months (± 21 days) until death, loss to follow-up, or until study termination by the Sponsor. All patients will be followed for survival and new anti-cancer therapy (including targeted therapy and immunotherapy) information unless the patient requests to be withdrawn from follow-up; this request must be documented in the source documents and signed by the investigator. If the patient withdraws from study, the study staff may use a public information source (e.g., county records) to obtain information about survival status.

[X] Progression Scan.

[y] Local laboratory assessments from each cycle must be reviewed prior to study treatment administration for each cycle.

Appendix 3
Contact of external laboratories

This appendix includes the contact data of the investigators/ external laboratories that are going to provide information to be used in the study.

- Tumor responses according to HCC mRECIST, and EASL

Dr. [redacted]
Email: [redacted]
Hospital Clínic i Provincial
Barcelona
Spain

- Contact information for radiomic analyses::

[redacted]
Email: [redacted]
Vall d'Hebron Institute of Oncology
Barcelona
Spain

- Contact information for biomarker analyses:

Dr. [redacted]
Email: [redacted]
[redacted]
Clinica Universidad de Navarra
Pamplona
Spain

Appendix 4
Hepatic Adverse Events

Hepatic Adverse Event	SOC Code	HLGT Code	HLT Code	PT Code
Hepatobiliary disorders	10019805			
Hepatobiliary investigations		10019809		
Hepatobiliary therapeutic procedures		10019818		
Hepatobiliary neoplasms malignant and unspecified		10019815		
Hepatic and biliary neoplasms benign		10019813		
Gastrointestinal haemorrhages NEC		10017959		
Hepatobiliary and spleen infections			10064462	
Gastrointestinal and hepatobiliary procedural complications			10017927	
Thrombocytopenias			10043555	
Oesophageal varices			10030209	
Oesophageal therapeutic procedures			10030179	
Gastrointestinal haemorrhages			10052742	

Gastrointestinal haemorrhages NEC			10017959	
Platelet count decreased				10035528
Ascites				10003445
Bacterascites				10068547
Biliary ascites				10074150
Haemorrhagic ascites				10059766
Malignant ascites				10025538
Hepatic encephalopathy				10019660
Hepatic encephalopathy prophylaxis				10066599
Spontaneous bacterial peritonitis				10061135
Oesophageal varices haemorrhage				10030210
Upper gastrointestinal haemorrhage				10046274
Gastric varices haemorrhage				10057572
Oesophageal haemorrhage				10030172

Bleeding varicose vein				10005144
Haematemesis				10018830

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