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TITLE: Improving Response to Immunotherapy in Patients with Advanced Hepatocellular Carcinoma and Chronic Hepatitis C Virus Infection with Direct-Acting Antiviral Therapy

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1.0 TRIAL SUMMARY

Title	Improving Response to Immunotherapy in Patients with Advanced Hepatocellular Carcinoma and Chronic Hepatitis C Virus Infection with Direct-Acting Antiviral Therapy
Trial Phase	This is a Phase IV study/hypothesis-generating study
Clinical Indication	Hepatocellular carcinoma (HCC)
Trial Type	Interventional
Drugs (all commercially available)	HCC Agents: Atezolizumab + Bevacizumab (Atezo/Bev) Direct-Acting Antiviral Agents (DAAs): Sofosbuvir + velpatasvir, or sofosbuvir + velpatasvir + voxilaprevir
Type of control	No treatment control
Route of administration	Atezolizumab + Bevacizumab: Intravenous DAAs: Oral
Trial Blinding	Unblinded Open-label
Eligibility	Male/female subjects with hepatitis C virus (HCV) genotype GT1 through GT6 with advanced HCC with no curative option will be enrolled in this trial.
Treatment Groups	There will be only 1 treatment group within this trial. Patients will receive atezolizumab (1200 mg) plus bevacizumab (15 mg per kilogram of body weight) intravenously every 3 weeks combined with any of the following DAA regimens: 1) sofosbuvir (400 mg) + velpatasvir (100 mg), or 2) sofosbuvir (400 mg) + velpatasvir (100 mg) + voxilaprevir (100 mg) administered once daily or twice daily, orally, for 12 to 24 weeks depending on the HCV GT and history of cirrhosis and DAA treatment failure. Ribavirin (RBV) will be combined with DAAs in cases of previous DAA treatment failure. RBV will be administered weight-based as follows <75 kg: 1 g/day in 2 divided dose and ≥75 kg: 1.2 g/day in 2 divided doses.
Number of trial subjects	Approximately 15 subjects will be enrolled.
Estimated enrollment period	48 months
Estimated duration of the trial	It is estimated that the trial will require approximately 24 months from the time the first patient signs the informed consent form (ICF) until the last subject's last study-related phone call or visit.
Duration of Participation	Each subject will participate in the trial from the time the subject signs the ICF through the final contact. After a screening phase of up to 30 days, each patient will receive Atezo/Bev beginning on Day 1 of each 3-week dosing cycle. Patients will receive Atezo/Bev until unacceptable toxic effects occurred or there is a loss of clinical benefit. Patients could continue treatment beyond disease progression if the investigator observes evidence of clinical benefit and if symptoms and signs indicating unequivocal disease progression are absent. Any DAA combination above will be allowed. Dose modifications are not permitted with Atezo/Bev or DAAs. RBV dose reduction will be permitted. Treatment will continue until progressive disease, unacceptable adverse events (AEs), and intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdrawal of consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, or administrative reasons requiring cessation of treatment. Patients who discontinued for reasons other than progressive disease will have post-treatment follow-up visits for monitoring disease status until progressive disease, initiating a non-study cancer treatment, withdrawing consent from

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	study participation, or becoming lost to follow-up. All subjects will be followed (by telephone or clinic visit [in person or telemedicine]) for overall survival until death, withdrawal of consent from study participation, or the end of the study. After the end of the study treatment, each subject will be followed for 24 weeks days for adverse event monitoring. Serious AEs (SAEs) will be collected for 24 weeks after the end of treatment or 4 weeks after the end of treatment if the subject initiates new anticancer therapy or has premature discontinuation of study treatment, whichever is earlier. Virologic response (sustained virologic response [SVR]) will be collected for 12 after the end of all HCV study therapy.
The estimated average length of treatment per subject	Patients will be treated with the study drugs until unacceptable toxic effects occur or there is a loss of clinical benefit. Patients could continue treatment beyond disease progression if the investigator observed evidence of clinical benefit and if symptoms and signs indicating unequivocal disease progression are absent.

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2.0 TRIAL DESIGN

2.1 Trial Design

This is a pilot/ hypothesis-generating study investigating the combination of immune checkpoint therapy (ICT) atezolizumab + bevacizumab (Atezo/Bev) for patients with advanced hepatocellular carcinoma (HCC) with chronic hepatitis C virus (HCV) infection who either progressed on or after previously systemically treated HCC. Patients will receive Atezo/Bev combined with any of the following direct-acting antiviral agents (DAAs) such as 1) Sofosbuvir + velpatasvir, or 2) sofosbuvir + velpatasvir + voxilaprevir. These DAAs are not known to be anti-cancer therapies. Ribavirin (RBV) will be combined with DAAs in cases of previous DAA treatment failure. To be eligible for the study, subjects must have chronic HCV infection with genotype (GT) 1 through 6 and Child-Pugh class A liver disease. All drugs used in this trial (Atezo/Bev plus sofosbuvir/velpatasvir, or sofosbuvir/velpatasvir/voxilaprevir +/- RBV) are commercially available and will be administered and monitored according to the standard of care (SOC) practice.[1,2]

These trial aims are to 1) determine whether HCV clearance with DAA therapy enhances response to ICT in patients with HCC and chronic HCV infection, and 2) determine whether HCV clearance with DAA therapy enhances the immune response against liver cancer cells by reversing the exhaustion of the overall T cells and virus-specific T cells in patients with HCC and chronic HCV infection. The inclusion and exclusion criteria will be the same for each combination of Atezo/Bev and DAAs unless stipulated by one of the added agents. To be eligible, patients must receive DAAs plus Atezo/Bev as first-line systemic therapy or subsequent line therapy if documented objective radiographic progression after stopping treatment with systemic anti-HCC treatment (other than ICT), progression on anti-HCC therapy other than ICT, or intolerance to anti-HCC therapy (other than ICT), and have a disease not amenable to a curative treatment approach (e.g., transplant, surgery, or ablation). Intolerance to anti-HCC therapy is defined as: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.0) Grade ≥2 drug-related adverse event(s) (AE[s]) which both a) persisted despite comprehensive supportive therapy according to institutional standards and b) persisted or recurred after anti-HCC therapy interruption of at least 7 days and a dose reduction and resulted in the subject requesting or the physician recommending discontinuation due to the toxicity. To be eligible, subjects must have at least 1 measurable lesion that is confirmed by M.D. Anderson radiology faculty or M.D. Anderson study collaborator from radiology per Response Evaluation Criterin in Solid Tumors (RECIST) 1.1, Child-Pugh status score of A, an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1, and life expectancy of greater than 3 months. To be eligible for DAA treatment, the patients must have chronic HCV GT1 through GT6 and Child-Pugh class A liver disease.

Patients will be required to provide a tumor tissue sample at enrollment and after DAA treatment to support the correlative endpoints of the study. Approximately 15 patients will be allocated to receive standard of care treatment with atezolizumab (1200 mg) plus bevacizumab (15 mg per kilogram of body weight) intravenously every 3 weeks combined with any of the following DAA regimens: 1) sofosbuvir (400 mg) + velpatasvir (100 mg), or 2) sofosbuvir

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(400 mg) + velpatasvir (100 mg) + voxilaprevir (100 mg) administered once daily or twice daily for 12 to 24 weeks depending on the HCV GT and history of cirrhosis and DAA treatment failure. Ribavirin (RBV) will be combined with DAAs in cases of previous DAA treatment failure. RBV will be administered weight-based as follows <75 kg: 1 g/day in 2 divided doses and ≥75 kg: 1.2 g/day in 2 divided doses.

The overarching goal of this proposal is to determine the effect of HCV cure after DAA therapy on the response of patients with HCC to ICT.

The primary aim of this trial is to determine whether HCV clearance with DAA therapy enhances response to ICT in patients with HCC and chronic HCV infection and the primary objective under this aim is to estimate the objective response rate with Atezo/Bev and DAAs. The objective response rate to ICT is defined as the proportion of confirmed complete or partial responses after receiving DAAs. Starting with screening, all imaging assessments will be determined at our institution using RECIST 1.1. Imaging assessments will be performed every 12 weeks (Q12W) calculated from the date of starting Atezo/Bev and independent of treatment delays. RECIST 1.1 will be used for treatment decisions until the first radiologic evidence of progressive disease (PD).

Patients may continue to be treated with Atezo/Bev and DAAs until PD is confirmed by RECIST, unacceptable AEs, an intercurrent illness that prevents further administration of treatment, the investigator decides to withdraw the subject, subject withdraws consent, pregnancy, noncompliance with trial treatment or procedure requirements, or administrative reasons. Patients who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (Q12W; 84 ± 7 days). All patients will be followed Q12W for overall survival (OS) until death, withdrawal of consent from participation in the study, or the end of the study, whichever comes first.

Patients should complete 12 to 24 weeks of dosing of DAAs before the cessation of antivirals. Patients who attain a complete response (CR) by 2 tumor imaging assessments at least 4 weeks apart and who have received at least 8 treatments (approximately 6 months of therapy) with Atezo/Bev may discontinue treatment at the discretion of the investigator after receiving at least 2 treatments beyond the initial determination of a CR.

Adverse events (AEs) will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI CTCAE version 4.0. After the end of the combination treatment with Atezo/Bev and DAA treatment, each subject will be followed for 24 weeks for AE monitoring. Serious AEs (SAEs) will be collected for 24 weeks after the end of treatment or 4 weeks after the end of treatment if the subject initiates new anticancer therapy or has premature discontinuation of study treatment, whichever is earlier. Virologic responses will be collected for 12 and 24 weeks after the end of DAA therapy.

This study will be conducted in conformance with Good Clinical Practices (GCP).

2.2 Determine the immune response against liver cancer cells after DAAs

We plan on performing multiple experiments to determine whether HCV clearance with DAA

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therapy enhances the immune response against liver cancer cells by reversing the exhaustion of the overall T cells and virus-specific T cells in patients with HCC and chronic HCV infection. Lymphocyte infiltrates, in particular tumor-infiltrating CD8+ T cells, have been associated with improved survival and lower relapse rates after liver resection.[3]

Multiple lymphocyte and myeloid cell subsets (e.g., CD4+, CD8+, regulatory T cells [Tregs], natural killer [NK] cells, macrophages, dendritic cells [DCs], and myeloid-derived suppressor cells [MDSCs]) will be characterized using high-dimensional multi-parametric flow cytometry to detect and quantify the frequencies of cells exhibiting proliferation, cytotoxicity, and cytokine production, as well as changes in the expression levels of immune checkpoint signaling receptors and activation markers (e.g., PD-1, CTLA-4, Lag3, ICOS, and 4-1BB). The CD8+ T-cell response will also be correlated with activated T-cell subsets identified by multiplex immunofluorescence (mIF) analysis of tumor biopsy samples obtained before and after DAA treatment. Using this strategy, we reported earlier on the kinetics of changes in different immune cell subsets from blood and cytobrush samples from cervical cancer patients undergoing standard-of-care chemoradiation treatment.[4]

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart - Section 6.0. Details of each procedure are provided in Section 7.0 – Trial Procedures.

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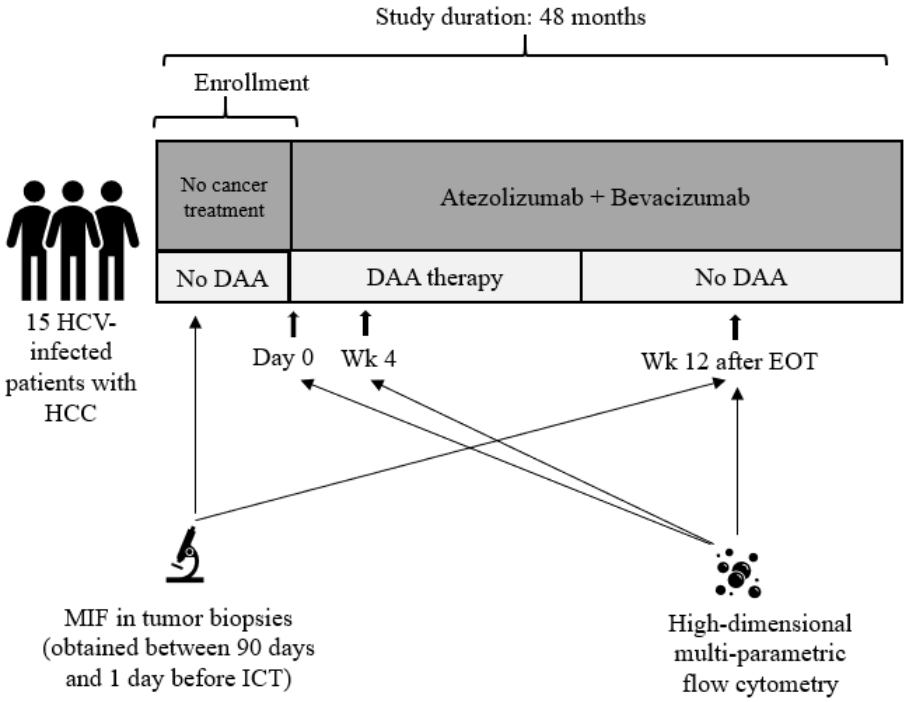
2.3 Trial Diagram

Figure 1. Study Schema



Abbreviations: DAAs, direct-acting antivirals; EOT, end of treatment; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ICT, immune checkpoint therapy; MIF, multiplex immunofluorescence analysis.

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

The overarching goal of this proposal is to determine the effect of HCV cure after DAA therapy on the response of patients with HCC to ICT. We hypothesize that DAA treatment in HCV-infected patients with HCC synergizes with concomitant ICT and improves oncologic outcomes via enhanced CD8+ T-cell responses by reversing the exhaustion of tumor-specific T cells that are associated with chronic HCV infection.

3.1 Primary Objective(s)

3.1.1. Estimate the objective response rate (ORR) to ICT and estimate the sustained virologic response (SVR) in patients with HCC and chronic HCV infection using HCV clearance with DAA therapy.

3.1.2. Estimate the change of the overall T cells and virus-specific T cells before and after the antiviral treatment in patients with HCC and chronic HCV infection using HCV clearance with DAA therapy.

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3.2 Secondary objective:

3.2.1. Collect the safety profile of the treatment and estimate the time-to-event variable such as overall survival (OS) and progression free survival (PFS).

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1.1 Background on HCC

Liver cancer is the third leading cause of cancer deaths in the world and has one of the most rapidly rising mortality rates of any cancer in the United States (US).[5,6] Despite advances in early detection, liver transplantation, and liver-directed therapies, about 70% of HCC patients present with advanced disease with no curative option. HCC is resistant to most traditional chemotherapy agents, and the median survival for patients with advanced disease is typically 6 to 9 months without therapy.

4.1.2 Background on immunotherapy for HCC

Immune checkpoint therapy (ICT) seems to be particularly effective in patients with a T-cell-recruiting tumor microenvironment.[7] The antitumor effect of ICT is mediated by CD8+ T cells, but only 20–30% of all HCC patients have an immunologically active tumor microenvironment.[8] Among patients with HCC, the overall response rate to ICT is only between 17%-33% (overall response rate of 27-30% on combination therapies, e.g., IMbrave150 study regimen of Atezolizumab + Bevacizumab), and median overall survival (OS) rate after ICT is only between 13.9-19.2 months.[9] *There is an urgent need to improve the rate of response to ICT in patients with HCC.*

4.1.2 Background on Infections, including HCV infection, and immune response

Infectious pathogens or their components have been used to augment weak antitumor responses and to reprogram the tumor microenvironment to increase immune-mediated responses and improve cancer outcomes.[10] DAA therapy improves the OS of HCC patients,[11] and clinical trials have shown that patients with HCV-associated HCC appear to be more sensitive to ICT but the reason remains unknown.[12]

Chronic HCV infection is associated with T-cell exhaustion, which has been reversed through DAA therapy or checkpoint inhibition after DAA treatment. Chronic HCV infection is associated with a lower frequency of HCV-specific CD8+ T cells and an increase in the number of so-called exhausted T cells, which exhibit impaired proliferation, IFN-γ production, and cytotoxicity.[13-15] The use of DAAs has been associated with a remarkable HCV cure rate, and with enhanced hepatic and systemic immune responses.[16-19] Studies have shown a decline in the number of peripheral exhausted T cells,[18] specific restorations of the systemic immune response in terms of proliferating HCV-specific CD8+ T cells,[20] and augmentation of HCV-specific immunity[18] in patients in whom DAA treatment resulted in a sustained virologic response. Of note, in a study that failed to show restoration of HCV-

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specific CD8+ T-cell phenotypes and functional responses after DAA treatment, *in vitro* blockade of the PD-1/PD-L1 pathway resulted in enhanced proliferation of some HCV-specific CD8+ T cells after HCV clearance.[19] This finding suggests that HCV-specific CD8+ T cells that could not restore their proliferative capacity upon clearance of HCV can do so through immune checkpoint inhibition.[19] *These findings form the basis of the proposed study's approach: combining ICT and DAAs*.

4.1.3 Pharmaceutical and Therapeutic Background on ICT treatment of HCC

In 2017 the FDA granted accelerated approval to nivolumab for the treatment of HCC in patients who have been previously treated with sorafenib. Approval was based on a 154-patient subgroup of CHECKMATE-040,[21] a multicenter, open-label trial conducted in patients with HCC and Child-Pugh A cirrhosis who progressed on or were intolerant to sorafenib. In addition to including patients without active hepatitis viral infection, the trial enrolled patients with either active HBV (31%) or HCV (21%) but not those with active co-infection with HBV and HCV or with hepatitis D virus infection. Patients received nivolumab 3 mg/kg by intravenous infusion every 2 weeks. The confirmed overall response rate, as assessed by blinded independent central review using RECIST 1.1, was 14.3% (95% CI: 9.2, 20.8), with 3 complete responses and 19 partial responses. Response duration ranged from 3.2 to 38.2+ months; 91% of responders had responses lasting 6 months or longer and 55% had responses lasting 12 months or longer.

The IMbrave150 trial showed significantly better overall survival and progression-free survival outcomes with Atezo/Bev than with sorafenib in patients with unresectable hepatocellular carcinoma who had received no previous systemic treatment. The confirmed objective response rates were 27.3% (95% CI, 22.5 to 32.5) with Atezo/Bev and 11.9% (95% CI, 7.4 to 18.0) with sorafenib, according to independent assessment with RECIST 1.1 (P<0.001), and 33.2% (95% CI, 28.1 to 38.6) and 13.3% (95% CI, 8.4 to 19.6), respectively, according to hepatocellular carcinoma–specific mRECIST (P<0.001).[22]. Atezo/Bev is now recommended as first-line or subsequent-line systemic therapy for patients with HCC.[1]

Despite these advances, continued investigation of additional agents for advanced HCC patients without a curative option remains crucial.

4.1.4. Background on HCV

4.1.4.1 HCV Epidemiology and Natural History

Globally, an estimated 58 million people have chronic HCV infection, with about 1.5 million new infections occurring per year, and approximately 80% of these will progress to chronic infection.[23]. HCV is also the most common bloodborne pathogen in the United States, chronically affecting approximately 2.4 million Americans.[24]

Long-term complications of chronic HCV infection develop in chronically infected individuals for several years to decades, including cirrhosis, end-stage liver disease, and HCC.[24]

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HCV has 6 major GTs, which can each be split into multiple subtypes. The global distribution of HCV GTs is diverse, which reflects differences in epidemiology, modes of transmission and ethnic variability. HCV GT1, GT2, and GT3 have a fairly broad geographical distribution, whereas HCV GT4, GT5, and GT6 are generally confined to specific geographical regions.[24]

4.1.4.2 Overview of HCV Therapy

Interferon-free regimens combining 2 or 3 direct-acting antiviral drugs (DAAs) with or without ribavirin are now the standard of care treatment for HCV.[24] DAAs target specific nonstructural viral proteins involved in the replication cycle of HCV and include NS3/4A protease inhibitors (glecaprevir, voxilaprevir), NS5B nucleos(t)idic (sofosbuvir) and non-nucleos(t)idic (dasabuvir) polymerase inhibitors, and NS5A replication complex inhibitors (ledipasvir, velpatasvir, pibrentasvir).[2] The combinations are given for 8 to 24 weeks, according to baseline factors such as fibrosis stage, GT and subtype baseline viral load, prior therapeutic history of the patient (naïve or experienced), and pre-existing and SVR rates greater than 90% with good tolerance.[2] Safety and SVR rates are similar in clinical trials and real-life studies, usually higher than 95% in the per-protocol analysis.[2] In cancer patients, we have observed SVR12 rates over 80% to 90%, nearly matching the response rates in the general population of treatment-naïve or treatment-experienced patients.[25,26]

Pharmaceutical and Therapeutic Background of Atezolizumab / Bevacizumab

4.1.5.1 Overview

Several cancer immunotherapies that target the PD-L1–PD-1 pathway (i.e., checkpoint inhibitors) are currently being evaluated in patients with HCC.[9] Atezolizumab selectively targets PD-L1 to prevent interaction with receptors PD-1 and B7-1, thus reversing T-cell suppression. Bevacizumab is a monoclonal antibody that targets VEGF, inhibits angiogenesis and tumor growth, and showed response rates of 13 to 14% in single-agent phase 2 studies in patients with advanced liver cancer. A phase 1b study of atezolizumab plus bevacizumab in patients with untreated unresectable hepatocellular carcinoma showed an acceptable side-effect profile and promising antitumor activity, with an objective response rate of 36% and a median progression-free survival of 7 months.[27]

A global, open-label, phase 3 trial, patients with unresectable HCC who had not previously received systemic treatment (IMbrave150 trial), showed significantly better OS and PFS outcomes with Atezo/Bev than with sorafenib in patients with unresectable HCC who had received no previous systemic treatment. The confirmed objective response rates were 27.3% (95% CI, 22.5 to 32.5) with Atezo/Bev and 11.9% (95% CI, 7.4 to 18.0) with sorafenib, according to independent assessment with RECIST 1.1 (P<0.001), and 33.2% (95% CI, 28.1 to 38.6) and 13.3% (95% CI, 8.4 to 19.6), respectively, according to HCC–specific mRECIST (P<0.001).[22]. In May 2020, following results from the phase III IMbrave150 trial, a new standard of care for advanced and unresectableHCCwas approved by the FDA: atezolizumab (an anti-PD-L1 ICT) plus bevacizumab (an anti-VEGF monoclonal antibody).[9] Atezo/Bev is now recommended as first-line or subsequent-line systemic therapy for patients with HCC.[1]

4.1.5.2 Preclinical and Clinical Trial Data

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DAA therapy in HCV-infected cancer patients improves oncologic outcomes. DAA therapy improves the OS of HCC patients.[11] We found that HCV-infected patients with oropharyngeal cancers who received antiviral treatment against HCV, most of them treated with DAAs, had better 5-year OS and 5-year progression-free survival rates than untreated patients.[28] We speculate that the improved tumor response in these patients was associated with elevated CD8+ T-cell infiltration, as has been described in patients with squamous cell carcinoma of the head and neck.[29]

DAA treatment invigorates the CD8+ T cells with the specific restoration of a systemic immune response marked by proliferative HCV-specific CD8+ T cells[20] or augmentation of HCV-specific immunity.[18] ICT is known to assist in enhancing antitumor effector immunity.[30] Therefore, the combination of DAA and ICT should provide synergistic immune activation and thereby produce better oncologic outcomes. We aim to determine whether HCV eradication following DAA therapy enhances the response to ICT in patients with HCC and chronic HCV infection. *We hypothesize that DAA therapy in HCV-infected patients with HCC synergizes with concomitant ICT and improves oncologic outcomes via enhanced CD8+ T-cell responses by reversing the exhaustion of tumor-specific T cells that is associated with chronic HCV infection.*

Completion of the proposed research would markedly expand knowledge of the systemic and local CD8+ T-cell responses associated with DAA therapy and have major patient-care implications, as HCV-infected patients with HCC would be started on DAA treatment as early as possible to improve the HCC response to treatment with ICT. For years, DAAs were not recommended as early treatment in patients with active HCC given the risk of HCC progression.[31,32] Data from our center and large studies in the US and Europe,[33-35] no longer support the theory of HCC progression on DAAs. Our proposed research will test innovative hypotheses and use innovative approaches to generate new insights into how the reversal of the exhaustion of the HCV-specific CD8+ T cell response[16] associated with HCV clearance can lead to better ICT response. This project is novel as there are no recommendations regarding the timing of DAAs in HCV-infected patients with HCC who will receive ICT.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Hepatocellular carcinoma

HCC is often driven by inflammation of various types, including viral infections. In a mouse model of HCC, blockade of PD-1 with immunostimulatory monoclonal antibodies extended survival.[36] HCC patients with higher tumor expression of PD-L1 have a significantly poorer prognosis than patients with lower expression, and tumor expression of PD-L1 is also an independent predictor for postoperative recurrence in HCC.[37] In addition, high expression levels of PD-1, both in TILs and peripheral blood mononuclear cells (PBMCs) also correlate with increased stage and recurrence in HCC patients after surgical resection.[38]

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A T-cell-recruiting tumor microenvironment exhibits robust antigen presentation and T-cell activation that can develop a tumor-specific CD8+ T-cell response able to eliminate cancer cells, generate systemic tumor-specific immunity, and form long-term antitumor immune memory.[39-41] We will study whether a novel approach converts immunologically inactive HCCs into ones with a T-cell-recruiting microenvironment.

4.2.2 HCV infection, DAAs, and Selected Subject Population

Chronic HCV infection is a major public health problem associated with a substantial medical, social, and economic burden. Despite the approval of several DAAs by the FDA, a great proportion of HCV-infected patients remains undiagnosed as routine screening is not yet common in most countries and is not widely implemented in the United States, and infected patients may not have any symptoms for many years after being infected.[24]

DAA therapy against HCV is associated not only with a high cure rate but also with enhanced hepatic and systemic immune responses.[16-19] DAA treatment has been associated with a decline in the number of peripheral exhausted T cells,[18] restoration of a systemic immune response with proliferative HCV-specific CD8+ T cells,[20] and augmentation of HCV-specific immunity.[18]

Immunotherapy has been studied for the treatment of HCV infection and in chronic HCV patients with HCC. In a recent study from our group on 51 HCV-infected patients with solid tumors, including 17 patients (33%) with HCC, ICI was safe in this patient population.[42] However, treating concomitantly HCV infection in the setting of active ICT in HCC has not been studied to date.

We hypothesize that DAA treatment in HCV-infected patients with HCC synergizes with concomitant ICT and improves oncologic outcomes via enhanced CD8+ T-cell responses by reversing the exhaustion of tumor-specific T cells that is associated with chronic HCV infection. This will be a novel approach to ICT in HCC patients that may lead to improved HCC outcomes in patients with concomitant HCV infection. We anticipate that the information generated from the proposed work will support the prompt initiation of DAAs with HCC immunotherapy treatment in HCV-infected patients to improve ICT response. Further, the information gained will allow us to design studies in patients with different malignancies receiving ICT to determine the impact of HCV clearance, particularly among patients with immunologically inactive tumors such as lung cancer, glioblastomas, and ovarian, prostate, pancreatic, and breast cancers.

4.2.3 Rationale for Dose Selection/Regimen/Modification

4.2.3.1 Atezolizumab/Bevacizumab Dose

The planned dose of atezolizumab is 1200 mg plus bevacizumab (15 mg per kilogram of body weight) intravenously every 3 weeks as used in clinical trials (IMbrave150)[22], and recommended on guidelines. Atezolizumab and bevacizumab are commercially available

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cancer treatment drugs. Management of their administration and potential adverse events will be conduced as per SOC procedures.

4.2.3.2 DAA Dose and Duration

DAA regimens used in this study include the following commercially available drugs

4.2.3.2.1 Sofosbuvir (400 mg) + velpatasvir (100 mg)

4.2.3.2.2 Sofosbuvir (400 mg) + velpatasvir (100 mg) + voxilaprevir (100 mg)

All these DAA regimens will be administered orally, once daily, or twice daily for 12 to 24 weeks depending on the HCV GT and history of cirrhosis and DAA treatment failure.

4.3.3.3 Ribavirin Dose and Duration

Ribavirin (RBV) will be combined with DAAs in cases of previous DAA treatment failure. RBV will be administered weight-based as follows <75 kg: 1 gram, orally daily in 2 divided doses and ≥75 kg: 1.2 gram orally daily in 2 divided doses.

4.2.4 Rationale for Endpoints

4.2.4.1 Primary Endpoints

4.2.4.1.1 Objective response rate to ICT

Objective response rate (ORR) to ICT is defined as the proportion of confirmed complete or partial responses after finishing the combination of Atezo/Bev and DAAs. ORR will be calculated using RECIST 1.1 as assessed by M.D. Anderson radiology and with confirmatory assessment as required per iRECIST at any time during the trial. Subjects with unknown or missing response information will be treated as non-responders.

The changes in CD8+ T-cell frequencies will be collected and summarized.

4.2.4.1.2 Sustained Virologic Response

The goal of therapy for chronic HCV infection is the eradication of the virus, which is typically measured as a Sustained Virologic Response (SVR). In our study, the virologic response (undetectable HCV RNA quantitative) at 12 weeks after the end of DAA therapy (SVR 12) will be measured and correlated to standard oncologic outcomes. SVR is an objective endpoint that signifies long-term clearance of HCV and is generally regarded as a “virological cure.” Based on numerous observational cohorts showing strong correlations between SVR and multiple clinically important outcomes, such as the development of HCC, end-stage liver complications, and mortality.[2] The FDA examined the correlation between SVR12 and SVR24 in over 13,000 subjects pooled from multiple clinical trials of Peg-IFN-based regimens and found a high rate

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of concordance between SVR12 and SVR24; sensitivity and specificity forSVR12 were 99% and 98%, respectively. Therefore, the FDA has concluded that SVR12 is suitable as a primary endpoint for regulatory approval of DAAs.[43]

4.2.4.2 Secondary/Exploratory Endpoints

This study will also utilize standard endpoints used in oncology studies. Secondary/exploratory endpoints will include PFS, and OS as assessed per RECIST 1.1.

1) Progression-free survival (PFS), is defined as the time from the first dose of study treatment to the first documented progressive disease (PD) according to RECIST 1.1 and with confirmatory assessment, as required per iRECIST, or death due to any cause, whichever occurs first. If a subject does not have a documented date of progression or death, PFS will be censored at the date of the last adequate assessment.
2) Overall survival (OS), is defined as the time from the date of the first dose of study to the date of death due to any cause. Censoring will be performed using the date of last known contact for those who are alive at the time of analysis.

4.2.4.3 Safety Endpoints

Safety parameters commonly used for evaluating investigational systemic anticancer treatments are included as safety endpoints including, but not limited to, the incidence of, causality, and outcome of adverse events (AEs)/serious adverse events (SAEs); and changes in vital signs and laboratory values. Adverse events will be assessed according to NCI Common Terminology Criteria for Adverse Events, version 4.0.

4.2.4.4 Immune Response after DAA Research

DAA treatment invigorates the CD8+ T cells with the specific restoration of a systemic immune response marked by proliferative HCV-specific CD8+ T cells[20] or augmentation of HCV-specific immunity.[18] Cancer immunotherapies represent an important and novel class of antitumor agents. ICT is known to assist in enhancing antitumor effector immunity.[30] Therefore, the combination of DAA and ICT should provide synergistic immune activation and thereby produce better oncologic outcomes.

We hypothesized that DAA treatment enhances the immune response against liver cancer cells by reversing the exhaustion of overall T cells and virus-specific T cells associated with chronic HCV infection.

Preliminary data in the literature show that lymphocyte infiltrates, in particular tumor-infiltrating CD8+ T cells, have been associated with improved survival and lower relapse rates after liver resection.[3]

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Translational Molecular Pathology Immuno-Profiling Lab (TMP-IL): The biomarker analyses will be conducted in TMP-IL directed by Dr. Cara Haymaker, at the Department of Translational Molecular Pathology (TMP), MD Anderson Cancer Center, chaired by Dr. Ignacio Wistuba, Houston, TX. All biospecimens will be collected using the *Biorepository NCI Best Practices*, and standard operating procedures (SOPs). All specimens and derivatives are given de-identified numbers.

Sample Collection

1. Core needle biopsy (CNB) samples: A new fresh CNB will be obtained from liver tissue in all patients prior to treatment (15 samples), and 12 weeks (+/- 4 weeks) after the end of DAA treatment. At least 5 cores will be obtained from the CNB procedure. The CNB cores (~6-8 mm in length) will be processed as FFPE or fresh frozen specimens.

   - Cores 1 to 3: Immediate and overnight fixation in 10% buffered formalin for paraffin embedding, usually within 20-24 hour after fixation. For biopsies performed on Friday, fixation time may extend to 48 hours (FFPE samples).

   - Cores 4 and 5: Flash freezing in liquid nitrogen.

   The CNB specimens will be examined for quality control and pathological characterization before being utilized for immune-profiling, as well as for DNA, RNA, protein extractions, and flow cytometry. These samples will be stored at -80°C.

2. Blood specimens: Whole blood (60 mL, 4 x 10ml Green top Heparin tubes; 1 x10 ml EDTA purple top tube and 1 x10 Streck tube) containing ethylenediaminetetraacetic acid will be collected on 3 different occasions, day 0 (start of DAA treatment), week 4 of DAA treatment, and week 12 after the end of DAA treatment, using the same blood draw used to collect other blood samples as part of the standard-of-care laboratory studies (see study schema in Figure 1). The timing of the collection of blood samples and their storage and shipment will follow the standardized processes of MD Anderson's Laboratory Medicine Reference Laboratory. Peripheral blood mononuclear cells (PBMCs) will be processed by the MDACC institutional tissue bank and cryopreserved for future flow cytometry in the Haymaker Lab.[44-46]

Investigations may include but are not limited to:

Blood Analysis

Whole blood will be collected on 3 different occasions from patients (Figure 1).

Flow cytometry: Multiple lymphocyte and myeloid cell subsets (e.g., CD4+, CD8+, regulatory T cells [Tregs], natural killer [NK] cells, macrophages, dendritic cells [DCs], and myeloid-derived suppressor cells [MDSCs]) will be characterized using high-dimensional multi-parametric flow cytometry to detect and quantify the frequencies of cells exhibiting proliferation, cytotoxicity, and cytokine production, as well as changes in the expression levels of immune checkpoint signaling receptors and activation markers (e.g., PD-1, CTLA-4, Lag3, ICOS, and 4-1BB). The CD8+ T-cell response will also be correlated with activated T-cell

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subsets identified by multiplex immunofluorescence (mIF) analysis of tumor biopsy samples obtained before and after DAA treatment. Using this strategy, we reported earlier on the kinetics of changes in different immune cell subsets from blood and cytobrush samples from cervical cancer patients undergoing standard-of-care chemoradiation treatment.[4]

HCV Markers

Blood will also be obtained for HCV viral load using the COBAS assay. HCV RNA in serum will be quantified using the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems, Branchburg, NJ), which has a quantification range of 15 to 100,000,000 IU/mL (1.18 $\log_{10}$ to 8.00 $\log_{10}$ IU/mL).

Tissue Analysis

Tissue immune monitoring will be conducted done at the following time points:

A new fresh CNB will be obtained from liver tissue in all patients 1) prior to DAA treatment , and 2) after the end of DAA treatment.

The analyses will be performed at M.D. Anderson Translational Immunoprofiling Laboratory Moonshots Platform (TMP-IL). Assays may include but are not be limited to:

Multiplex Immunofluorescence Analysis (MIF)

MIF will be performed on tumor core samples utilizing immune marker panels to assess intratumoral T-cell activation. mIF image analysis uses a tyramide signal amplification methodology and the Opal workflow and chemistry, which allows simultaneous staining of multiple biomarkers within a single paraffin-embedded tissue section.[47] Localization of tumor-infiltrating lymphocytes and engagement of the PD-1/PD-L1 axis will be assessed using mIF staining with a validated panel consisting of CD68, PD-L1, CD3, CD8, PD-1, FoxP3, Ki67, pancytokeratin, and DAPI (Figure 2). Tumor-cell presence will be assessed using hematoxylin-eosin before mIF staining. In addition, T helper cell subsets will be assessed using a validated panel including CD3, CD8, Tbet, GATA3, FoxP3, and DAPI.

For the analysis, the scanning and image capture will be performed with a multispectral microscope (Vectra/Polaris, Akoya Biosciences) and specialized image analysis software (InForm, Akoya Biosciences) that permits the identification of co-expressed markers included in each panel. The tissue will be compartmentalized into the tumor and stromal compartments using key markers to identify tumor cells (e.g., pan-cytokeratin for carcinomas) with the tissue segmentation tool in InForm. The final results will be consolidated using the Phenoptr Reports & Phenoptr package (Akoya Biosciences).

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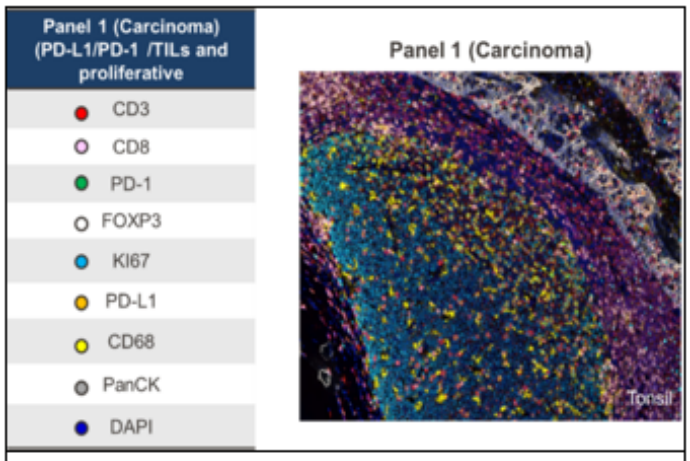

Figure 2. mIF panel design for baseline and on-treatment biopsies. Left, markers included in this validated panel. Right, representative image of a tonsillar carcinoma stained with this panel.

4.3 Benefit/Risk

Patients in clinical trials may not receive direct benefit from treatment during participation, as clinical trials are designed to provide information about the safety and effectiveness of an investigational medicine. However, data using similar drugs in HCC and other cancers suggests that some subjects may benefit from therapy.

Additional details regarding specific benefits and risks for subjects participating in this clinical trial may be found in the accompanying Informed Consent document.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Male and female HCV-infected patients with advanced HCC with no curative option of at least 18 years of age will be enrolled in this trial.

5.1.2 Patient Inclusion Criteria

To be eligible for participation in this trial, the patient must:

1. Be willing and able to provide written informed consent for the trial.
2. Be at least 18 years of age on the day of signing informed consent.
3. Ability to comply with the study protocol, in the investigator's judgment
4. Have histologically or cytologically confirmed diagnosis of HCC (fibrolamellar and mixed hepatocellular/cholangiocarcinoma subtypes are not eligible) based on pathology report.
5. Disease that is not amenable to curative surgical and/or locoregional therapies, or progressive disease after surgical and /or locoregional therapies
6. No prior systemic therapy for HCC.
7. Patients who received prior local therapy (e.g., radiofrequency ablation, percutaneous ethanol or acetic acid injection, cryoablation, high-intensity focused ultrasound, transarterial chemoembolization, transarterial embolization, etc.) are eligible provided the target lesion(s) have not been previously treated with local therapy or the target

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lesion(s) within the field of local therapy have subsequently progressed in accordance with RECIST version 1.1.

8. ECOG Performance Status of 0 or 1
9. Have a detectable HCV RNA quantitative based on the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems, Branchburg, NJ) at the time of screening.
10. Have documented chronic HCV GT1 through GT6 including evidence of mixed genotype infection:
    a. Positive for anti-HCV antibody, HCV RNA, or any of the above HCV GTs at least 3 months before screening (HCV RNA and HCV GT must be confirmed by screening lab results) OR
    b. Positive for anti-HCV antibody or HCV RNA at the time of screening with a liver biopsy consistent with chronic HCV infection (or a liver biopsy performed before enrollment with evidence of chronic HCV disease, such as the presence of fibrosis) or a Fibroscan performed within 12 months of Day 1 of this study with a result of >12.5 kPa or a FibroSure® (Fibrotest®) performed during Screening with a score of ≥0.75 or aspartate aminotransferase (AST): platelet ratio index (APRI) of >2. APRI formula: AST ÷ lab upper limit of normal (ULN) for AST × 100 ÷ (APRI calculation to be provided by the central laboratory.)
11. Have a Child-Pugh A liver score at screening or within 14 days of the first dose of the study drug.
12. Have liver disease staging assessment as follows:

    Cirrhosis is defined as any one of the following

    a. A liver biopsy performed prior to Day 1 of this study showing cirrhosis (F4)
    b. Fibroscan performed within 12 calendar months of Day 1 of this study showing cirrhosis with result >12.5 kPa
    c. A FibroSure® (Fibrotest®) performed during Screening with a score of ≥0.75 or an aspartate aminotransferase (AST): platelet ratio index (APRI) of >2. APRI formula: AST ÷ lab upper limit of normal (ULN) for AST × 100 ÷ (APRI calculation to be provided by the central laboratory.)

    Absence of cirrhosis is defined as any one of the following:

    a. Liver biopsy performed within 24 months of Day 1 of this study showing absence of cirrhosis
    b. Fibroscan performed within 12 months of Day 1 of this study with a result of ≤12.5 kPa
    c. A Fibrosure (Fibrotest) score of ≤0.27 or AST to Platelet Ratio Index (APRI) of ≤1 during Screening

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Fibroscan cut-off of 12.5 kPa has a positive predictive value of 90% and a sensitivity of 95% for ≥F3. Based on box and whisker plot of interquartile distribution >12.5 kPa will exclude the majority of subjects with metavir F3 fibrosis. In the absence of a definitive diagnosis of presence or absence of cirrhosis by the above criteria, a liver biopsy is required. Liver biopsy results supersede the results obtained by Fibroscan, Fibrosure or Fibrotest.

13. Have a predicted life expectancy of greater than 3 months.
14. Have measurable disease based on RECIST 1.1 as confirmed by M.D. Anderson radiology. Target lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.

    Note: the same image acquisition and processing parameters should be used throughout the study for a given subject.
15. Patients with a past or resolved hepatitis B virus (HBV) infection, defined as having a negative HBsAg test and a positive total hepatitis B core antibody (HBcAb) test and negative HBV DNA test at screening, are eligible for the study.
16. Female patients of childbearing potential must have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of trial medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
17. Male patients of childbearing potential must agree to use an adequate method of contraception as outlined in Section 5.5.3 - Contraception, starting with the first dose of trial therapy through 120 days after the last dose of trial therapy.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
18. Be willing to use an adequate method of contraception for the course of the study through 120 days after the last dose of study medication (male and female subjects of childbearing potential [Section 5.5.3]). Acceptable methods of contraception are as follows:

    Single method (one of the following is acceptable):

    a. intrauterine device (IUD)
    b. vasectomy of a female subject's male partner
    c. contraceptive rod implanted into the skin

    Combination method (requires the use of 2 of the following):

    d. diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
    e. cervical cap with spermicide (nulliparous women only)
    f. contraceptive sponge (nulliparous women only)
    g. male condom or female condom (cannot be used together)

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h. hormonal contraceptive: oral contraceptive pill (estrogen/ progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

Note: Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and Institutional Review Boards (IRBs)/Ethics Review Committees (ERCs). Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

19. Patients treated with RBV must agree to double barrier birth control from Day 1 to 6 months following last dose of study therapy or they are excluded from this trial.
20. Have adequate organ function as defined in Table 1. Specimens must be collected within 14 days before the start of trial treatment.

Table 1 Adequate Organ Function Laboratory Tests

System	Laboratory Value
Hematological	
Absolute neutrophil count	≥1500/μL
Platelets	≥100,000/μL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine **OR** Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN **OR** ≥30 mL/min for subject with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for subjects with liver metastases)
Coagulation	
INR or PT/aPTT	≤1.5 × ULN unless subject is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
Abbreviations: ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; EPO = erythropoietin; GFR = glomerular filtration rate; INR = international normalized ratio; PT = prothrombin time; PTT = partial thromboplastin time; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; ULN = upper limit of normal.	

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5.1.3 Patient Exclusion Criteria

1. Is currently participating in or has participated in a trial of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of trial treatment.

   Note: Patients must have recovered from all AEs due to previously therapies to ≤ Grade 1 or baseline. Subjects with ≤ Grade 2 neuropathy may be eligible Note: Subjects who have entered the follow-up phase of an investigational trial may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

   Note: Patients who have entered the follow-up phase of an investigational trial may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

2. Has had esophageal or gastric variceal bleeding within the last 6 months. All subjects will be screened for esophageal varices, unless such screening has been performed in the past 12 months before first dose of treatment. If varices are present, they should be treated according to institutional standards before starting study treatment.
3. Subjects with persistent alanine aminotransferase (ALT) >5 × ULN at screening are not eligible for enrollment.
4. Subjects with persistent Total Bilirubin (Tbil) >2.0 mg/dL at screening are not eligible for enrollment
5. Subjects with clinically apparent ascites or encephalopathy, or untreated varices are not eligible for enrollment. Subjects with Child-Pugh class B and C liver disease are also ineligible.
6. Portal vein invasion at the main portal branch (Vp4), inferior vena cava, or cardiac involvement of HCC based on imaging.
7. Has had encephalopathy in the last 6 months. Subjects on rifaximin or lactulose to control their encephalopathy are not allowed.
8. Had a solid organ or hematologic transplant.
9. Had prior systemic therapy for HCC other than sorafenib and/or regorafenib, or intercurrent local therapy to the liver tumor between sorafenib and/or regorafenib and study drug.
10. Has evidence of history of chronic active hepatitis not caused by HCV, including but not limited to untreated active HBV (see criteria below under criterion 27), drug-induced hepatitis that is not resolved clinically, and autoimmune hepatitis.
11. Has an active autoimmune disease that has required systemic treatment in past 2 years (ie, with use of disease-modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.

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12. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial drug.
13. Has received locoregional therapy to the liver (TACE, TAE, radiation, radioembolization, or ablation) or surgery to the liver or other site within 6 weeks before the first dose of the study drug. Minor surgery must have occurred at least 7 days before the first dose of study treatment (Cycle 1, Day 1). Subjects must have recovered adequately (i.e., Grade ≤1 or baseline) from the toxicity and/or complications from any intervention before starting therapy.
14. Has a known history of an additional malignancy, except if the participant has undergonepotentially curative therapy with no evidence of that disease recurrence for 5 years since initiation of that therapy.

    a. Note: *The time requirement does not apply to participants who underwent successful definitive resection of basal cell carcinoma of the skin, superficial bladder cancer,squamous cell carcinoma of the skin, in situ cervical cancer, in situ breast cancer, or other in situ cancers.*

15. Has radiographically detectable (even if asymptomatic and/or previously treated) central nervous system (CNS) metastases and/or carcinomatous meningitis as assessed by local site investigator.
16. Has a history of (non-infectious) pneumonitis that required treatment with steroids or has current pneumonitis.
17. Has an active infection requiring systemic therapy.
18. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator, including dialysis.
19. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
20. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment.
21. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T cell receptor (eg, CTLA-4, OX-40, CD137)..
22. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
23. Has a known history of human immunodeficiency virus (HIV) infection.
24. Has a known history of active tuberculosis (TB; *Bacillus tuberculosis*).
25. Has received a live vaccine within 30 days before the first dose of trial drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella,

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varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

26. Has untreated active HBV.

    Note: Antiviral therapy for HBV must be given for at least 3 months before the first dose of the study drug, and HBV viral load must be less than 100 IU/mL before the first dose of the study drug. Those on active HBV therapy with viral loads under 100 IU/mL should stay on the same therapy throughout the study treatment. HBsAg, HBsAb, anti-HBe, anti-HBc, and HBV DNA must be measured at baseline and during the study. Those subjects who are anti-HBc (+) and negative for HBsAg and HBV DNA do not require HBV prophylaxis but need monitoring with HBsAg, HBsAb, anti-HBe, anti-HBc, and HBV DNA

27. Has received a live vaccine within 30 days of the planned start of study therapy (Cycle 1, Day 1). Note: The killed virus vaccines used for seasonal influenza vaccines for injection are allowed; however intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
28. Subjects with a history of significant or unstable cardiac disease are excluded due to the hemolytic anemia associated with RBV. Subjects with proven coronary artery disease or angina.
29. Has received prior first-line therapy within 14 days of the first dose of study medication.
30. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
31. Has known psychiatric or substance abuse disorders that would interfere with cooperating with the requirements of the trial.

5.2 Trial Treatments

The treatment to be used in this trial are outlined in **Table** 2, **Table** 3, and **Table** 4.

Table 2 Trial Treatments

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Sofosbuvir + velpatasvir	Tablet: sofosbuvir (400 mg) and velpatasvir (100 mg)	qday	PO	Daily	SOC

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Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Sofosbuvir + velpatasvir + voxilaprevir	Tablet: sofosbuvir (400 mg) and velpatasvir (100 mg) and voxilaprevir (100 mg)	qday	PO	Daily	SOC
RBV	Capsule: 200 mg See Table 4	qday	PO	Daily	SOC
Abbreviations: IV = intravenous; PO = oral; Q3W = every 3 weeks; qday = once daily; RBV = ribavirin; SOC = standard of care.					

The duration of DAA is dependent upon HCV GTs and presence or absence of resistance polymorphisms. Please see **Table** 3.

Table 3 DAA Trial Treatment Duration With or Without Cirrhosis[2]

Patient Population	Treatment	Duration
Treatment-Naive Genotype 1a Patients Without Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 1a Patients With Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Patients Genotype 1b Without Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 1b Patients With Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 2 Patients Without Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 2 Patients With Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 3 Patients Without Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 3 Patients With Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg) for patients without baseline NS5A RAS Y93H for velpatasvir	12 weeks
Treatment-Naive Genotype 4 Patients Without Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 4 Patients With Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Treatment-Naive Genotype 5 or 6 Patients With and Without Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)	12 weeks
Sofosbuvir-Based Treatment Failures, With or Without Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg)[a]	12 weeks
Glecaprevir/Pibrentasvir Treatment Failures (All Genotypes), With or Without Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg)[b]	

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Sofosbuvir/Velpatasvir/Voxilaprevir Treatment Failures, With or Without Compensated Cirrhosis	Daily fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg) plus weight-based ribavirin	24 weeks

Abbreviations: GT = genotype; NS5A = non-structural protein 5A; PegIFN = pegylated interferon; RBV = ribavirin.
a Genotype 3: Add weight-based ribavirin if cirrhosis is present and there are no contraindications.
b For patients with compensated cirrhosis, addition of weight-based ribavirin is recommended.

In patients with CrCl greater than 50 mL per minute, RBV will be administered twice a day (BID) PO (in RBV containing arms) at a total daily dose of 1,000 mg to 1,200 mg based on subject weight on Day 1 (Table). RBV will be used for 12 to 24 weeks.

Table 4 Recommended Dosing for RBV in Combination Therapy for Subjects with CrCl Greater Than 50 mL Per Minute.

Body Weight in kg	RBV Dose	RBV capsules or tablets
<75 kg	1,000 mg/day in 2 divided doses	2 × 200 mg capsules or tablets A.M. 3 × 200 mg capsules or tablets P.M.
≥75 kg	1,200/mg day	3 × 200 mg capsules or tablets A.M. 3 × 200 mg capsules or tablets P.M.
Abbreviations: A.M. = ante meridian; P.M. = post meridian; RBV = ribavirin.		

Trial Treatment should begin within 3 days of allocation/assignment. However, every effort should be made to begin trial treatment on the day of allocation (ie, Cycle 1 Day 1).

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of trial treatments by the protocol and any applicable laws and regulations.

All trial treatments will be administered on an outpatient basis.

All products indicated in the Table are commercially available.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for the selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on the preparation and administration of DAAs, and RBV are provided in the Pharmacy Manual.

5.2.1.2 Dose Modification and Toxicity Management Guidelines for atezolizumab or bevacizumab

AEs associated with ICT exposure may represent an immunologic etiology. There will be no dose modifications for atezolizumab or bevacizumab in this study. Management of toxicity

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related to Atezo/Bev will be conducted per SOC of these commercially available cancer treatment drugs. These patients will be managed by Dr. Kaseb, director of the HCC program at MD Anderson Cancer Center, and by our experienced team in the Department of Gastrointestinal Medical Oncology.

5.2.1.3 DAAs Dose Modification (Escalation/Titration/Other)

Modification of the DAAs is not permitted except for hepatic events, SAEs, or laboratory abnormalities.

The DAAs used in this clinical trial have been approved by the FDA since 2013. Management of toxicity related to DAAs will be conducted per SOC of these commercially available antivirals. These patients will be managed by Dr. Torres, director of the HCV clinic at MD Anderson Cancer Center, and by our experienced team of the Department of Infectious Diseases.

5.2.1.4 RBV Dose Modification (Escalation/Titration/Other)

If DAAs are administered with RBV refer to the RBV prescribing information for a description of RBV-associated adverse reactions. Monotherapy RBV for HCV is not permitted in this trial.

The primary toxicity of RBV is hemolytic anemia, which was observed in approximately 10% of RBV/Intron A-treated subjects in clinical trials. While this study does not include Intron A treatment hemolytic anemia surveillance is still warranted. The anemia associated with RBV capsules occurs within 1 to 2 weeks of initiation of therapy. Because the initial drop in hemoglobin may be significant, it is advised that hemoglobin or hematocrit be obtained before the start of treatment and at Week 2 and Week 4 of therapy, or more frequently if clinically indicated. Subjects should then be followed as clinically appropriate Fatal and nonfatal myocardial infarctions have been reported in patients with anemia caused by RBV. Subjects should be assessed for the underlying cardiac disease before initiation of RBV therapy. Subjects with pre-existing cardiac disease should have electrocardiograms (ECGs) administered before treatment and should be appropriately monitored during therapy. If there is any deterioration of cardiovascular status, therapy should be suspended or discontinued. Because the cardiac disease may be worsened by drug-induced anemia, patients with a history of significant or unstable cardiac disease should not use RBV.

RBV should not be used in subjects with CrCl less than 50 mL/min. Subjects with impaired renal function and those over the age of 50 should be carefully monitored concerning development of anemia.

If severe AEs or laboratory abnormalities develop during the study, RBV can be modified based on the recommended guidelines provided in **Table 5** .

For subjects with a history of stable cardiovascular disease, a permanent dose reduction is required if the hemoglobin decreases by greater than or equal to 2 g/dL during any 4 weeks. In addition, for these cardiac history patients, if the hemoglobin remains less than 12 g/dL after 4

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weeks on a reduced dose, the patient should discontinue combination therapy. It is recommended that a patient whose hemoglobin level falls below 10 g/dL have his/her RBV dose modified or discontinued per 6.

Subjects exhibiting the following conditions should be closely monitored and may require dose reduction or discontinuation of RBV therapy. Refer to the prescribing information:

- Hemolytic anemia may occur with a significant initial drop inhemoglobin
- Pancreatitis
- Pulmonary infiltrates or pulmonary function impairment
- New or worsening ophthalmologic disorders
- Concomitant administration of azathioprine
- Severe decreases in neutrophil and platelet counts and hematologic, endocrine and hepatic abnormalities
- Dental/periodontal disorders with combination therapy

Table 5 Dose Reduction Rules for RBV

Laboratory Parameters	Reduce RBV if	Discontinue Therapy if
HGB without a history of cardiac disease	8.5 to <10 g/dl	< 8.5 g/dl
HGB with a history of stable cardiac disease	<2 g/dL decrease in hemoglobin during any 4-week period during treatment	<8.5 g/dL or <12 g/dL after 4 weeks of dose reduction

Abbreviations: HGB = hemoglobin; RBV = ribavirin

Table 6 Dose Modification for RBV

Any weight	1 x 200 mg capsule or tablet A.M. 2 x 200 mg capsule or tablets P.M.

Abbreviations: AM = ante meridian; FDD = full daily dose; PM = post meridian; RBV = ribavirin

Once RBV has been withheld due to either a laboratory abnormality or clinical adverse reaction, an attempt may be made to restart RBV at 600 mg daily and further increase the dose to 800 mg daily. However, it is not recommended that RBV be increased to the originally assigned dose (1,000 mg to 1,200 mg).

5.2.1.5 Dose Interruptions

If a subject misses a dose of DAAs and it is less than 8 hours before the next dose, the missed dose should be skipped and the normal dosing schedule resumed. Patients should not double the next dose to compensate for what has been missed.

If for any reason DAAs need to be interrupted they can be interrupted for up to 3 days. If DAAs are interrupted for more than 3 days, consult HCV experts for that patient. RBV is not to be taken as monotherapy in the absence of DAAs.

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If Atezolizumab plus Bevacizumab treatment is interrupted then maintain the subject on DAAs (±RBV) unless due to severe liver toxicity. No dosage adjustment of DAAs is recommended in patients with mild hepatic impairment (Child-Pugh A).

If a patient misses a dose of RBV, then they should take the missed dose as soon as possible with food on the same day. If an entire day has gone by, then these missed doses should be skipped, and the normal dosing schedule should be resumed. Patients should not double the next dose to "make up" what has been missed.

If for any reason RBV needs to be interrupted (e.g. for safety reasons), if possible, it should be restarted within 3 days.

5.2.2 Timing of Dose Administration

Day 1 of treatment should begin on the day of starting DAAs on patients receiving Atezo/Bev.

DAAs and Atezo/Bev will be administered per SOC.[1,2]

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the investigators and patients will know the treatment administered.

5.3 Randomization or Treatment Allocation

This is a non-randomized trial single-arm study. Patients will be enrolled based upon inclusion and exclusion criteria.

5.4 Stratification

No stratification based on age, sex, or other characteristics will be used in this trial

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

5.5.1 Acceptable or Concomitant Medications/Vaccinations

All treatments necessary for a patient's welfare may be administered at the discretion of the investigator in keeping with the SOC for atezo/bev or DAAs with or without RBV.

5.5.2 Prohibited Concomitant Medications

Per SOC, patients are prohibited from receiving therapies in the risk category "X" to be avoided with the study drugs atezo/bev or DAAs with or without RBV.

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5.5.3 Contraception

Reproductive considerations are applicable to both males and females only in subjects receiving RBV. For atezo/bev, these considerations are only relevant to female of childbearing potential. Atezo/bev and RBV may have adverse effects on a fetus in utero.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they meet 1 of the following criteria:

- She is postmenopausal, defined as at least 12 months with no menses without an alternative medical cause. In women <45 years of age who are not using hormonal contraception or hormonal replacement therapy, a high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- She had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy, or bilateral tubal ligation/occlusion, at least 6 weeks before screening.
- She has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving the trial drug and for 120 days after the last dose of trial drug by complying with 1 of the following:

- Practice abstinence from heterosexual activity.

  Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and IRBs/ERCs. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.
- Use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

- Single method (1 of the following is acceptable):
  - IUD
  - Vasectomy of a female subject's male partner
  - Contraceptive rod implanted into the skin
- Combination method (requires use of 2 of the following):

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- Diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- Cervical cap with spermicide (nulliparous women only)
- Contraceptive sponge (nulliparous women only)
- Male condom or female condom (cannot be used together)
- Hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the trial medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the trial. In order to participate in the trial, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of trial medication initiation (or 14 days prior to the initiation of trial medication for oral contraception) throughout the trial period up to 180 days (Arm B) after the last dose of trial medication. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the trial.

5.5.4 Pregnancy

If a patient inadvertently becomes pregnant while on treatment with Atezo/bev and RBV, the patient will be immediately discontinued from trial treatment. Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant during the study or within 5 months after the last dose of atezolizumab or 6 months after the last dose of bevacizumab or DAAs, or 9 months after the last dose of RBV. The investigator should counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until the conclusion of the pregnancy. Any serious AEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported per SOC.

5.5.5 Pregnancies in Female Partners of Male Patients

Male patients will be instructed through the Informed Consent Form to immediately inform the investigator if their partner becomes pregnant during the study or within 6 months after the last dose of bevacizumab or DAAs or within 9 months after the last dose of RBV. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male patient exposed to study treatment. An investigator who is contacted by the male patient or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the investigator and/or obstetrician.

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5.5.6 Discontinuation of Treatment

Discontinuation of study treatment does not represent a withdrawal from the study.

As certain data on clinical events beyond study treatment discontinuation may be important to the study, they must be collected through the subject's last scheduled follow-up, even if the subject has discontinued study treatment.

Subjects may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a subject may be discontinued from study treatment by the investigator if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

5.6 Beginning and End of the Trial

The overall trial begins when the first subject signs the informed consent form (ICF). The overall trial ends when the last subject completes the last study-related phone call or visit, discontinues from the trial or is lost to follow-up (i.e. the subject is unable to be contacted by the investigator).

5.7 Clinical Criteria for Early Trial Termination

The clinical trial may be terminated early if the extent (incidence and/or severity) of emerging effects/clinical endpoints is such that the risk/benefit ratio to the trial population as a whole is unacceptable based on the prinicipal investigators' discretion.

5.8 Data and Safety Monitoring Committees

MD Anderson's Data and Safety Monitoring Committees will be responsible for monitoring this trial. This trial will adhere to institutional data safety monitoring plans.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart Initial Treatment with DAAs on Patients on Atezolizumab + Bevacizumab

Trial Period:	**Screening Phase**	**Period of combination treatment with DAAs and Atezolizumab + Bevacizumab (Weeks)**				**DAA Untreated Follow Up (Weeks)**				
						Follow-up			**Premature Discontinuation**	
Treatment Title:	**Screening**	**B**	**2**	**4**	**EOT**	+4	+12	+24	4	12
Week (approximate)	**0**	**X**	**X**	**X**	**X**	X	X	X	X	X
Administrative Procedures										
ICF	X									
Inclusion/exclusion criteria	X									
Demographics and medical history	X									
Prior and concomitant medication review	X	X	X	X	X	X		X		
Study medication adherence		X	X	X	X					
Survival status		X	X	X	X	X		X	X	X
Clinical Procedures/Assessments										
Review AEs	X	X	X	X	X	X		X	X	
Full physical examination	X					X		X		
Directed physical examination		X	X	X	X					
Child-Pugh score	X									
Height, weight, and vital signs (T, P, RR, BP)[c]	X	X	X	X	X	X				
Laboratory Assessments										
Pregnancy test (blood or urine)	X									

Trial Period:	**Screening Phase**	**Period of combination treatment with DAAs and Atezolizumab + Bevacizumab (Weeks)**				**DAA Untreated Follow Up (Weeks)**				
						Follow-up			**Premature Discontinuation**	
Treatment Title:	**Screening**	**B**	**2**	**4**	**EOT**	+4	+12	+24	4	12
Week (approximate)	**0**	**X**	**X**	**X**	**X**	X	X	X	X	X
PT/INR	X[e]		X	X	X	X		X		
CBC with differential	X[e]	X	X	X	X	X	X	X		
Chemistry panel	X[e]	X	X	X	X	X	X	X		
Liver panel[a]	X[e]	X	X	X	X	X	X	X	X	X
Fibrotest or APRI	X									
AFP	X				X		X	X		
Viral testing										
HCV Ab	X									
HCV genotype	X									
HCV viral load	X	X	X	X	X	X	X	X	X	X
Anti-HBc, HBsAg, anti-HBs	X									
If Anti-HBc + and HBsAg +: HBsAg and HBV viral load		X	X	X	X	X	X	X		
If Anti-HBc + and HBsAg -: HBsAg and HBV viral load		X	X	X	X	X	X	X		
Plasma for HCV viral resistance testing[b]		X					X[s]			
Laboratory Immune Assessments										
Blood for immune analysis[c]		X		X			X			
Liver tissue Collection[d]	X						X			
Liver tissue for immune analysis	X						X			
Efficacy Measurements										

Trial Period:	**Screening Phase**	**Period of combination treatment with DAAs and Atezolizumab + Bevacizumab (Weeks)**				**DAA Untreated Follow Up (Weeks)**				
						Follow-up			**Premature Discontinuation**	
Treatment Title:	**Screening**	**B**	**2**	**4**	**EOT**	+4	+12	+24	4	12
Week (approximate)	**0**	**X**	**X**	**X**	**X**	X	X	X	X	X
Tumor imaging[e]	X				X		X			

Abbreviations: AEs = adverse events; AFP = alpha fetoprotein; APRI = aspartate aminotransferase:platelet ratio index; BP = blood pressure; CBC = complete blood count; DC = discontinuation; EOT- end of treatment (DAA); HBV = hepatitis B virus; HBcAg = hepatitis B core antigen; HBsAb = anti-HBsAg antibody; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; ICF = informed consent form; INR = international normalized ratio; RR = respiratory rate; T = temperature; PT = prothrombin time.

a. Liver panel (albumin, ALT, AST, total and direct bilirubin, and alkaline phosphatase).

b. Blood samples will be collected for HCV resistant testing at baseline on selected cases of difficult-to-treat HCV genotypes or treatment failure.

c. Blood samples will be obtained at baseline, at Week 4 of DAAs, Week 12 after EOT. There will be up to a total of 45 blood samples (15 patients × 3 samples = 45 blood samples), which will be collected in Green top Heparin tubes, EDTA purple top tubes and Streck tubes.

d. Liver biopsies will be required pre-treatment with DAAs, and week 12 after the end of DAA treatment. This sample should be drawn for planned multiplex immunofluorescence (MIF). Pre-treatment or baseline biopsy can be obtained between 90 days and 1 day before combination treatment

e. The following imaging studies are required at baseline: CT abdomen with or without contrast; MRI of abdomen and pelvis with or without contrast unless patient has a contraindication for MRI or severely claustrophobic.

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7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject or each subject's legally acceptable representative before participating in a clinical trial. If there are changes to the subject's status during the trial (e.g., health or age of majority requirements), the investigator or qualified designee must ensure the appropriate consent is in place.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial. Remote consenting will not be utilized in this study.

The initial ICF, any subsequent revised written ICF and any written information provided to the subject must receive the IRB approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed promptly if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB requirements, applicable laws and regulations.

In this study, we will adhere to the Office of the Clinical Research SOP 04: Informed Consent Process.

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8.0 STATISTICAL ANALYSIS PLAN

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, but before any final database lock, changes are made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended.

8.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized in Table 6. The comprehensive plan is provided in Sections 8.2 to 8.13.

Table 1 Key Elements of the Statistical Analysis Plan

Study Design Overview	Improving Response to Immunotherapy in Patients with Advanced Hepatocellular Carcinoma and Chronic Hepatitis C Virus Infection with Direct-Acting Antiviral Therapy
Treatment Assignment	This is a pilot/ hypothesis-generating study
Analysis Populations	Efficacy: All Subjects as Treated (ASaT) Safety: ASaT
Primary Endpoints	ORR, SVR, overall T cells and virus-specific T cells
Statistical Methods for Key Efficacy Analyses	Objective Response Rate (ORR) based on RECIST 1.1 on patients on Atezo/Bev receiving DAAs. The ORR is defined as the proportion of responders with a confirmed complete response or partial response. The sustained virologic response (SVR). The response rate and SVR will be estimated with 90% exact confidence interval (CI). Descriptive statistics such as mean, median, and standard deviation, etc. in terms of T cells or other virus-specific T cell measurements.
Statistical Methods for Key Safety Analyses	Counts and percentages of subjects with AEs will be provided.
Interim Analysis	No planned interim analysis for this pilot study.
Multiplicity	Multiplicity adjustment is planned in this pilot study.
Sample Size and Power	The planned sample size is 15 subjects

8.2 Responsibility for Analyses

The statistical analysis of the data obtained from this study will be the responsibility of the Biostatistics department of MD Anderson.

This trial is being conducted as a non-randomized, open-label study, i.e., patients, and investigators will be aware of patient treatment assignments after each patient is enrolled and treatment is assigned.

8.3 Hypotheses/Estimation

The objectives and hypotheses of the study are stated in Section 3.

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8.4 Analysis Endpoints

Efficacy and safety endpoints that will be evaluated are listed below.

8.4.1 Exploratory Efficacy Endpoints

8.4.1.1 Primary Endpoints

- Objective response rate (ORR) to ICT: defined as the proportion of confirmed complete or partial response after finishing the combination of Atezo/Bev and DAAs. ORR will be calculated using RECIST 1.1 as assessed by M.D. Anderson radiology and with confirmatory assessment as required per iRECIST at any time during the trial. Subjects with unknown or missing response information will be treated as non-responders.

- Sustained Virologic Response (SVR): the virologic response (undetectable HCV RNA quantitative) at 12 weeks after the end of DAA therapy (SVR 12) will be measured and correlated to standard oncologic outcomes (Section 4.2.4.1.1).

- The changes in CD8+ T-cell frequencies will be collected and summarized.

8.4.1.2 Secondary Exploratory/Efficacy Endpoints

This study will also utilize standard endpoints used in oncology studies.
Secondary/exploratory endpoints will include PFS, and OS as assessed per RECIST 1.1.

- Progression-free survival (PFS), defined as the time from the first dose of study treatment to the first documented progressive disease (PD) according to RECIST 1.1 and with confirmatory assessment, as required per iRECIST, or death due to any cause, whichever occurs first. If a subject does not have a documented date of progression or death, PFS will be censored at the date of the last adequate assessment.

- Overall survival (OS), defined as the time from the date of the first dose of study to the date of death due to any cause. Censoring will be performed using the date of last known contact for those who are alive at the time of analysis.

8.4.2 Safety Endpoints

- Safety parameters commonly used for evaluating investigational systemic anticancer treatments are included as safety endpoints including, but not limited to, the incidence of, causality, and outcome of adverse events (AEs)/serious adverse events (SAEs); and changes in vital signs and laboratory values. Adverse events will be assessed according to NCI Common Terminology Criteria for Adverse Events, version 4.0. Safety endpoints are described in Section 4.2.4.3.

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8.5 Analysis Populations

Eligible patients enter the study after enrolment.

8.5.1 Efficacy Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of ORR, SVR, PFS, and OS. The ASaT population consists of all patients who received at least one dose of study treatment.

Details on the approach to handling missing data are provided in Table 8.

8.5.2 Safety Analysis Populations

The ASaT population will be used for the analysis of safety data in this study. The ASaT population consists of all patients who received at least one dose of study treatment.

At least one laboratory or vital sign measurement obtained after at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

8.6 Statistical Methods

8.6.1 Statistical Methods and Data Analysis

This section describes the statistical methods that address the primary and secondary objectives.

The ORR and SVR will be estimated with 90% exact confidence interval (CI). With 15 patients, assuming the response rate is 27% (4/15), the 90% exact confidence interval (CI) is (10%, 51%). Table 7 listed more scenario regarding difference response rates. We would also characterize the change in the phenotypic and functional status of the systemic and local CD8+ T cells between before and after DAA therapy and summarize the time-to-event outcomes. Descriptive statistics such as the mean, standard deviation will be summarized in terms of the changes in CD8+ T-cell frequencies. Changes in immune cell frequencies will be explored between DAA responders and non-responders using Wilcoxon rank-sum test or Fisher's Exact test as appropriate. When multiple comparisons are made, we will report P-values both before and after false discover rate adjustments. Time-to-event variables such as overall survival (OS) and progression-free survival (PFS) will be estimated using Kaplan-Meier methods.

Table 2 Analysis Strategy for Efficacy Variables

Total evaluable patients	Number of responders	Response rate	Lower 90% CI*	Upper 90% CI*
15	0	0%	<1%	18%
15	1	7%	<1%	27%
15	2	13%	2%	36%
15	3	20%	6%	44%

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Total evaluable patients	Number of responders	Response rate	Lower 90% CI*	Upper 90% CI*
15	4	27%	10%	51%
15	5	33%	14%	58%
15	6	40%	19%	64%
15	7	47%	24%	70%
15	8	53%	30%	76%
15	9	60%	36%	81%
15	10	67%	43%	86%
15	>10**	>67%	>43%	>86%

* Exact confidence interval computed by the method of Clopper and Pearson (Biometrika 26:404-413, 1934)
**Actual response rate with corresponding CI will be calculated. Exact confidence interval was calculated for all responses.

The efficacy in biomarker subgroups will be summarized by descriptive statistics.

Table 3 Statistical Methods Per Endpoint Analysis

Endpoint/Variable (Description, Time Point)	Statistical Method	Analysis Population	Missing Data Approach
Primary Endpoint			
ORR	the 90% exact confidence interval estimate	ASaT	Subjects with missing data are considered non-responders
Key Secondary/Exploratory Endpoints			
PFS – RECIST 1.1 by M.D. Anderson radiology	Summary statistics using Kaplan-Meier method	ASaT	Censored at last assessment
OS	Summary statistics using Kaplan-Meier method	ASaT	Censored at last known alive date

8.6.2 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs, laboratory tests, and vital signs.

Counts and percentages of subjects with AEs will be provided. Analysis of AEs will be descriptive.

8.6.3 Demographic and Baseline Characteristics

The number and percentage of subjects screened, the primary reasons for screening failure and the primary reasons for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized either by descriptive statistics or categorical tables for all enrolled subjects.

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8.7 Interim Analyses

No Interim analysis is planned.

8.8 Multiplicity

Multiplicity adjustment is planned in this study.

8.9 Sample Size

In this study, approximately 15 subjects will be enrolled.

8.10 Subgroup Analyses and Effect of Baseline Factors

No subgroup analysis is planned.

8.11 Compliance (Medication Adherence)

No drug accountability data is planned.

Data Security/Confidentiality

Participant confidentiality and privacy is strictly held in trust by the participating investigator, their staff, the safety and oversight monitor(s), and the sponsor(s) and funding agency. This confidentiality is extended to the data being collected as part of this study. Data that could be used to identify a specific study participant will be held in strict confidence within the research team. No personally identifiable information from the study will be released to any unauthorized third party without prior written approval of the sponsor/funding agency, as applicable.

All research activities will be conducted in as private a setting as possible.

Access to Study Records

Study records may be accessed by IRB approved study personnel, or authorized inspectors. The study monitor, other authorized representatives of the sponsor or funding agency, representatives of the Institutional Review Board (IRB), regulatory agencies or representatives from companies or organizations supplying the product, may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Methods of Storage of Study Records

All data collected from MD Anderson Cancer Center (MDACC) sources will be maintained on a password protected server compliant with HIPAA. Study staff will have role based restricted access to directories and files on the server, according to project responsibilities. Only those with data entry permissions can add records. The PI or a delegate will review the conditions under which data will be released to recipient- investigators. Each application for

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use will need IRB approval and consents, if appropriate. The level of identifiability will determine the process for review and approval as well as the way information is shared.

Any study data or records maintained in paper documents will be stored in the offices of the PI or other delegated study staff, in a locked cabinet or other comparable controlled environment, and will be accessible only to authorized study team members or authorized inspectors.

Duration of Study Record Storage

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor/funding agency requirements.

Sharing of Study Records

There are no plans to share study data with entities external to MD Anderson Cancer Center, aside from authorized inspectors as applicable (i.e. authorized representatives of the sponsor or funding agency, representatives of the Institutional Review Board (IRB), regulatory agencies or representatives from companies or organizations supplying the product). If data will be shared, IRB approval will be sought, and applicable inter-institutional agreements executed, prior to data sharing.

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