STUDY PROTOCOL MANIFEST-HCC-RO

A MULTI-CENTRIC, RETROSPECTIVE, REAL-WORLD STUDY ON FIRST-LINE THERAPY WITH ATEZOLIZUMAB PLUS BEVACIZUMAB FOR ADVANCED OR UNRESECTABLE HEPATOCELLULAR CARCINOMA IN ROMANIAN POPULATION

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The study will be conducted as specified in this protocol and in compliance with Good Clinical Practice (GCP) and other applicable regulatory requirements

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GLOSSARY OF TERMS

Abbreviation	Term
AE	Adverse Events
ALBI	Albumin-bilirubin Score
ALP	Alkaline Phosphatase
AFP	Alfa-Fetoprotein
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATZ	Atezolizumab
ATZ plus BEV	Atezolizumab plus Bevacizumab
BCLC	Barcelona Clinic Liver Cancer
BEV	Bevacizumab
BMI	Body Mass Index
CBR	Clinical Benefit Rate
CI	Confidence Interval
CP A, B, C	Child-Pugh A, B or C
CR	Complete Response
CRO	Contract Research Organization
CSP	Clinical Study Protocol
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease Control Rate
DOR	Duration of Response
DP	Disease Progression
EASL	European Association for the Study of the Liver
EBL	Endoscopic Band Ligation
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EGD	Esophagogastroduodenoscopy
EHS	Extrahepatic Spread
GGT	Gamma Glutaryl Transferase
HBsAg	Hepatitis B Virus Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus

HR	Hazard Ratio
pHTA	Portal Hypertension
iCCA	Intrahepatic Cholangiocarcinoma
ICI	Immune Checkpoint Inhibitor
IHC	Immunohistochemistry
INR	Internationalized Ratio
ITT	Intention-to-Treat
MA	Marketing Authorization
MELD	Model for End-Stage Liver Disease
mRECIST 1.1.	Modified Response Evaluation Criteria in Solid Tumors, version 1.1
MRI	Magnetic Resonance Imaging
MVI	Microvascular Invasion
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
ORR	Objective Response Rate
OS	Overall Survival
PFS	Progression-Free Survival
PI	Principal Investigator
PR	Partial Response
PP	Per-Protocol
PPS	Post Progression Survival
RFS	Recurrence Free Survival
RWD	Real World Data
RWS	Real World study
SIRT	Selective Internal Radiotherapy
SmPC	Summary of Product Characteristic
SD	Stable Disease
TACE	Transarterial Chemoembolization
T2DM	Type 2 Diabetes Mellitus
TEAE	Treatment-Emergent Adverse Event
TBL	Total Bilirubin
TTP	Time to Progression
uHCC	Unresected Hepatocellular Carcinoma
US	Ultrasound
VEGF	Vascular Endothelial Growth Factor

1 GENERAL

1.1 Background

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most frequent cause of cancer-related death globally. (1,2)

Patients with cirrhosis are at highest risk of developing this malignant disease, and the association between liver cirrhosis and HCC is accountable for a high rate of treatment failure. Survival data reported from Europe and United States show that HCC is associated with a very poor prognosis, with the 5-year survival around 5% in most countries. (3)

The geographic variability in the incidence of primary liver cancer is largely explained by the distribution and the natural history of the hepatitis B and C viruses. Etiologic factors for HCC tend to differentiate in developing countries. (4)

With the incidence of obesity, type II diabetes mellitus (T2DM) and metabolic syndrome continuing to increase in Europe, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which can lead to fibrosis and cirrhosis, may become the most common cause of HCC in developed countries in the near future. (5,6)

While early-stage HCC can be treated with resection, liver transplantation, or ablation, the majority of patients are diagnosed with unresectable disease, which is associated with a poor prognosis. (7)

Immuno-oncology-based regimens have emerged as the standard of care in the palliative systemic treatment of HCC, significantly improving survival outcomes compared to traditional targeted therapies. (8,9) Statistically significant survival benefits were seen with atezolizumab (ATZ) plus bevacizumab (BEV) and sintilimab-bevacizumab biosimilar as well as durvalumab-tremelimumab and camrelizumab-rivoceranib combinations, as new data continues to emerge. (9)

The combination of immune checkpoint inhibitors (ICI) and anti-VEGF agents has demonstrated superior efficacy in advanced disease, starting with IMbrave050 and leading to their widespread adoption in the HCC arsenal. (10)

IMbrave050

In *The Lancet*, Shukui Qin and colleagues reported the first results of their interim analysis of the IMbrave050 trial, comparing ATZ plus BEV versus active surveillance after curative-intent resection or ablation in HCC, and reporting positive RFS, becoming the first trial to do so. (11) The study recruited adult patients (N=668) with high-risk surgically resected or ablated HCC, admitted from 134 hospitals and medical centers in 26 countries in four WHO regions (European region, region of the Americas, South-East Asia region, and Western Pacific region). Patients were randomly assigned to receive intravenous 1200 mg atezolizumab plus 15 mg/kg bevacizumab every 3 weeks for 17 cycles (12 months) or to active surveillance.

Results showed that the combination therapy significantly reduced the risk of recurrence compared to active surveillance. A clinically meaningful improvement in RFS was observed, supporting the potential role of immunotherapy in the adjuvant setting. However, overall survival (OS) data were immature at that time.

The AE profile revealed that careful monitoring remains essential, especially for immune-related toxicities and VEGF-associated risks such as bleeding and hypertension.

The study provided early evidence of the overall survival (OS) benefit, suggesting a trend toward improved survival with ATZ plus BEV.

IMbrave150

IMbrave150 (NCT03434379), a global, open-label, phase 3 trial, enrolled patients with unresectable HCC who had not previously received systemic treatment were randomly assigned in a 2:1 ratio to receive either ATZ plus BEV or sorafenib until unacceptable toxic effects occurred or there was a loss of clinical benefit. The co-primary end points were OS and PFS in the intention-to-treat (ITT) population, as assessed at an independent review facility according to RECIST 1.1. (12) In patients with unresectable HCC, ATZ plus BEV resulted in better OS and PFS outcomes than sorafenib.

On 20 September 2017, Tecentriq® (atezolizumab) was approved in Europe (EMEA/H/C/004143) for several cancer indications, including its landmark approval for HCC, as first-line treatment of unresectable or metastatic HCC in combination with BEV, based on the results of IMbrave150 trial, which demonstrated superior OS and PFS compared to sorafenib.

Cheng et colleagues reported the comprehensive results after updated efficacy and safety data from IMbrave150 (NCT03434379). After longer follow-up, ATZ plus BEV maintained clinically meaningful survival benefits over sorafenib and had a safety profile consistent with the primary analysis. (13)

On 01 August 2022, treatment with Tecentriq was reimbursed in Romania, according to the amended list of therapeutic protocols issued by the Ministry of Health and National Health Insurance System.

Several registries and national databases (e.g., GIDEON, REFLECT, Flatiron Health, real-world cohorts in Japan, Europe, and the U.S.) have provided early insights into real-world outcomes of ATZ plus BEV. (14–17)

This retrospective, multicentric study will be coordinated by Dr. Adrian Radu Vidra (Lead Investigator) from Regional Institute of Gastroenterology and Hepatology “Prof. Dr. Octavian Fodor” Cluj-Napoca (Coordinating Site) to further investigate the clinical and demographic profile of patients receiving first-line treatment with atezolizumab and bevacizumab in the real-world setting of clinical practice from Romania.

1.2 Identification of the Clinical Study Protocol

1.2.1 Title of the Clinical Study Protocol

A Multi-Centric, Retrospective, Real-World Study on First-Line Therapy with Atezolizumab Plus Bevacizumab for Advanced or Unresectable Hepatocellular Carcinoma in Romanian Population **(MANIFEST-HCC-RO).**

1.2.2 Reference number identifying the specific clinical study, if any;

This study and its details are registered in clinicaltrials.gov with ID number NCT06903663.

1.2.3 Version or date of the Clinical Study Protocol

The present protocol is CSP version 1.0 dated 10 Jun 2025.

1.2.4 Summary of the revision history in the case of amendments

Not yet applicable.

1.3 Sponsor

The Sponsor of this present study is **Dr. Adrian Radu Vidra** from **Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" Cluj-Napoca**.

The contact details of the Sponsor are the following:

Sponsor Name and Leading Institution:	**Dr. Adrian Radu Vidra** **Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" Cluj-Napoca**
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During the study a Contract Research Organization (CRO) will represent and will perform on behalf of the Sponsor several study-related activities, including regulatory submissions. The contact details of the Contract Research Organization are the following:

CRO Name:	**MDX Research SRL**
Contact Name:	**Ramona Petrita** Chief Scientific Officer
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1.4 Principal Investigator, Coordinating Investigator and Investigation Site(s)

1.4.1 Principal Investigator of the Coordinator Centre

This study will be conducted in 4 investigational centers located in Romania.

The Coordinating Principal Investigator is **Dr. Adrian Radu Vidra** (last name: Vidra).

The role of the Coordinating Principal Investigator is to implement and manage the day-to-day conduct of the study as well as ensure data integrity and the rights, safety and well-being of the participants involved in the study.

Dr. Adrian Radu Vidra is a Senior Medical Oncologist at Oncology Department from **Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" Cluj-Napoca**, currently focusing on the research in HCC therapies. As an active member of European Society of Medical Oncology (ESMO), Dr. Adrian Radu Vidra currently hold the title of Chair of the ESMO-Practicing Oncologist Working Group. He plays a crucial role in representing the interests of oncologists who are actively engaged in clinical practice, fosters collaboration between oncologists, policymakers, and healthcare organizations and strengthens links between academic research and real-world clinical practice.

1.4.2 Involved Centers

This study will be multicentric. As it is an Investigator - Initiated study, Dr. Adrian Radu Vidra will coordinate and lead the study. All 4 clinical sites are listed below:

The Investigator and involved center details are the following:

1	Name:	**ADRIAN RADU VIDRA, MD**
	Role:	Lead Investigator
	Coordinating Centre:	**Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" Cluj-Napoca** Department of Medical Oncology, Regional Institute of Gastroenterology and Hepatology Prof. Dr. Octavian Fodor, 400162 Cluj-Napoca, Romania
2	Name:	**CRISTINA OPREAN, MD**
	Role:	Principal Investigator
	Centre:	**Centrul De Oncologie Oncohelp** **Department Of Medical Oncology** 57-59 Ciprian Porumbescu Street, 300239 Timisoara, Romania
3	Name:	**VLAD-ADRIAN AFRĂSÂNIE, MD**
	Role:	Principal Investigator
	Centre:	**Institutul Regional De Oncologie Iasi** **Department Of Medical Oncology** 2-4 General Henri Mathias Berthelot Street, 700483 Iasi, Romania
4	Name:	**VLAD CROITORU, MD**
	Role:	Principal Investigator
	Centre:	**Institutul Clinic Fundeni** **Compartment Of Medical Oncology** 258 Soseaua Fundeni, District 2, 022328 Bucharest, Romania

1.5 Rationale

Advances in diagnostic and therapeutic tools, along with improved understanding of their application, are transforming HCC patients' treatment. Integrating these innovations into clinical practice presents challenges and necessitates guidance.

ATZ plus BEV has been approved as a front-line treatment for unresected HCC, following the results of IMBrave150. (12) However, the inclusion criteria used in clinical trials do not support the use of novel therapies in several real-world scenarios involving underrepresented subgroups, such as patients with unpreserved liver function, other comorbid conditions, a history of solid-organ transplantation, autoimmune disorders and those with a high risk of bleeding. (18)

Several real-world studies and meta-analysis proved their usefulness in guiding the clinicians towards the choice of the therapeutic intervention and optimizing the surveillance of high-risk individuals for HCC. (19–21)

Results from a German real-world cohort published in 2023 by *Jost-Brinkmann* and colleagues report that ATZ plus BEV showed good efficacy and safety in patients with uHCC and partially advanced liver cirrhosis in a real-world setting. Moreover, a new biomarker, the ROC-optimized neutrophil-to-lymphocyte ratio (NLR) was the strongest independent predictor of ORR and PFS. In patients with advanced cirrhosis Child-Pugh (CP) B, liver function was significantly better preserved with immunotherapy compared to no immunotherapy. (22) Patients with CP type B cirrhosis showed similar ORR but shorter OS and PFS compared to patients with preserved liver function. (22)(23)

A key challenge remains to identify those patients who may benefit the most from the combination treatment.

Hepatocellular carcinoma (HCC) represents a significant public health burden in Romania due to its strong association with chronic liver diseases such as hepatitis B and C infections, alcohol-related liver disease, and metabolic dysfunction-associated steatohepatitis (MASH). Romania has one of the highest prevalence rates of viral hepatitis in Europe, which contributes to the increasing incidence of HCC. Understanding the real-world characteristics of Romanian HCC patients can help identify specific risk factors, comorbidities, and treatment gaps unique to this population. A real-world study in HCC patients in Romania is crucial for bridging knowledge gaps between clinical trials and actual patient care. By deeply analyzing baseline characteristics, the study can drive improvements in early detection, treatment personalization, and healthcare policy, ultimately enhancing patient outcomes.

1.6 Risk-Benefit Assessment

The available information suggests that the ATZ plus BEV is safe and effective as first-line systemic therapy for patients with uHCC and advanced HCC. The overall efficacy and safety of the combination has been well established in the literature and from the various clinical studies. (21,24–28)

This combination has since replaced tyrosine kinase inhibitors (TKIs) as the standard for many patients. The combination significantly increases OS (median 19.2 months vs. 13.4 months with sorafenib) and objective response rates (30% vs. 11%), offering better disease control.

Some patients achieve durable responses, which were rarely seen with previous systemic therapies.

1.7 Overall synopsis of the Clinical Study Protocol

Title	A Multi-Centric, Retrospective, Real-World Study on First-Line Therapy with Atezolizumab Plus Bevacizumab for Advanced or Unresectable Hepatocellular Carcinoma in Romanian Population
Protocol code	(MANIFEST-HCC-RO)
Version	1.0 – 10 June 2025
NCT no.	NCT06903663
Type	Non-interventional, multicentric, observational, post-marketing study
Sponsor	**Dr. Adrian Radu Vidra**
Objectives	**Primary Objectives** The primary objective of our study is to identify pathological and clinical features of HCC that can influence patients' response to treatment. **Secondary Objectives** The secondary objectives of our study include: • Overall survival (OS): Time from treatment initiation with ATZ plus BEV to death from any cause. • Progression-free survival (PFS): Time from treatment initiation to documented disease progression or death. • Objective response rate (ORR) and disease control rate (DCR). • Duration of response (DR): Time from first documented response to progression or death. **Safety Objectives** Safety assessment will consist of monitoring the incidence of all adverse events observed following the treatment with ATZ plus BEV, graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5. Safety events will be stratified by type of events, grades, seriousness and will be collected at time points defined in the study flowchart.
Outcomes	• Descriptive epidemiology of patients treated with ATZ plus BEV • PFS and OS, according to viral and non-viral etiology in the selected cohort Time Frame: 12 months] • Disease Control Rate (DCR) in participants [Time Frame: 12 months] DCR will be calculated per modified Response Evaluation Criteria in Solid Tumors (mRECIST) V1.1 criterion, as the proportion of patients with best overall response to protocol therapy of complete response (CR), partial response (PR) or stable disease (SD) that is maintained for at least 12 weeks.

	• Progression Free Survival (PFS) will be defined as the time from the onset of the treatment with ATZ/BEV until disease progression or death from any cause. Time Frame: 12 months] • Overall Survival (OS) Time Frame: 12 months] • OS will be defined as the elapsed time from the first onset of the treatment with ATZ/BEV to death from any cause. For surviving patients, follow-up will be censored at the date of last contact (or last date known to be alive). Follow-up for OS will be performed at 12 months • Objective Response Rate (ORR) Time Frame: 12 months] ORR will be defined as the proportion of the patients with a confirmed CR or PR, as per mRECIST V1.1 criterion. • Duration of Response (DOR) Time Frame: 12 months] DOR will be defined as the elapsed time from documented tumor response to documented disease progression or death from any cause. The cut-off date is 31 December 2025.
Design	Allocation: Non-randomized intervention Model: Single Group Assignment Masking: Open-label Primary Purpose: Retrospective collection of data for patients treated with the combination ATZ plus BEV
Inclusion criteria	Eligible patients should meet the following inclusion criteria: 1. Adult women and men (≥ 18 years of age) with proven initial diagnosis of HCC with evidence of loco-regional recurrent or advanced disease not amenable to resection. 2. At least one cycle of therapy with ATZ plus BEV. 3. Eligible individuals will be required to have Baseline (pre-index) EGD available. 4. No prior systemic therapy for HCC. 5. Evaluable disease as defined per modified Response Evaluation Criteria in Solid Tumors (mRECIST) V1.1 criterion (At least 1 evaluable CT result over the entire available index and post-index period, excluding the baseline). 6. Patients identified from the patient registry in the hospital with treatment charts between April 1, 2022- December 31, 2024 (index date). 7. Eligible individuals will be required to have data available prior to the index date (pre-index period; baseline characteristics).
Exclusion criteria	Participants with the following characteristics are excluded: 1. A diagnosis of any other primary cancer prior to the index date 2. ATZ plus BEV treatment as part of a clinical trial 3. ATZ or BEV given off-label 4. Patients with incomplete medical records

	5. Patients receiving other investigational drugs 6. History of hepatic encephalopathy 7. Patients with brain metastasis 8. HBV and HBC co-infection
Number of Centers	4 geographically different centers located in Romania (Cluj-Napoca, Iasi, Timisoara and Bucharest).
Ethical Board	The retrospective analysis will begin after the favorable opinion from the Ethics Committees of the individual centers is obtained.
Sample Size	100 patients
Treatment	Retrospective collection of data for patients treated with the combination ATZ plus BEV.
Statistical analysis	The present study is a retrospective cohort study. The data cut-off date is 31 December 2025. The primary study endpoint is Demographic and Clinical characteristics analyzed by descriptive statistics. Secondary endpoints include OS, PFS, DoR, DCR, ORR and safety. Subgroup analyses based on baseline characteristics (e.g., prior therapies, ECOG performance) will be performed. Data on baseline characteristics, radiological response, treatment patterns and adverse events will be summarized using descriptive statistics. Continuous variables will be presented as median and full range, and categorical variables will be reported as frequencies and percentages. Median duration of therapy was defined as the time from the first administration until the last administration of the drugs ATZ plus BEV. Treatment exposure will be calculated as follows: **Number of days exposed = date of last dose minus date of first dose plus 1 day minus periods of interruptions.** Patients with at least one staging imaging assessment will be evaluated for radiological response. Vital status for all patients will be recorded. Where possible, death and reason for death (disease progression or death from any cause) will be captured. PFS is defined as the time from the date of the first therapy administration of ATZ plus BEV until radiological disease progression or death, whatever occurred first. Patients still alive and without radiologically confirmed progression at the date of the last contact or data cut-off will be censored. OS will be defined as the time from the start of the treatment with atezolizumab and bevacizumab until the date of death. Survival curves will be calculated with the Kaplan–Meier method and compared by means of the log-rank test.

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
Demography data (age, race, height, weight, ethnicity, gender, environment)		X												
Eligibility criteria		X												
Smoking history		X												
Alcohol consumption history		X												
Medical history		X												
Clinical Data at Therapy Onset	HCC Diagnosis Date	X												
	BCLC Classification													
	Histopathology / Imaging date													
	ECOG Performance Status:													
	Cirrhosis Grading													
	Cirrhosis Etiology (Viral, Alcoholic, Non-Alcoholic, etc)													
	Esophageal Varices grading and prophylaxis status													
	Varices Prophylaxis													
	Viremia Status													

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
	Concomitant Antiviral Treatment:													
	Previous Antiviral Treatment:													
	Resection (number of tumors)													
	Tumor size													
	Microvascular invasion													
	Minor macrovascular invasion (Vp1/Vp2)													
	Extrahepatic spread (EHS)													
	Poor tumor differentiation (grade 3 or 4)													
	Ablation (number of tumors)													
Treatment Initiation Date (Month, Year)		X	X	X	X	X	X	X	X	X	X	X	X	X
Number of Administered Cycles			X	X	X	X	X	X	X	X	X	X	X	X
Time Interval from Diagnosis to Systemic Therapy Initiation		X												
Treatment discontinuation and vital status														X
Date of disease progression														X
History of Subsequent Treatments (if applicable) after Progression														X
Adverse events of interest			X	X	X	X	X	X	X	X	X	X	X	X

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
Lab tests	Complete blood count incl Hemoglobin, Leukocytes, Platelets	X	X	X	X	X	X	X	X	X	X	X	X	X
	Serum creatinine (SCr)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Urea	X	X	X	X	X	X	X	X	X	X	X	X	X
	Hepatic function (ALT + AST)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Total bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X
	Direct bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X
	Alkaline phosphatase (ALP)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Gamma GT	X	X	X	X	X	X	X	X	X	X	X	X	X
	Serum Alpha-Fetoprotein (AFP)	X	X	X	X	X	X	X	X	X	X	X	X	X
	INR	X	X	X	X	X	X	X	X	X	X	X	X	X
	Albumin	X	X	X	X	X	X	X	X	X	X	X	X	X
Child-Pugh Score		X												
CT scan (mRECIST 1.1 evaluation)		X	X	X	X	X	X	X	X	X	X	X	X	X

2 STUDY OBJECTIVES

Primary Objectives

The primary objective of our study is to describe pathological and clinical features of HCC that can influence patients' response to treatment.

Secondary Objectives

The secondary objectives of our study include:

- Overall survival (OS): Time from treatment initiation to death from any cause.
- Progression-free survival (PFS): Time from treatment initiation to documented disease progression or death.
- Objective response rate (ORR) and disease control rate (DCR).
- Duration of response (DR): Time from first documented response to progression or death.

Safety Objectives

Safety assessment will consist of monitoring the incidence of all adverse events observed following the treatment with ATZ plus BEV, graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5. Safety events will be stratified by type of events, grades, seriousness and will be collected at time points defined in the study flowchart.

2.1 Primary Outcome

The defined primary study outcome is describing the pathological and clinical features of patients treated with ATZ+BEV in a real-life setting.

History of the disease, including etiology of liver disease, BCLC stage, MVI, EHS, Child-Pugh Score, ALBI grade, MELD, Betablocker medication, Prior treatment, Resection, Local ablation, TACE/SIRT, BMI, ALT, AST, TBL, Albumin, INR, AFP will be collected and presented.

2.2 Secondary Outcomes

The defined study secondary outcomes are:

- PFS and OS, according to viral and non-viral etiology in the selected cohort [Time Frame: 12 months]
- Disease Control Rate (DCR) in participants [Time Frame: 12 months]

DCR will be calculated per modified Response Evaluation Criteria in Solid Tumors (mRECIST) V1.1 criterion, as the proportion of patients with best overall response to protocol therapy of complete response (CR), partial response (PR) or stable disease (SD) that is maintained for at least 12 weeks

- Overall Survival (OS) [Time Frame: 12 months
- OS will be defined as the elapsed time from onset of the treatment to death from any cause. For surviving patients, follow-up will be censored at the date of last contact (or last date known to be alive). Follow-up for OS will be performed at 12 months.
- Objective Response Rate (ORR) [Time Frame: 12 months]

ORR will be defined as the proportion of the patients with a confirmed CR or PR, as per mRECIST V1.1 criterion.

- Duration of Response (DOR) [Time Frame: 12 months

DOR will be defined as the elapsed time from documented tumor response to documented disease progression.
The data cut-off date is 31 December 2025.

2.3 Safety Endpoints

Safety assessment will consist of monitoring the incidence of all adverse events observed following the treatment with ATZ plus BEV, graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5. Safety events will be stratified by type of events, grades, seriousness and will be collected at time points defined in the study flowchart.

3 JUSTIFICATIONS FOR THE DESIGN OF THE STUDY

This study will use an observational cohort design. This study design will capture data on patients retrospectively initiated on therapy with atezolizumab-bevacizumab in multiple Institutions across Romania. Prescription data from patients with confirmed HCC between 2022 and 2024 will be reviewed. The final cohort size is estimated to be around 100 patients. The present study may contribute in validating the efficacy and safety of the ATZ plus BEV outside the controlled environment of clinical trials, particularly in Eastern European, Caucasian patient populations.

4 STUDY POPULATION

4.1 Inclusion criteria

Eligible patients should meet the following inclusion criteria:

1. Adult women and men (≥ 18 years of age) with proven initial diagnosis of HCC with evidence of loco-regional recurrent or advanced disease not amenable to resection.
2. At least one cycle of therapy with ATZ plus BEV.
3. Eligible individuals will be required to have Baseline (pre-index) EGD available.
4. No prior systemic therapy for HCC.
5. Evaluable disease as defined per modified Response Evaluation Criteria in Solid Tumors (mRECIST) V1.1 criterion (At least 1 evaluable CT result over the entire available index and post-index period, excluding the baseline).
6. Patients identified from the patient registry in the hospital with treatment charts between April 1, 2022- December 31, 2024 (index date).
7. Eligible individuals will be required to have data available prior to the index date (pre-index period; baseline characteristics).

4.2 Exclusion criteria

The following participants are excluded from the present study:

1. A diagnosis of any other primary cancer prior to the index date
2. ATZ plus BEV treatment as part of a clinical trial
3. ATZ or BEV given off-label
4. Patients with incomplete medical records
5. Patients receiving other investigational drugs
6. History of hepatic encephalopathy
7. Patients with brain metastasis
8. HBV and HBC co-infection

4.3 Planned Sample Size

An approximate number of **100** patients will be included in the present study.

4.4 Participant Identification

Each patient enrolled will be identified by a five-digit study code and patient initials (first name followed by family name). These will be the only identifying elements and will be used only for the purposes of this study.

Site 01 (Lead PI: Dr. Adrian Radu Vidra) — the first participant will be assigned study code **01001**, with subsequent participants numbered sequentially up to **01099.**

Site 02 (PI: Dr. Cristina Oprean) — the first participant will be assigned study code **02001**, with sequential numbering up to **02099**.

Site 03 (PI: Dr. Vlad-Adrian Afrăsânie) — the first participant will be assigned study code **03001**, with sequential numbering up to **03099**.

Site 04 (PI: Dr. Vlad Croitoru) — the first participant will be assigned study code **04001**, with sequential numbering up to **04099**.

5 STUDY DRUG

5.1 Administration, mode and mechanism of action

Duration

Retrospective collection of data for patients treated with the combination ATZ plus BEV

Discontinuation of treatment

Collection of data for final treatment discontinuation will include:

- CT/MRI assessment
- Clinical progression
    - Child-Pugh classification
        - total bilirubin
        - serum levels of albumin
        - International Normalized Ratio (INR)
        - presence (and severity) of ascites
        - presence (and severity) of encephalopathy
    - ECOG performance status
    - Hepatic function assessment: gamma GT, AST, ALT

5.2 Synopsis of safety data

The combination of ATZ plus BEV has demonstrated good tolerability in the treatment of advanced HCC. (29)

The RWS conducted by *Tovoli et colleagues* showed that the most common TEAE included fatigue (42.3%), hypertension (28.2%), anorexia (18.6%), and diarrhea (17.2%). The most common treatment-

related Grade 3-4 AEs were hypertension (7.0%), digestive non-variceal bleeding (3.8%), increased aminotransferases (3.2%), and variceal bleeding (2.5%).(30)

Other key safety findings include: (31,32)

- Adverse Events (AEs): The most commonly reported AEs include hypertension, proteinuria, fatigue, diarrhea, decreased appetite, and infusion-related reactions.
- Serious Adverse Events (SAEs): Grade 3-4 SAEs such as gastrointestinal bleeding, hepatotoxicity, and thromboembolic events have been observed in some patients.
- Immune-Related Adverse Events (irAEs): Potential irAEs include pneumonitis, hepatitis, endocrinopathies, and colitis, requiring close monitoring and immunosuppressive interventions if necessary.
- Hemorrhagic Risks: Due to bevacizumab's anti-angiogenic properties, an increased risk of bleeding, including gastrointestinal hemorrhage and epistaxis, has been reported.
- Hypertension and Proteinuria: Bevacizumab-related hypertension is a common AE and requires management with antihypertensives. Proteinuria may necessitate dose modifications or treatment discontinuation in severe cases.
- Hepatic Toxicity: In HCC patients, liver function tests should be closely monitored due to the risk of increased transaminases and bilirubin levels.

The *TALENTop study* found that active HBV infection did not compromise the safety of ATZ plus BEV therapy. These findings could support that, with concurrent use of oral antiviral therapy, patients with active HBV infection should be included in the list of patients deemed suitable for this treatment. (33)

5.3 Compliance

The present retrospective study shall be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. These principles protect the rights, safety and well-being of human participants, which are the most important considerations and shall prevail over interests of science and society. These principles shall be understood, observed, and applied at every step in the observational, retrospective cohort study by the Investigator, the Investigator's nominated staff, the Sponsor personnel and the Contract Research Organization personnel.

Prior to commencing the study, the Sponsor shall obtain documentation of the EC's approval/favorable opinion. The present study shall not begin until the required approval/favorable opinion from the EC or regulatory authority have been obtained.

The Sponsor may end this trial in any of the collaborating clinical centers for any of the following reasons:

- The clinical site cannot include the adequate number of participants;
- Serious and/or persistent non-compliance with the protocol;
- Careless or premeditated false data in the electronic CRF;
- Non-compliance with GCP, Standard Operating Procedures or regulatory requirements;
- Inadequate cooperation with the Sponsor;
- The Principal Investigator requires a discontinuation of the trial;
- Lack of data confidentiality.

If the trial is prematurely ended or suspended for any reason, EC and Regulatory Authorities will be informed promptly.

5.5 Previous and Concomitant Medications

All concomitant medication recorded in the patient charts will be captured for all enrolled patients with number of prescriptions (date and dose) and duration (start/stop dates).

6 VARIABLES AND METHODS OF ASSESSMENT

6.1 Efficacy Variables

The quality and completeness of the collected data will be evaluated prior to data analysis. If a participant is missing information for one or more variables, even after the resolution of its query, the missing data will not be replaced. If a participant has been involved in violation of inclusion/exclusion criteria, the respective data will be excluded from the analysis.

The response was assessed by CT or MRI scan evaluated in order to evaluate best response rate and duration of response during the treatment. We will also evaluate the number and type of subsequent treatment lines and the OS.

The primary study endpoint is Pathological and Clinical characteristics analyzed by descriptive statistics. Secondary endpoints include OS, PFS, DoR, DCR, ORR and safety. Subgroup analyses based on baseline characteristics (e.g., prior therapies, ECOG performance) will be performed. Overall Survival (OS), will be defined as the elapsed time from ATZ plus BEV treatment onset to death from any cause. Objective Response Rate (ORR), will be defined as the proportion of the patients with a confirmed CR or PR, as per mRECIST V1.1 criterion.

Duration of Response (DOR), will be defined as the elapsed time from documented tumor response to documented disease progression.

The data cut-off date is 28 February 2026.

6.2 Safety Variables

All-grade AEs will be collected and summarized.

6.2.1 Adverse Events

NCI Common Terminology Criteria for Adverse Events V5.0 (CTCAE) is widely accepted as the standard classification and severity grading scale for adverse events in cancer therapy, clinical trials and other oncology settings. (37)

6.2.2 Collection of Adverse Events

Specific warnings and precautions are listed in the Summary of Product Characteristics (SmPC).

6.3 Definitions

An adverse event is an unexpected medical problem that happens during treatment with a drug or other therapy. Adverse events may be mild, moderate, or severe, and may be caused by something other than the drug or therapy being given.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a drug, without any judgment about causality or relationship to the drug.

An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

6.3.1 Assessment of Adverse Events

6.3.1.1 Seriousness

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- a congenital anomaly/birth defect.

6.3.1.2 Intensity

Investigators should assess the severity of AEs according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

The Severity Grades are as follows:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL) (Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

6.4. Recording Adverse Events

The Sponsor shall implement and maintain in the EDC system an Adverse Event module to ensure that the capturing of all events will be provided by the Investigator to the Sponsor immediately.

The Investigator is responsible for reporting all adverse events that occur during the study. The minimum required information is:

- Date of onset;
- The severity of the event (Mild/Moderate/Severe);
- If the event is serious (SAE);
- The causality assessment with the drug;

- Any other medical interventions performed by Investigator (e.g. changing concomitant medications);
- Status of the event at the moment of the report (Ongoing, Resolved);
- If resolved, stop date of the event.

6.4.1 Reporting of Deaths

All subjects must be followed for survival status until death, and information relating to the death (e.g., date and primary cause) should be obtained and recorded. Also, at the end of the study the investigators should mark for every participant the ongoing status: alive or deceased.

6.5 Laboratory Variables

The data will be collected retrospectively from the medical chart of each patient.

Laboratory assessments were collected at every 3 months as per standard of care and will include:

- **Hematology:** Hemoglobin, Platelets, Leukocytes
- **Clinical chemistry:** Creatinine, Urea, Total Bilirubin, Albumin, INR
- **Liver enzymes:** Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Gamma-glutamyl transferase, Serum Alpha-Fetoprotein.

The laboratory variables window is every 3 months +/- 2 weeks, for patients with HCC who had undergone least one cycle with ATZ plus BEV between the index dates.

6.6 Demographics and Baseline Characteristics

6.6.1 Participant Demography

Demographic data are performed at Screening Visit. Subject demography consists of:

- Age at screening;
- Race;
- Ethnicity;
- Gender;
- Weight;
- Height;
- BMI;
- Environment.

6.6.2 Disease History

The current evaluation of underlying liver function is based on the Child-Pugh classification, while the BCLC staging guide clinical practice according to three main prognostic variables: tumor status (number, size, vascular invasion, extrahepatic localization), liver function (Child-Pugh score) and ECOG performance status. (38–40) Decompensation of liver disease (jaundice, ascites, encephalopathy) reflects non-preserved liver function irrespective of the Child-Pugh or model for end-stage liver disease (MELD) score for which several improvements have been proposed, but compensated liver function could be stratified with additional granularity by using the albumin-bilirubin (ALBI) score, while also adding alpha-fetoprotein (AFP) concentration, irrespective of tumor burden.

Collection of BCLC score

- **BCLC score 0/A (Early-stage HCC)**

Very early stage (0) HCC is defined as a solitary HCC ≤2 cm without vascular invasion or extrahepatic spread and early stage (A) as a solitary HCC irrespective of size or as a multifocal HCC up to 3 nodules (none of them >3 cm) without macrovascular invasion or extrahepatic spread, in patients with preserved liver function and ECOG group PS 0–1. Recently, the ALBI (albumin-bilirubin) score has been developed as an HCC-specific improvement of the Child-Pugh score to reflect the severity and hence the prognosis of cirrhosis. Indeed, the limitations of the Child-Pugh scoring system include the subjectivity of assessment of ascites and encephalopathy, the interdependence of the five parameters, the absence of some important prognostic parameters such as renal function, and the lack of specificity for different disease etiologies.

- **BCLC score B (Intermediate -stage HCC)**

The BCLC B stage comprises a heterogeneous group of patients with preserved liver function Child–Pugh class A or class B liver function and large and/or multifocal HCC (defined as more than three tumors regardless of size, two to three tumors >3 cm in maximal diameter, or one single unresectable tumor >5 cm), and without cancer-related symptoms, macrovascular invasion, or EHS.

- **BCLC Score C (Advanced -stage HCC)**

The existence of portal or supra-hepatic macroscopic vascular invasion and/or extra-hepatic metastatic disease with preserved liver function.

- **BCLC Score D (Terminal -stage HCC)**

End-stage Liber function (PS 3-4) and any tumor burden.

Child-Pugh Score (41)

The Child–Pugh score remains the most widely used score in evaluating liver functional reserve in patients with liver cirrhosis. The Child-Pugh scoring system (also known as the Child-Pugh-Turcotte score) was designed to predict mortality in cirrhosis patients. Originally conceptualized by Child and Turcotte in 1964 to guide the selection of patients who would benefit from elective surgery for portal decompression, it broke down patients into three categories:

A - good hepatic function,

B - moderately impaired hepatic function, and

C - advanced hepatic dysfunction.

Their original scoring system used five clinical and laboratory criteria to categorize patients: serum bilirubin, serum albumin, ascites, neurological disorder, and clinical nutrition status, as follows:

- **Encephalopathy: None = 1 point, Grade 1 and 2 = 2 points, Grade 3 and 4 = 3 points**
- **Ascites: None = 1 point, slight = 2 points, moderate = 3 points**
- **Bilirubin: under 2 mg/dL = 1 point, 2 to 3 mg/dL = 2 points, over 3 mg/dL= 3 points**
- **Albumin: greater than 3.5 g/dL = 1 point, 2.8 to 3.5 g/dL = 2 points, less than 2.8 g/dL = 3 points**
- **Prothrombin Time* (sec prolonged): less than 4 sec = 1 point, 4 to 6 sec = 2 points, over 6 sec = 3 points**

***Frequently INR will be used as a substitute for PT, with INR under 1.7 = 1 point, INR 1.7 to 2.2 = 2 points, INR above 2.2 = 3 points.**

The severity of cirrhosis is scored as follows:

Child-Pugh A (score 5 to 6)

A Child-Pugh score of 5 to 6 corresponds to a Child-Pugh grade of A and indicates a well-functioning liver (compensated cirrhosis).

Child-Pugh B (score 7 to 9)

A Child-Pugh score of 7 to 9 corresponds to a Child-Pugh grade of B and indicates significantly compromised liver function.

Child-Pugh C (score 10 to 15)

A Child-Pugh score of 10 to 15 corresponds to a Child-Pugh grade of C and indicates decompensation of the liver (decompensated cirrhosis).

The Child-Pugh score should be reassessed if the patient's clinical condition improves or deteriorates.

6.6.3 Medical History

For the documentation of medical history, any previous and concomitant diseases before screening will be entered in the EDC.

7 STUDY CONDUCT

This study will be conducted in compliance with Good Clinical Practice (GCP) and other applicable regulatory requirements. These principles protect the rights, safety and well-being of human participants, which are the most important considerations and shall prevail over interests of science and society. These principles shall be understood, observed, and applied at every step in the study by the Investigator, the Investigator's nominated staff, the Sponsor personnel and the Contract Research Organization personnel.

All parties involved in the conduct of the study shall share the responsibility for its ethical conduct in accordance with their respective roles in the study.

7.1 Retrospective Data Parameters

During April 2022 - December 2024 the medical charts & medical databases will be analyzed in order to search for potential eligible participants.

Data will be collected through the institution's electronic and paper medical record database and medical charts. Patients with HCC who had undergone least one cycle with ATZ plus BEV between the index dates will be included. Patients' data including history of the disease, treatment course, laboratory results, and radiological datawill be collected retrospectively from patients' files.

Clinical characteristics, such as age, height, weight, BMI, ECOG status, Child-Pugh score, BCLC stage (including description for portal invasion, vascular invasion or multicentric HCC), cirrhosis etiology (Viral, Alcoholic, NASH, NAFDL), and viral status will be collected for all patients.

Treatment characteristics will include the start date and stop date for doses ATZ and BEV. Treatment with the two drugs and discontinuation were performed according to the recommendations of the manufacturer and at the discretion of the treating physician.

Radiological response was recorded by CT or MRI at baseline, 6–12 weeks after treatment initiation, and every 3 months thereafter according to the local guidelines. Tumor response was assessed according to RECIST V1.1. (42) Adverse effects were recorded at every visit and graded according to CTCAE V5. The data cut-off date is 31 December 2025.

A synopsis of prior and baseline comorbidities and medication use should also be collected.

Points for consideration in evaluation of reported events:

1. the temporal relationship (time to onset);
2. the clinical and pathological characteristics of the event;
3. the pharmacological plausibility based on previous knowledge of the drug and the therapeutic class if appropriate;
4. whether the event was previously reported as an adverse reaction in clinical trials or post-marketing;
5. any possible role of concomitant medications or medications taken prior to the event;
6. the role of the underlying or concurrent illnesses;
7. the effect of de-challenge or dose reduction;
8. the effect of re-challenge or dose increase;
9. patient’s characteristics, including previous medical history, such as history of drug allergies, presence of renal or hepatic impairment, etc.;
10. the possibility of drug interactions

7.2 Schedule of Assessments

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
Demography data (age, race, height, weight, ethnicity, gender, environment)		X												
Eligibility criteria		X												
Smoking history		X												
Alcohol consumption history		X												
Medical history		X												
Clinical Data at Therapy Onset	HCC Diagnosis Date	X												
	BCLC Classification													
	Histopathology / Imaging date													
	ECOG Performance Status:													
	Cirrhosis Grading													
	Cirrhosis Etiology (Viral, Alcoholic, Non-Alcoholic, etc)													
	Esophageal Varices grading and prophylaxis status													
	Varices Prophylaxis													

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
	Viremia Status													
	Concomitant Antiviral Treatment													
	Previous Antiviral Treatment													
	Resection													
	Tumor size													
	Microvascular invasion													
	Minor macrovascular invasion (Vp1/Vp2)													
	Extrahepatic spread)													
	Poor tumor differentiation (grade 3 or 4)													
	Ablation (number of tumors)													
Treatment Initiation Date (Month, Year)		X	X	X	X	X	X	X	X	X	X	X	X	X
Number of Administered Cycles			X	X	X	X	X	X	X	X	X	X	X	X
Time Interval from Diagnosis to Systemic Therapy Initiation		X												
Treatment discontinuation and vital status														X
Date of disease progression														X
History of Subsequent Treatments (if applicable) after Progression														X
Adverse events of interest			X	X	X	X	X	X	X	X	X	X	X	X

PROCEDURE \ EVENT		Date of diagnosis	Follow-up 3M	Follow-up 6M	Follow-up 9M	Follow-up 12M	Follow-up 15M	Follow-up 18M	Follow-up 21M	Follow-up 24M	Follow-up 27M	Follow-up 30M	Follow-up 33M	Follow-up 36M
Visit code		DD	FU3	FU6	FU9	FU12	FU15	FU18	FU21	FU24	FU27	FU30	FU33	FU36
Lab tests	Complete blood count incl Hemoglobin, Leukocytes, Platelets	X	X	X	X	X	X	X	X	X	X	X	X	X
	Serum creatinine (SCr)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Urea	X	X	X	X	X	X	X	X	X	X	X	X	X
	Hepatic function (ALT + AST)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Total bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X
	Direct bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X
	Alkaline phosphatase (ALP)	X	X	X	X	X	X	X	X	X	X	X	X	X
	Gamma GT	X	X	X	X	X	X	X	X	X	X	X	X	X
	Serum Alpha-Fetoprotein (AFP)	X	X	X	X	X	X	X	X	X	X	X	X	X
	INR	X	X	X	X	X	X	X	X	X	X	X	X	X
	Albumin	X	X	X	X	X	X	X	X	X	X	X	X	X
Child-Pugh Score		X												
CT scan (mRECIST 1.1 evaluation)		X	X	X	X	X	X	X	X	X	X	X	X	X

Table 1. Schedule of assessments

8 STATISTICAL METHODS

8.1 Study Participants

The trial will include around 100 participants with histologically confirmed HCC not amenable to surgery and/or locoregional therapies, no previous systemic therapy for HCC, ECOG status, Child-Pugh classification. If present, varices were to be treated per local standard of care prior to enrolment. Atezolizumab 1200 mg/bevacizumab 15 mg/kg were given Q3W until unacceptable toxicity or loss of clinical benefit. Patients will be identified by means of data extracted from the mentioned centers (please refer to Section 1.4.2).

Key inclusion and exclusion criteria are defined in Sections 4.1 and 4.2.

8.1.1 Disposition of Participants

The number and percentage of participants that completed at least 1 treatment cycle. Reasons for discontinuation from the treatment period as recorded on the electronic case report form (eCRF) will be summarized (number and percentage) by treatment group for all participants. Participants excluded from the safety and efficacy populations or other analysis populations will be listed.

8.2 General Considerations

Continuous data will be summarized by treatment group using descriptive statistics (number [n], mean, median, standard deviation [SD], minimum, and maximum). Categorical data will be summarized by treatment group using frequency tables (frequencies and percentages).

The safety evaluable individuals will include Intention-to-Treat (ITT) cohort consisting of all the participants who received ATZ pus BEV.

The efficacy analysis will be done in Per-Protocol (PP) population, which includes only participants who did not record any major protocol deviations.

The quality and completeness of the collected data will be evaluated prior to data analysis. If a subject is missing information for one or more variables, even after the resolution of its query, the missing data will not be replaced. If a participant has been involved in violation of inclusion/exclusion criteria, the respective data will be excluded from the analysis.

8.3 Determination of Sample Size

For this retrospective study, a sample size of approximately 100 patients was taken into consideration.

8.4 Demographics, Medical History, Baseline Characteristics, and Concomitant Medications

The data will be collected retrospectively from the medical chart and medical database of each patient of the outpatient unit. We will include demographic data and history of disease.

Demographic and other baseline characteristics, including gender, age, race, ethnicity, environment, medical history will be summarized for the safety set using descriptive statistics.

Clinical characteristics, such as age, height, weight, BMI, ECOG status, Child-Pugh score, BCLC stage (including description for portal invasion, vascular invasion or multicentric HCC), cirrhosis etiology (Viral, Alcoholic, NASH, NAFDL), and viral status will be collected for all patients, where available in the chart.

Descriptive summaries will include frequency tables for all categorical response variables and number, mean, SD, minimum, and maximum for all continuous variables.

Prior, concomitant, and follow-up medication use will be summarized descriptively by the number and percentage of participants receiving each medication within each therapeutic class. Multiple use of the same medication by a participant will be counted only once.

All concomitant medications will be noted by the Investigator during the study.

8.5 Treatment Compliance

Treatment compliance for the safety population will be summarized in terms of treatment duration, median time of treatment, median number of cycles. Median duration of therapy is defined as the time from the first administration until the last administration of the drugs. Treatment exposure will be calculated as follows:

Number of days exposed = date of last dose minus date of first dose plus 1 day minus periods of interruptions.

8.6 Hypothesis to be Tested

Being a retrospective, real world data study, no formal hypothesis is tested. Study objectives are to assess if the epidemiology and response-rate of HCC patients treated with ATZ+BEV in real-life settings in Romania are comparable with those reported in scientific literature.

8.7 Statistical Methods

The data cut-off date is 31 December 2025.

Data on baseline characteristics, radiological response, treatment patterns and adverse events will be summarized using descriptive statistics. Continuous variables will be presented shown as median and full range, and categorical variables will be reported as frequencies and percentages.

Median duration of therapy was defined as the time from the first administration until the last administration of the drugs. Treatment exposure will be calculated as follows:

Number of days exposed = date of last dose minus date of first dose plus 1 day minus periods of interruptions.

Patients with at least one staging imaging assessment will be evaluated for radiological response.

Vital status for all patients will be recorded. Where possible, death and reason for death (disease progression or death from any cause) will be captured. PFS is defined as the time from the date of the first therapy administration until radiological disease progression or death, whatever occurred first. Patients still alive and without radiologically confirmed progression at the date of the last contact or data cut-off will be censored.

OS will be defined as the time from the start of the treatment with ATZ plus BEV until the date of death.

Survival curves will be calculated with the Kaplan–Meier method and compared by means of the log-rank test.

9 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 Ethics Committee

This study will be conducted in accordance with the International Ethical Guidelines for Biomedical Research prepared by the Council for International Organizations of Medical Sciences in collaboration with the World Health Organization, the 75th WMA General Assembly, Helsinki, Finland, October 2024**.** (43)

These principles protect the rights, safety and well-being of human participants, which are the most important considerations and shall prevail over interests of science and society. These principles shall be understood, observed, and applied at every step in the study by the Investigator, the Investigator's nominated staff, the Sponsor personnel and the CRO personnel.

Individual consent for this retrospective study was waived.

9.2 Electronic Case Report Forms and Source Documentation

All data generated by the site personnel will be captured electronically at the study site using eCRFs. Data from external sources (such as laboratory data) will be entered in EDC system or imported and combined into the final analysis datasets. Once the eCRF clinical data have been submitted to the central server at the independent data center, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail. If additional corrections are needed, the responsible monitor or data manager will raise a query in the EDC system. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and the time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the eCRF page will be frozen. The specific procedures to be used for data entry and query resolution using the EDC system will be provided as a written training material. In addition, site personnel will receive training on the EDC system performed by the CRO personnel. As per GCP, training and ongoing monitoring will minimize the site variation.

All data obtained during this study should be entered into the EDC system promptly. All source documents from which EDC entries are derived should be placed in the participant's file. Measurements for which source documents are usually available include laboratory assessments.

9.3 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the local Institution EC in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before any data is collected. This protocol is to be followed exactly. To alter the protocol, amendments must be written, and approvals received from the appropriate EC prior to implementation. Administrative changes may be made to the protocol without the need for a formal amendment. The Protocol and all amendments will be distributed to all protocol recipients, with appropriate instructions by The Lead Investigator.

9.4 Publication Policy

The pseudo anonymization of study participants must be maintained. Participants will be identified in the EDC system and on documents submitted to the CRO by their participant number, initials and/or birth date, and not by name. Documents that identify the participant must be maintained in confidence by the Investigator. In order to allow patients to assess results from the present study, and to allow for effective supervision by the Member State concerned, the start of the clinical trial should be notified to the Competent Authority. In accordance with international standards, the results of the clinical trial should be reported within one year from the end of the clinical trial. The Sponsor should submit a

summary of the results of the observational study together with a summary that is understandable to a layperson, and the study report.

The study and its results will be recorded in clinicaltrials.gov.

By signing the study protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, Regulatory Authorities will be notified of the Investigator's name, address, qualifications and extent of involvement.

9.5 Study Sponsorship

The study is being undertaken by the Sponsor as part of the Lead Institution research program. The Lead Investigator initiated the observational study and received an unsolicited grant from Roche Holding AG, in order to cover the costs for the clinical operations and regulatory activities during the study initiation and development. Roche Holding AG had no role in study design, data collection, data analysis, data interpretation, or writing of the CSP.

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SIGNATURE PAGE (SPONSOR)

Statement of Agreement	I have read, understand, and agree to conduct the study entitled "A Multi-Centric, Retrospective, Real-World Study on First-Line Therapy with Atezolizumab Plus Bevacizumab for Advanced or Unresectable Hepatocellular Carcinoma in Romanian Population (MANIFEST-HCC-RO)" in accordance with the Clinical Study Protocol (Version 1.0 dated 10 June 2025). I agree to provide comprehensive study oversight and to conduct the study according to ICH GCP and all applicable regulations.

_____ _____

Sponsor's Name Date

_____

Sponsor's Signature

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Statement of Agreement	I have read, understand, and agree to conduct the study entitled "A Multi-Centric, Retrospective, Real-World Study on First-Line Therapy with Atezolizumab Plus Bevacizumab for Advanced or Unresectable Hepatocellular Carcinoma in Romanian Population (MANIFEST-HCC-RO)" in accordance with the Clinical Study Protocol (Version 1.0 dated 10 June 2025). I agree to provide oversight to all individuals who assist me in the conduct of this study. I am responsible for providing them with full information regarding the study conduct, and the obligations of confidentiality.

_____ _____

Principal Investigator's Name Date

_____

Principal Investigator's Signature

APPENDICES

A1. MODIFIED RECIST (MRECIST) ASSESSMENT FOR HEPATOCELLULAR CARCINOMA

IMAGE ACQUISITION

Optimization of image acquisition protocols and consistency in the use of the same protocol throughout follow-up examinations are key for proper application of mRECIST. Patients can be followed with either contrast-enhanced spiral computed tomography (CT)—preferably with use of multislice scanners—or contrast-enhanced dynamic magnetic resonance imaging (MRI). The administration of intravenous contrast is recommended for all CT or MRI studies if not medically contraindicated. In contrast-enhanced studies, it is mandatory to obtain a dual-phase imaging of the liver. Every effort should be made to time the contrast administration so that high-quality arterial phase imaging is obtained throughout the liver on the first run, and high-quality portal venous-phase imaging is obtained throughout the liver on the second run. Delayed imaging obtained in the equilibrium phase may be useful, but it is not mandatory and should be done only if it is part of clinical practice. For multidetector CT scanners that can acquire very thin slices, it is necessary to keep in mind that it is mandatory to use contiguous slices for image read and interpretation, to avoid missing small lesions. For example, the analysis of contiguous slices with traditional 5 mm thickness and 5 mm reconstruction interval is acceptable; however, the analysis of 2.5 mm thickness slices at 5 mm intervals is not acceptable.

IMAGE INTERPRETATION

To properly use the proposed mRECIST for HCC to assess response rates and time to progression in HCC clinical trials and to ensure comparability across studies, uniform image acquisition parameters, rigorous quality control, and independent blinded multireader assessments are mandatory. Therefore, the expert panel recommended adopting a centralized radiologic review for image interpretation rather than base the assessment on the image evaluation performed by local Investigator. Independent radiologists will be responsible for performing qualitative and quantitative assessments of imaging data. They will assess baseline imaging to determine the overall tumor burden and use this as a comparator for subsequent measurements. Tumor response will then be determined for each follow-up imaging time point. Overall response assessment includes, according to RECIST, evaluation of target lesions response, nontarget lesions response, and new lesions.

ASSESSMENT OF TUMOR LESIONS AT BASELINE

According to RECIST, tumor lesions are categorized at baseline as follows:

- measurable (lesions that can be accurately measured in at least one dimension as ≥1 cm with a spiral CT scan) or
- non-measurable (all other lesions, including small lesions (longest diameter <1 cm with spiral CT scan) and truly non-measurable lesions).

It is important to point out that only well-delineated, arterially enhancing lesions can be selected as target lesions for mRECIST. This may not be the case of infiltrative-type HCC. Infiltrative-type HCC should be considered as a nontarget lesion when the mass shows ill-defined borders and therefore does not appear to be suitable for accurate and repeat measurements. HCC lesions previously treated with locoregional or systemic treatments may or may not be considered as suitable to be

selected as target lesions for mRECIST: if the lesion shows a well-delineated area of viable (contrast enhancement in the arterial phase) tumor that is at least 1 cm in longest diameter, then it can be selected as a target lesion. In contrast, if the lesion is poorly demarcated or exhibits atypical enhancement as a result of the previous intervention, then it cannot be selected as a target lesion for mRECIST.

To be selected as a target lesion using mRECIST, an HCC lesion should meet all the following criteria:

- The lesion can be classified as a RECIST measurable lesion (i.e., the lesion can be accurately measured in at least one dimension as 1 cm or more).
- The lesion is suitable for repeat measurement.
- The lesion shows intratumoral arterial enhancement on contrast-enhanced CT or MRI.

RECIST	mRECIST
CR = Disappearance of all target lesions	**CR = Disappearance of any intratumoral arterial enhancement in all target lesions**
PR = At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of the diameters of target lesions	**PR = At least a 30% decrease in the sum of diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions**
SD = Any cases that do not qualify for either partial response or progressive disease	**SD = Any cases that do not qualify for either partial response or progressive disease**
PD = An increase of at least 20% in the sum of the diameters of target lesions, taking as reference the smallest sum of the diameters of target lesions recorded since treatment started	**PD = An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started**

Table 2 Assessment of Target Lesion Response: Conventional RECIST and mRECIST Assessment for HCC Following the AASLD-JNCI Guideline (52)

The measurement of the longest diameter of the viable tumor may be challenging in lesions showing partial internal necrosis (Figure 1). The following points should be taken into account in such cases:

- The measurement of the viable tumor should be performed on CT or MRI obtained in the arterial phase, when the contrast between viable vascularized tumor tissue and non-enhancing necrotic tissue is the highest;
- The longest diameter of the viable tumor is not necessarily located in the same scan plane in which the baseline diameter was measured: a thorough careful evaluation of the CT or MRI scans is required;
- The measurement of the viable tumor diameter should not include any major intervening areas of necrosis.

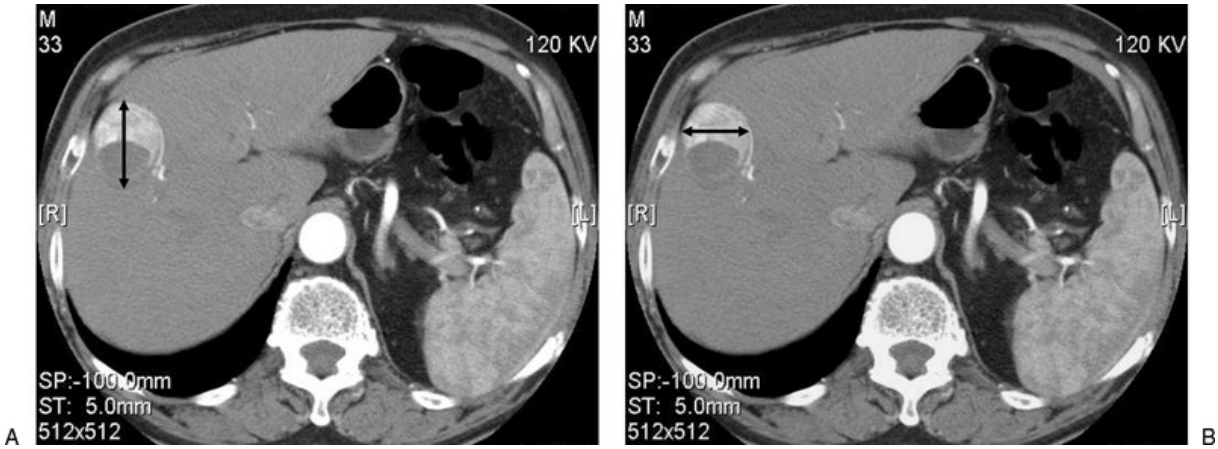


Figure 1. Application of mRECIST assessment for hepatocellular carcinoma (HCC). Target tumor response measurements on arterial phase computed tomography (CT) scans. (A) Measurement of longest overall tumor diameter according to conventional RECIST, and (B) measurement of longest viable tumor diameter according to mRECIST for HCC.