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Title: A Pilot Study of Tremelimumab – A Monoclonal Antibody against CTLA-4 – in combination with Trans-Arterial Catheter Chemoembolization (TACE), Radiofrequency Ablation (RFA), or Cryoablation in Subjects with Hepatocellular Carcinoma (HCC) or Biliary Tract Carcinomas (BTC)

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Investigators Roles: *A. Obtain information by intervening or interacting with living individuals for research purposes*

B. Obtaining identifiable private information about living individuals

C. Obtaining the voluntary informed consent of individuals to be subjects

D. Makes decisions about subject eligibility

E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes

F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes

G. Some/all research activities performed outside NIH

Investigational Agents:

Drug Name:	Tremelimumab
IND Number:	117537
Sponsor:	Center for Cancer Research, National Cancer Institute
Manufacturer:	MedImmune, Inc.

PRÉCIS

Background:

- Worldwide, hepatocellular carcinoma (HCC) is the fifth most common malignancy with a median survival of 6-9 months. For patients with advanced disease sorafenib is the only approved drug and this has limited benefit.
- Tremelimumab is a monoclonal antibody against CTLA4. Anti-CTLA4 therapy has been shown to enhance anti-tumor immunity by blocking tumor-induced immune suppression of cytotoxic T cells.
- Various tumor ablative procedures and techniques have been shown to result in immunogenic cell death and induction of a peripheral immune response. Both trans-arterial catheter chemoembolization (TACE) and radiofrequency ablation (RFA) have been shown to do this, as well as cryoablation and external beam radiation
- The underlying hypothesis of this study is that the effect of anti-CTLA4 treatment can be enhanced by TACE or RFA in patients with advanced hepatocellular carcinoma. We will also evaluate this in the context of cryoablation and radiation in HCC and RFA in cholangiocarcinoma.

Objective:

- To assess the safety and feasibility of combining Tremelimumab with trans-arterial catheter chemoembolization (TACE) radiofrequency ablation (RFA) or cryoablation in patients with advanced HCC.

Eligibility:

- Histologically or cytologically confirmed diagnosis of HCC or biliary tract carcinoma (cohort E).
- Childs-Pugh A/B7 cirrhosis only is allowed. If patient does not have cirrhosis, this limitation does not apply.
- Barcelona Clinic Liver Cancer (BCLC) Stage B and C patients.
- Patients must have disease that is not amenable to potentially curative resection, radiofrequency ablation, or liver transplantation.

Design:

- The proposed study is a pilot study of Tremelimumab - at a planned dose 10mg/kg/IV q 4 weekly x 6 doses and then 1 dose every 12 weeks - in patients with advanced HCC or cholangiocarcinoma treated with ablative therapy for 2 years or until disease progression (as per immune-related RECIST criteria) whichever comes first.
- The main study population is Cohort A as described below and these patients will be treated with either RFA or TACE. The decision on which treatment to administer (i.e. RFA vs. TACE) will be made in consultation with the interventional radiologists. The reason for this is that there are technical considerations (size and location of lesion, main portal vein occlusion etc.) which would make one procedure preferable over the other.
- We will also enroll an additional cohort of up to N=20 patients (Cohort B) with liver-confined disease for whom TACE is indicated as standard of care. These patients will

receive TACE in combination with Tremelimumab using the same schema. They will not receive RFA.

- For Cohort A (BCLC Stage C patients): TACE or radiofrequency ablation (RFA) will be performed once only.
- For Cohort B (BCLC Stage B patients): TACE may be repeated (as per standard of care) on months 3, 7, and 13, and q6 months thereafter (if indicated).
- Cohort B will only be recruited following completion of the dose-escalation portion of Cohort A.
- Dose escalation: Initially three patients will be treated at 3.5mg/kg (with subsequent dosing also at 3.5mg/kg) prior to enrolling to the10mg/kg dose level. If a single patient experiences dose-limiting toxicity (DLT) related to the combination of TACE/RFA and Tremelimumab this cohort will be expanded to N=6 as per a standard 3+3 design. If 3.5mg/kg is established to be safe we will proceed to the 10mg/kg dose level.
- Cohorts A+B: Following dose escalation up to N=40 patients will be treated at 10mg/kg (20 patients in Cohort A and 20 in Cohort B).
- Subjects will no longer enroll onto Cohort C effective with amendment I.
- Cohorts D-E: N=Up to 30 patients will be treated at 10mg/kg.
    - Evaluation period for Dose-limiting Toxicities (DLT) will extend for the first 8 weeks of the study. Restaging CT /MRI scan every 8 weeks to evaluate TTP in target lesion. (Both standard RECIST and Immune-related Response Criteria [irRC] will be employed).
    - Once safety is established for the initial concept of combining anti-CTLA4 with ablative procedures (RFA and TACE), we will similarly explore feasibility of tremelimumab at the same dose and schedule in 2 additional cohorts (denoted D-E) evaluating tremelimumab in combination with cryoablation in HCC (cohort D) and with RFA in a cohort of patients with biliary tract carcinoma (cohort E). For these additional cohorts the dose (10mg/kg) and schedule of tremelimumab will be as per cohorts A and B. The timing of the interventional procedure will also be identical.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary:

To assess the safety and feasibility of combining Tremelimumab with trans-arterial catheter chemoembolization (TACE) or radiofrequency ablation (RFA) in patients with advanced HCC.

1.1.2 Secondary:

To evaluate clinical indicators of efficacy (response rate, time to tumor progression, overall survival) in patients with advanced HCC undergoing TACE or radiofrequency ablation (RFA) in combination with Tremelimumab.

1.1.3 Exploratory:

1.1.3.1 To evaluate changes in immune parameters as well as pharmacokinetics in the peripheral blood of patients with advanced HCC undergoing TACE or radiofrequency ablation (RFA) in combination with Tremelimumab

1.1.3.2 Following amendment F we will evaluate safety and feasibility of combining Tremelimumab with cryoablation in patients with advanced HCC and radiofrequency ablation (RFA) in advanced intrahepatic cholangiocarcinoma.

1.1.3.3 To evaluate changes in immune parameters as well as pharmacokinetics in the peripheral blood of patients with advanced HCC or intrahepatic cholangiocarcinoma undergoing TACE, radiofrequency ablation (RFA) or cryoablation in combination with Tremelimumab.

1.2 BACKGROUND AND RATIONALE

1.2.1 HCC and the current therapeutic paradigm (radiofrequency ablation [RFA] trans-arterial catheter chemoembolization [TACE] and sorafenib)

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common malignancy with a median survival of 6-9 months[1]. The therapeutic paradigm – including the possible interventions and their indications – is outlined below. The approach to management has traditionally comprised of loco-regional strategies: surgery (partial resection or transplantation) or interventional radiologic procedures, such as chemoembolization or ablative techniques. Recently, sorafenib has been added to this paradigm and it is the only systemic drug therapy which has demonstrated a survival benefit in modern randomized studies.

Table 1: Therapeutic paradigm for HCC

Intervention	Indication	Potentially curative
Partial Hepatectomy	Limited liver disease with	Yes

	good reserve	
Transplantation	1 lesion <5cm or 3 < 3cm	Yes
RFA	Lesion to be ablated at least 1cm and not greater than 5cm.	Yes
TACE	Liver confined; no portal vein thrombosis	No
Sorafenib	Metastatic disease	No

The most widely used staging system in HCC is the Barcelona Clinic Liver Cancer (BCLC) Staging system, the major advantage of which is that in addition to staging patients it also directs management[2]. The BCLC staging system is shown in Figure 1 below.

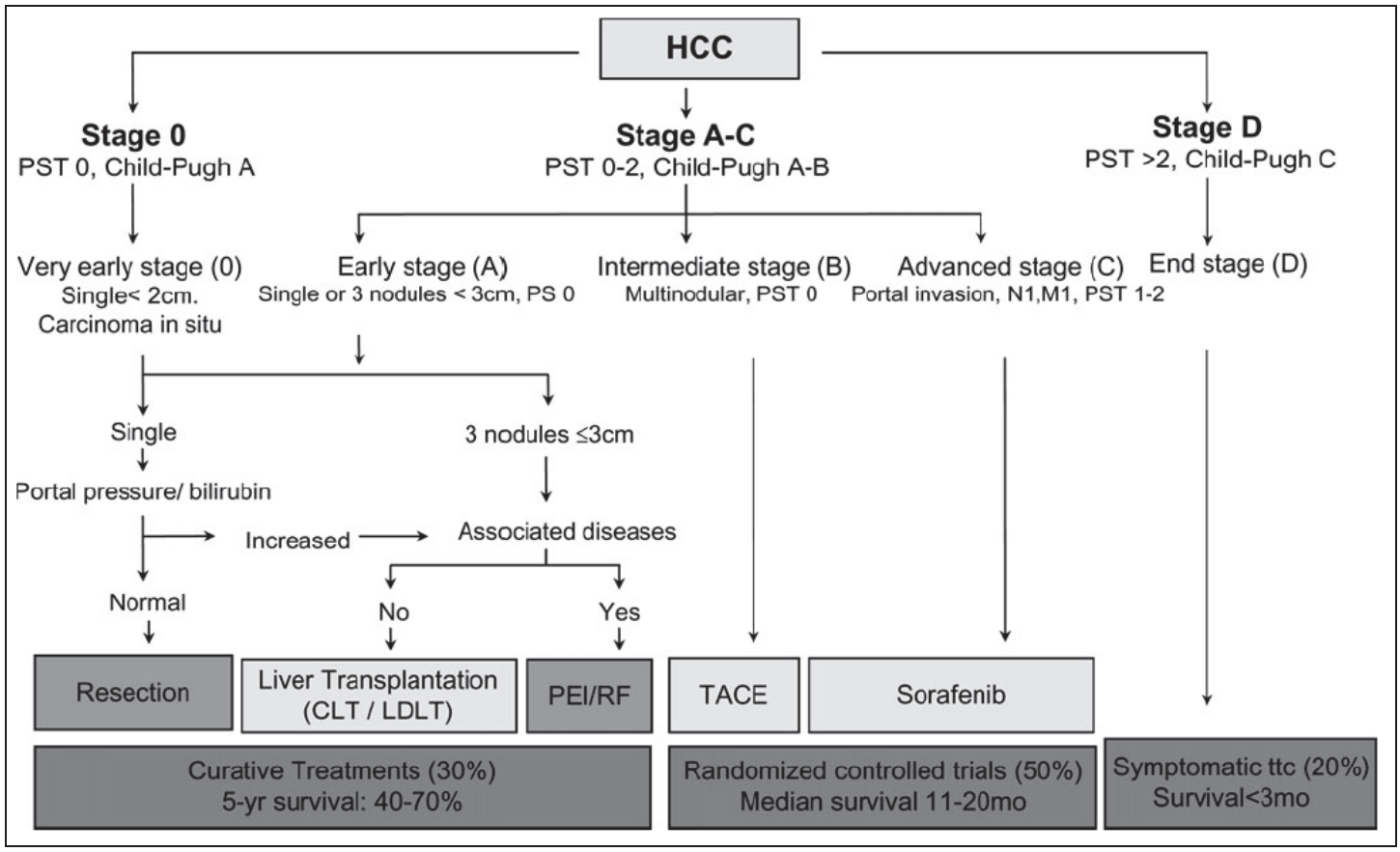


Figure 1: Barcelona Clinic Liver Cancer (BCLC) staging classification and treatment schedule.

For the proposed study we will not include patients for whom surgical resection or transplantation is a possibility. We will also exclude patients with isolated lesions for whom

RFA alone is possible, given the curative potential of that intervention. The target population will be those with advanced disease for whom the only available interventions are TACE or sorafenib.

1.2.2 Recent advances in immune-based approaches in solid tumor malignancies

The past two years have seen progress for immune-based approaches in solid tumor malignancies, with FDA approvals for these approaches in prostate cancer and melanoma[3-5]. In melanoma, anti-CTLA4 therapy has been shown to demonstrate a median survival benefit in two separate phase 3 studies, both of which were associated with long-term disease control in approximately one-fifth of patients. More recently, anti-PD1 therapy has demonstrated a similar degree of clinical activity not only in melanoma and kidney cancer but also in lung cancer, a disease type previously thought to be refractory to an immune approach. Appreciation of the role in developing tumors of immune-evasion has also been evidenced by its inclusion as one of the (updated) hallmarks of cancer[6].

Both anti-PD1 and anti-CTLA4 therapy enhance anti-tumor immunity by blocking tumor induced immune suppression of cytotoxic T cells and therefore exaggerating the immune activation that must first occur of its own accord and is thought to be the result of tumor neo-antigens formed as a tumor progresses. The role of CTLA4 in inhibiting T-cell activation is represented in the schematic in Figure 2 below. CTLA4-mediated immune checkpoint is induced in T cells at the time of their initial response to antigen. The level of CTLA4 induction depends on the amplitude of the initial T cell receptor (TCR)-mediated signaling. After the TCR is triggered by antigen encounter, CTLA4 is transported to the cell surface. The stronger the stimulation through the TCR (and CD28), the greater the amount of CTLA4 that is deposited on the T cell surface. Therefore, CTLA4 functions as a signal dampener to maintain a consistent level of T cell activation in the face of widely varying concentrations and affinities of ligand for the TCR.

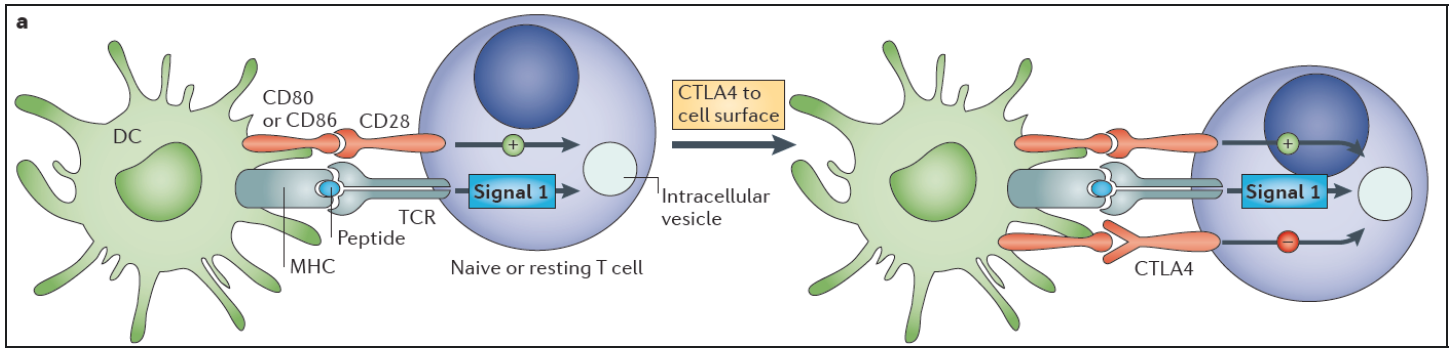


Figure 2: CTLA4-mediated immune checkpoint is induced in T cells at the time of their initial response to antigen. (Pardoll. Nat Rev Cancer. 2012[7])

Whilst these studies demonstrate the potential for anti-CTLA4 (or anti PD1) therapy to enhance the anti-tumor immune response already in process, it clearly does not work in the majority of patients. The question is whether the initial immune activation stage can be enhanced. The potential for this was intriguingly demonstrated in a recent publication by Postow et al who

reported a case of the so-called abscopal effect in a patient with melanoma treated with ipilimumab and radiotherapy[8]. Temporal associations were noted between tumor shrinkage and antibody responses to the cancer-testis antigen NY-ESO-1 in addition to changes in peripheral-blood immune cells, and increases in antibody responses to other antigens after a patient being treated with anti-CTLA4 was then treated with external beam radiotherapy. The enhanced effect from anti-CTLA4 seen after radiation appears to have been due to a boost effect of tumor cell death and immune stimulation following deliverance of a tumor antigen load. It is this enhanced effect we wish to study in the context of interventional procedures for HCC.

1.2.3 Immune response to interventional radiological treatments

The aim of our proposed study is to enhance the antitumor immune response which has been shown to occur following interventional radiological treatments. The attractive aspect of this approach – especially in a disease as prevalent as HCC – is that it has the potential for enhancing the effect of treatments (both RFA and TACE) which have already been approved, have demonstrated survival benefit in randomized studies and are commonly performed throughout the community. Following RFA and TACE necrotic tumor results in the release of tumor antigens which are taken up by antigen-presenting cells (mainly dendritic cells) and which activate a tumor-specific immune response[9]. Ablated tumor tissue has been shown to promote dendritic cell maturation (and resultant T-cell stimulatory properties)[10,11]. This antigen release is potentially significant because, although ablative procedures are very effective in eradicating visible lesions, a tumor-specific immune response may prevent recurrent disease in addition to treating distant metastases. In other words, RFA and TACE have the potential to turn a patient's tumor into an endogenous vaccine.

Several studies have documented an increase in peripheral antitumor immunity following interventional radiological procedures, predominantly RFA[10,12-15]. This has been demonstrated both in animal models and in human studies[12,14,15]. Ayaru et al. evaluated the immune response in HCC patients (N=10) undergoing TACE[16]. They found that AFP-specific CD4-T cell responses were significantly expanded during ($p < 0.0001$) and after embolisation ($p < 0.002$). The development of higher frequencies of AFP-specific CD4 T cells after treatment were significantly associated with the induction of >50% necrosis of tumor and an improved clinical outcome ($p < 0.007$). Mizukoshi et al. measured cytotoxic T cell response to tumor associated antigens (TAA) in HCC patients undergoing treatment[17]. They measured the frequency of TAA-specific T cells before and after HCC treatment by ELISPOT assay in 12 cases who received TACE, RFA or chemotherapy. The frequency of TAA-specific T cells was found to be increased in all patients. Furthermore, incubation of T cells with CTLA-4 antibodies resulted in an increase of the number of TAA-specific T cells. Dromi et al. evaluated the influence of subtotal RFA on tumor-specific immune response in a murine urothelial carcinoma (MB49) tumor model[18]. RFA resulted in enhanced systemic antitumor T-cell immune responses and tumor regression that was associated with increased dendritic cell infiltration into the remaining viable, non-ablated tumor.

Hansler et al. assessed the specific cytotoxic T cell response in patients with malignant liver tumors (both metastatic colon cancer and HCC) which were treated with RFA. Significant tumor-specific cytotoxic T-cell stimulation was seen, with a dramatically increased tumor specific cytolytic activity of CD8(+) T cells against autologous liver and tumor lysate after RFA[12]. Hiroishi et al. analyzed tumor-specific CD8(+) T-cell responses in twenty patients with HCC undergoing loco-regional therapy (either RFA or TACE)[13]. They found that the CD8(+) T-

cell response was increased and, on multivariate analysis, the magnitude of the immune response was the only significant prognostic factor for a prolonged tumor-free interval (hazard ratio 0.342, P = 0.022).

Despite this, only a few cases of spontaneous remission of metastases following RFA – the so-called abscopal effect – have been reported, presumably because this immune response by itself is too weak to be clinically significant[19-21]. A number of studies have tried to boost this anti-tumor immune response following ablation by combining with an immunomodulatory agent:

Waitz et al. provided preclinical proof-of-concept for this in a mouse model of prostate cancer using CTLA-4 blockade to augment the immune response from cryoablation[22]. The combination therapy resulted in >80% of intratumoral CD8+ T cells being antigen-specific. The effect on secondary responses was assessed by injection of the secondary tumor on the opposite flank of the mouse on Day 2 following ablation. The growth of secondary tumors was found to be unaffected by cryoablation alone, but the combination treatment (anti-CTLA4 + RFA) was sufficient to slow growth or trigger rejection. In addition, secondary tumors were highly infiltrated by $CD4^+$ T cells and $CD8^+$ T cells, and there was a significant increase in the ratio of intratumoral T effector cells to T regulatory cells, compared with either RFA or anti-CTLA4 alone. Johnson et al. sought to enhance the antitumor effect of RFA by combining it with huKS-IL2 immunocytokine [tumor-specific monoclonal antibody fused to interleukin-2 (IL2)] in mice bearing CT26-KS colon adenocarcinoma. Mice were treated with RFA, huKS-IL2 via intratumoral injection, or combination therapy. Treatment of mice bearing s.c. tumors with RFA and huKS-IL2 resulted in significantly greater tumor growth suppression and enhanced survival compared with mice treated with either RFA or huKS-IL2 alone. When sub-therapeutic regimens of RFA or huKS-IL2 were used, tumors progressed in all treated mice. In contrast, the combination of RFA and immunocytokine resulted in complete tumor resolution in 50% of mice. Treatment of a tumor with RFA and intratumoral huKS-IL2 also showed antitumor effects against a distant untreated tumor. Tumor-free mice after treatment with RFA and huKS-IL2 showed immunologic memory based on their ability to reject subsequent challenges of CT26-KS and the more aggressive parental CT26 tumors. Flow cytometry analysis of tumor-reactive T cells from mice with complete tumor resolution showed that treatment with RFA and huKS-IL2 resulted in a greater proportion of cytokine-producing CD4 T cells and CD8 T cells compared with mice treated with RFA or huKS-IL2 alone. den Brok et al. evaluated combination treatment of RFA plus TLR9 stimulation in B16 tumors and showed that the combination resulted in far more potent antitumor immune responses than either treatment modality alone[23].

1.2.4 Tremelimumab

Tremelimumab (formerly CP-675,206) is a human IgG2 mAb directed against CTLA-4.

Upon T cell activation, CTLA-4 expression acts to dampen immune responses by CTLA-4 relocation to the cell surface in order to modulate and eventually switch off T cell activation.

Tremelimumab blocks the inhibitory effect of CTLA-4, and therefore enhances T cell activation. The binding of CTLA-4 to its target ligands (B7.1 [CD80] and B7.2 [CD86]) provides a negative regulatory signal, which limits T cell activation. Blockade of B7 binding to CTLA-4 by anti-CTLA-4 antibodies also results in markedly enhanced T cell activation and antitumor activity in animal models, including killing of established murine solid tumors and induction of protective antitumor immunity.

Anti-CTLA-4 therapy has recently been shown to be a validated approach to cancer treatment by the approval of the mAb ipilimumab in 2011 for the treatment of patients with metastatic melanoma, based on 2 Phase III studies that demonstrated a significant improvement on OS in the first- and second-line settings. In general, tumor response rates to anti-CTLA-4 therapy are low, approximately 10%, but the durable response or stable disease seen in a proportion of patients can lead to a significant prolongation of OS. In a large, single-arm Phase II Tremelimumab trial in patients with advanced refractory and/or relapsed melanoma, objective responses (primary endpoint) following Tremelimumab 15 mg/kg Q3M were observed in 16 of 241 (6.6%) patients (95% CI: [3.84, 10.56]). Responses were durable (present at ≥6 months from enrollment) in all 16 responders. A Phase III, open-label, randomized study comparing Tremelimumab 15 mg/kg Q3M (Arm A) to either dacarbazine or temozolomide (Arm B) in patients with advanced melanoma was terminated following a pre-specified interim futility analysis. At the time of database lock, the median OS (primary endpoint) was 12.58 months in Arm A and 10.71 months in Arm B (HR=1.1416, p=0.1272).

The efficacy data for tremelimumab are consistent with those of the related – and FDA-approved- anti-CTLA-4 antibody ipilimumab: tumor response rates are generally low (approximately 10%) but the responses observed are generally durable. The ipilimumab melanoma data clearly demonstrate that a small proportion of patients with an objective response and a small impact on PFS rates can lead to a significant prolongation of OS, and support development of this class of agent in other tumors. Although Phase II and Phase III studies of tremelimumab in metastatic melanoma failed to meet the primary endpoints of response rate and OS, respectively, the data clearly indicate activity of tremelimumab in melanoma, with response rates and median OS similar to those observed in the ipilimumab trials. However, in contrast to tremelimumab, there are no safety data for the use of ipilimumab in HCC.

1.2.4.1 Clinical experience of Tremelimumab

Tremelimumab has been evaluated in a number of clinical studies – and over 1000 patients – and demonstrated manageable toxicities[24,25].

The efficacy data for Tremelimumab are consistent with those of the related anti-CTLA-4 antibody ipilimumab: tumor response rates are generally low (approximately 10%) but the responses observed are generally durable. The ipilimumab melanoma data clearly demonstrate that a small proportion of patients with an objective response and a small impact on PFS rates can lead to a significant prolongation of OS, and support development of this class of agent in other tumors. Although Phase II and Phase III studies of Tremelimumab in metastatic melanoma failed to meet the primary endpoints of response rate and OS, respectively, the data clearly indicate activity of Tremelimumab in melanoma, with response rates and median OS similar to those observed in the ipilimumab trials.

The AE profile of Tremelimumab is consistent with that of ipilimumab, and with the pharmacology of the target. To date, no tumor type or stage appears to be associated with unique AEs (except for vitiligo, which appears to be confined to patients with melanoma). Events reported at a frequency of ≥5% and assessed by the investigator as related to treatment (listed in descending order of frequency) were diarrhea, rash, pruritus, fatigue, nausea, vomiting, anorexia, headache, abdominal pain, and colitis.

1.2.4.2 Experience in Hepatitis/HCC

Tremelimumab has recently been shown to be safe in a study in HCC. Twenty-one patients with HCV-related HCC were treated with Tremelimumab intravenously at a dose of 15 mg/kg every 90 days for two cycles. Toxicity was manageable with reported treatment-related adverse events among 80% of patients. Grade 3 or higher adverse events included 1 case of pruritus, 1 case of purpura, and 5 cases of elevated transaminases. There was preliminary evidence of efficacy. In an intention-to-treat analysis, investigators observed a median overall survival of 7.5 months and time to progression of 6.4 months as well as a reduction of hepatitis C virus (HCV) in the patients' blood, which was also accompanied with objective enhancements of antiviral immunity. A significant and progressive decline in serum HCV viral load was observed (median values: baseline 3.78x10e5 copies/ml vs. day 120 3.02x10e4 copies/ml, $P = .02$; vs. day 210 1.69x10e3 copies/ml, $P = .04$).

1.2.5 Justification for dose and schedule

There is safety data from ~70 patients treated with 10 mg/kg Q4 weeks and currently an ongoing study is collecting additional data on this dose and schedule. The proposed dose and schedule is informed by safety and efficacy data on Tremelimumab, and by data showing a relationship between exposure and survival in the advanced melanoma studies with Tremelimumab. The PK of Tremelimumab has been characterized in various clinical studies with more than 1,000 patients treated with Tremelimumab to date. Tremelimumab has been administered at doses ranging from 3 to 15 mg/kg Q4W or Q12W. The PK of Tremelimumab was linear and dose-proportional. The disposition of Tremelimumab exhibits biphasic elimination, with a long terminal phase half-life of ~20 days. In an early Phase 1/2 study (A3671002; Camacho et al, 2009), patients with melanoma in the Phase 2 portion of the study were randomly assigned to Tremelimumab 15 mg/kg Q12W (n = 46) or Tremelimumab 10 mg/kg Q4W (n = 44). The overall AE frequency between the 2 schedules was comparable, albeit with fewer Grade 3-4 AEs (13%) in the less frequent dosing regimen (15 mg/kg Q12W) compared to the Q4W regimen (27%). The difference in Grade 3-4 AEs was largely due to differences in the incidence of ≥Grade 3 diarrhea (21% vs. 9%). However, all SAEs were manageable and reversible when appropriate intervention was applied. Efficacy results of this study showed similar response rates in each arm (10% for 10 mg/kg Q4W, 9% for 15 mg/kg Q12W) with no significant difference in OS.

However, a retrospective analysis of data from Phase 2 and Phase 3 melanoma studies with15 mg/kg Q12W Tremelimumab showed an improvement in the OS outcome for patients who achieved a higher exposure as measured by the area under the concentration-time curve from time 0 to 90 days (AUC90). Furthermore, pharmacokinetic simulations indicate that following Tremelimumab at 10 mg/kg Q4W, approximately 90% patients are expected to be above the target concentration (~30 μg/mL) compared to ~50% with 15 mg/kg every Q12W dosing regimen. Based on these data, MedImmune now proposes using a Tremelimumab dosing regimen of 10 mg/kg Q4W both for this study and across all other studies.

1.2.6 Rationale for Re-Induction

Re-treatment with Anti-CTLA-4 agent provide durable response or stable disease in patients with melanoma who initially respond to treatment but later progressed[26,27] with some subjects achieved better overall response after re-induction[4]. No new types of toxicities observed after re-

induction with safety profile consistent with induction profile.[26,27] These findings support re-treatment with immune checkpoint inhibitors after initial disease control.

RFA or TACE are modalities inducing localized cell death and enhancing antitumor immune response[28,29]. To date it is unknown what type of treatment, RFA vs TACE is more immunogenic. By allowing the same patient to undergo TACE and RFA we will produce different immune stimulation and possibly enhance antitumor immune response. In addition, benefit of re-treatment with TACE or RFA is to provide a similar immune boost as revaccination treatment in cancer vaccine clinical trials.

Combining TACE and RFA in HCC treatments has been atttemted in single centers small studies showing an improvement in OS without significant increase in side effects[30,31]. Larger randomized clinical trial of 189 patiens resulted in improvement in OS and recurrence free survival in RFA-Tace group relative to RFA group alone whithout significant differences in toxicity profiles.[32] Findings from these studies confrim tolerability of combination. For definite conclusions on efficacy of combined interventional management larger clinical trials are needed.

1.2.7 Rationale for Viral Hepatitis Studies and Specifically for Hepatitis B Surface Antigen and Hepatitis B Immune Monitoring

Anti-HCV specific immune responses as well as a reduction in Hepatitis C viral load have been described in in patients with chronic HCV infection and HCC (decline in serum HCV viral load from median values: basal 3.78x10e5 copies/ml to median values on day 120 of 3.02x10e4 copies/ml, p=0.02; vs. day 210 1.69x10e3 copies/ml, p=0.04); in addition three patients demonstrated a complete viral response for the duration of the follow-up[33].

The antiviral effect of CTLA-4 blockade in patients with chronic HBV infection is unknown. Hepatitis e antigen (HBeAg) is a marker of viral replication and infectivity that is usually associated with and active liver disease. Hepatitis B surface antigen (HBsAg) is a protein on the surface of the hepatitis B virus. Its presence identifies patients with chronic infection. CTLA-4 blockade in cynomolgus macaques enhanced antibody response to hepatitis B surface antigen (HBsAg)[34]. Interestingly, polymorphisms of CTLA-4 have been associated with chronic hepatitis B and C infection[35,36]. The mechanisms behind these events imply an existing link between CTLA-4 activity and hepatic viral pathogenesis that should be exploited in details for therapeutic benefit. The population of HCC patients with chronic hepatitis B virus (HBV) infection will provide an exceptional occasion to simultaneously test the anti-tumoral, immune and antiviral effect of tremelimumab.

1.2.8 Rationale for Amendment F

The purpose of amendment F is to add 3 additional patient cohorts (denoted C, D and E) of N=10 patients each to evaluate additional globally used and potentially immunomodulative procedures (cryoablation and external beam radiation) in patients with HCC. We would also like to evaluate RFA plus tremelimumab in biliary tract carcinoma. The justification for each of these cohorts is as follows:

*Cohort C: SBRT plus tremelimumab in HCC (**removed, effective with amendment I**).* Several studies have documented an increase in peripheral antitumor immunity following radiation. It may even be the case that an intact immune system is critical for RT to exert its maximal antitumor effect. This was suggested by a mouse model of melanoma in which single-fraction

RT slowed the growth of small tumors in immune-competent but not immune-deficient mice[37]. The same authors also showed that in an animal model of breast cancer ablative RT of a primary tumor prevented the growth of metastatic tumor colonies in the lung, an effect that was dependent on the presence of CD8+ T cells. Other animal models have likewise displayed augmentation of systemic antitumor immunity following local RT[38-40]. The underlying mechanism appears to be that RT-induced necrosis results in the exposure of tumor antigens, thereby increasing the pool of intracellular peptides for cross-presentation[41]. Radiation has been shown to augment MHC-I expression by tumors, which is critical for antigen recognition by cognate CD8+ TCRs and which is known to be diminished in tumors as one of their escape mechanisms[42]. Tumor antigen processing and presentation on MHC-I molecules is dependent on expression of a protein called highmobility group box 1 (HMGB-1), a "danger signal" which binds toll-like receptor 4 (TLR4) on dendritic cells. In a pivotal study Apetoh et al. demonstrated that RT causes dying tumor cells to release HMGB-1[43]. Intriguingly these authors also reported that in breast cancer patients undergoing radiation and chemotherapy the presence of a polymorphism in TLR4 – and by implication a less immunogenic drug-induced cell death – was associated with an inferior prognosis. Similarly, in esophageal cancer patients preoperative chemoradiation has been shown to increase cancer-specific T cell responses and serum levels of HMGB-1, the latter of which correlated with overall survival[44].

Despite this, only a few cases of spontaneous decrease of metastases following radiation – the so-called abscopal effect – have been reported. This is presumably because the immune response by itself is too weak to be clinically significant. A number of studies have tried to boost this anti-tumor immune response following ablation by combining with an immunomodulatory agent: Dewan et al. evaluated RT in combination with anti-CTLA-4 antibody in two separate mouse models of breast and colorectal carcinoma[45]. The authors found that the combination of anti-CTLA4 and RT achieved enhanced tumor response at the primary site (compared to either modality alone). in addition to an abscopal effect. (Interestingly this only occurred in fractionated versus single-dose RT). The frequency of CD8+ T cells showing tumor-specific IFN-gamma production was proportional to the absopal effect. Demaria et al. tested the combination of RT with CTLA-4 blockade in a breast cancer model (4T1) known to be poorly immunogenic[46]. Anti-CTLA4 alone did not have any effect on primary tumor growth or survival. RT was able to delay the growth of the primary irradiated tumor, but in the absence of anti-CTLA4 survival was similar to that of control mice. In contrast, mice treated with RT + anti-CTLA4 had a statistically significant survival advantage. The increased survival correlated with inhibition of lung metastases formation and required CD8+.

Administering radiation therapy to the liver is not standard or conventional. With newer techniques however, this has been shown to be a safe strategy and to have palliative benefit in an incurable population. For example, Dr. Citrin – one of our associate investigators – has previously conducted a study employing hepatic radiation in colorectal cancer[47]. In that study patients received a total radiation dose of 32 Gy to sites of metastatic disease in the liver, delivered in 8-Gy courses (4 separate 2-Gy fractions) beginning one day after each vaccine boost (days 22–25, 36–39, 50–53, 64–67). The treatment was well tolerated, with the most common toxicity being a transient dermatologic reaction as a result of the vaccine used in the study. There were no ≥ grade 3 toxicities attributable to the treatment and specifically there were no ≥ grade 2 hepatic toxicities. Other additional reports have documented the safety of SBRT to the liver at doses/schedules of 60 Gy/10 Fr and 50 Gy/4 Fr[48]; 36 to 60 Gy in 6 to 15 Gy per fraction (HCC, cholangio and colorectal cancer)[49]; single doses of 17 to 30 Gy (median dose 24 Gy)[50]; median

dose per fraction and total dose of 3.6 Gy (2.0-13.0 Gy) and 55 Gy (30-80 Gy) in N=26patients with colorectal cancer[51].

Cohort D: Cryoablation and tremelimumab in HCC. Whilst RFA is the more traditional ablative strategy in HCC there is a growing use of cryoablation globally. In addition there is a growing body of data which suggests that it may be more effective[52]. At a minimum it appears to be equivalent in terms of safety and efficacy, as recently shown in a recent multicenter randomized study comparing the outcomes of percutaneous cryoablation with RFA for the treatment of HCC[52]. Three hundred and sixty patients with Child-Pugh class A or B cirrhosis and one or two HCC lesions ≤ 4 cm, treatment naïve, without metastasis were randomly assigned to cryoablation (n=180) or RFA (n=180). The primary end-points were local tumor progression at 3 years after treatment, and safety. Local tumor progression rates at 1, 2, and 3 years were 3%, 7%, and 7% for cryoablation and 9%, 11%, and 11% for RFA, respectively (P=0.043). For lesions >3 cm in diameter, local tumor progression rate was significantly lower in cryoablation group versus RFA group (7.7% vs 18.2%, P=0.041). The 1-, 3-, and 5-year overall survival rates were 97%, 67% and 40%, for cryoablation and 97%, 66%, and 38% for RFA, respectively (P=0.747). The 1-, 3-, and 5-year tumor-free survival rates were 89%, 54%, and 35% in cryoablation group and 84%, 50%, and 34% in RFA group, respectively (P=0.628). Major complications occurred in seven patients (3.9%) following cryoablation and in six patients (3.3%) following RFA (P=0.776). The conclusions of this study were that cryoablation appeared to be just as safe and feasible as radiofrequency or microwave ablation in HCC and is very commonly used across the world. Intriguingly – for our purposes - there is evidence that cryoablation may be better in terms of immune stimulation compared to other ablative strategies[53].

Cohort E: RFA in biliary tract carcinoma: Biliary tract carcinoma (BTC) is the second most common hepatobiliary cancer. For the majority of patients who present with or develop metastatic/ unresectable disease the modest standard therapy comprises Gemcitabine in combination with cisplatin based on the ABC-02 trial which demonstrated a median overall survival benefit of 11.7 v 8.1 months (P<0.001) in favor of cisplatin–gemcitabine compared to gemcitabine alone[54]. There is no standard second-line treatment for biliary tract carcinomas.

The most compelling argument in favor of testing immune-based strategies (and anti-PD1 therapy in particular) in this disease is that chronic inflammation is the most common etiologic factor in the development of BTC. Recent studies have linked the increasing incidence of chronic hepatitis C virus infection with intrahepatic cholangiocarcinoma[55], which may explain the increasing incidence of the intrahepatic subtype [56]. In biliary tract cancer, the prevalence and prognostic relevance of infiltrating T-lymphocytes has been documented. Goeppert et al. evaluated immune cell infiltration in 375 cases of biliary tract cancer obtained following surgical resection[57]. Approximately half the patients had intraepithelial tumor infiltrating T cells (both CD4 and CD8) and this was associated with better survival. Further evidence of the importance of immune activation/suppression, this time specific to the PD1/PD-L1 axis, was provided by Ye et al. who evaluated the expression of B7-H1(PD-L1) and its receptor PD-1 in N=31 surgically resected cholangiocarcinoma tissues and the corresponding cancer adjacent tissues[58]. Expression of PD-L1 and PD-1 was found to be up-regulated in cholangiocarcinoma tissues compared with the cancer adjacent tissues. Tumor-related PD-L1 expression was significantly correlated with both tumor differentiation and pTNM stage and was inversely correlated with CD8+ tumor-infiltrating lymphocytes. Similarly, Nakakubo et al. investigated the significance of tumor-infiltrating immune cells in 110 surgically resected biliary tract cancer (gallbladder) specimens[59].

They found high levels of CD4+ T cell (51.1%), CD8+ T cell (37.8%), NL cell (33.3%), and dendritic cell (48.9%) infiltration. CD4+ and CD8+ T cell infiltration correlated with decreasing tumour invasion, and high numbers of infiltrating DCs correlated with decreasing lymph-node tumour metastasis. Furthermore, increased infiltration of CD4+ and CD8+ T cells and DCs exhibited a significant correlation with prolonged survival. Because of these data, and coupled to the data summarized earlier for immune stimulation following RFA, we wish to treat a cohort of biliary tract carcinoma patients.

1.2.9 Rationale for Amendment I

The purpose of amendment I is to remove Cohort C. Given a range of factors - the number of variables in this study, the finding of interesting activity with ablative methods (commonly used in HCC) and the relative infrequency of SBRT in this disease – it was decided both scientifically and for patient safety reasons to focus and refine our research efforts. At this point we will not enroll to Cohort C but preferentially enroll HCC patients to either cohort B of this protocol or our new protocol (16-C-0135). Should we decide to evaluate SBRT in combination with immune modulating therapy in this population in the future it will only be done after an IRB amendment.

1.2.10 Rationale for Amendment J

The purpose of amendment J is to stop further tremelimumab treatment beyond 2 years if there is no evidence of disease progression. Currently patients would continue to receive tremelimumab treatment every 12 weeks until disease progression. The justification for this is as follows:

1) Two-year-duration of immune checkpoint inhibitor treatment is adequate for disease treatment and control: early trials in melanoma allowed treatment until progression; then, 2-year duration was the maximal adopted by majority of clinical trials [60], [61], [62] and approved regimens. Such study design does not prevent patients from benefiting the most from immune checkpoint inhibitor treatment [63]. Effectively, the discontinuation of immune checkpoint inhibitor after a predetermined duration is not associated with an increased risk of acquired resistances such as that described with cytotoxic chemotherapy [63]. Few reports have examined the outcome in patients who achieved a response and stopped therapy after 2 to 3 years [64]. Among them, 61 (64%) stopped treatment after complete response and maintained their remission. Median duration of treatment was 23 months. Only 2 of the 61 patients who stopped treatment after complete response experienced disease progression. Many of those who did not achieve a complete response and whose disease progressed were able to respond to further immunotherapy [64]. The existence of the "tail on the curve" of survival in melanoma, and now other cancers, suggests that a subset of patients who discontinue treatment with one of these agents experience durable responses after therapy and do not need to be treated until progression. Presumably, the patient's immune system has been engaged and is still monitoring and effectively suppressing the cancer. Nevertheless, re-challenging the tumor with the same immune checkpoint inhibitor even after a long stable period demonstrated a greater response and lesser adverse events [65]. Thus, indefinite treatment may not be truly necessary, and a duration of not less than 1 year and not more than 2 years seems reasonable.

2) Prolonged immune checkpoint inhibitor exposure has greater risks for potentially life-threatening toxicities: despite important clinical benefits, immune checkpoint inhibition is associated with a unique spectrum of immune-related adverse events. Serious side effects are observed in less than 10% of patients, and typically emerge an average of 6 to 12 weeks after the

start of treatment [66],[67]. The risk of side effects increases with higher doses, and overall seems to increase with cumulative exposure. Moreover, as immunotherapies are being tested in combination, side effects tend to be additive. For example, the more common adverse effects are related to GI inflammation, which can be as minor as slight irritation or as severe as colitis, potentially even leading to perforation of the bowel. Those potentially life-threatening toxicities in the context of prolonged treatment may outweigh the benefits of indefinite immune checkpoint inhibitor therapy.

Given above discussed justifications, it is decided both scientifically and for patient safety reasons to focus and refine our research efforts. At this point we will discontinue tremelimumab administration after 2 years if patient is experiencing responding to the treatment over 2 years, including stable disease, partial or complete remission.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histopathological confirmation of hepatocellular carcinoma (HCC) or (Cohort E only) biliary tract carcinoma (BTC) by the Laboratory of Pathology of the NCI prior to entering this study OR histopathological confirmation of carcinoma in the setting of clinical and radiological characteristics which, together with the pathology, are highly suggestive of a diagnosis of HCC (or biliary tract carcinoma in Cohort E). Fibrolammelar variant is also allowed. For cohort E, the term BTC includes intra- or extrahepatic cholangiocarcinoma, gallbladder cancer or ampullary cancer, as long as there is an intrahepatic component amenable to RFA.

2.1.1.2 Patients must have disease that is not amenable to potentially curative resection, transplantation or ablation. For Cohorts A and D patients must have progressed on, been intolerant to, or refused prior sorafenib therapy. Cohort E patients must have received at least one line of chemotherapy for BTC.

2.1.1.3 Disease must be technically amenable to transhepatic arterial chemoembolization (TACE), radiofrequency ablation (RFA), or cryoablation. Each case will be discussed at GI tumor board with interventional radiology. Patients must have evaluable disease.

2.1.1.4 If liver cirrhosis is present, patient must have a Child-Pugh A/B7 classification (see Appendix B section **12.2**).

2.1.1.5 Age ≥18 years

2.1.1.6 Life expectancy of greater than 3 months.

2.1.1.7 ECOG performance status 0-2 (see Appendix A section **12.1**)

2.1.1.8 Patients must have normal organ and marrow function as defined below:

leukocytes	≥3,000/mcL
absolute neutrophil count	≥ 1,000/mcL
platelets	≥ 60,000/mcL
total bilirubin	If cirrhosis present: Part of Child Pugh

	requirement If no cirrhosis: Bili should be ≤ 2 xULN
Serum albumin	If cirrhosis present: Part of Child Pugh requirement If no cirrhosis: albumin should be ≥ 2.5g/dl
Patients are eligible with ALT or AST up to 5 x ULN.	
creatinine	<1.5X institution upper limit of normal OR
creatinine clearance	≥45 mL/min/1.73 m^2, as calculated below, for patients with creatinine levels above institutional normal

2.1.1.9 Patients must have recovered from any acute toxicity related to prior therapy, including surgery. Toxicity should be ≤ grade 1 or returned to baseline.

2.1.1.10 Patients must not have other invasive malignancies within the past 5 years (with the exception of non-melanoma skin cancers, non-invasive bladder cancer or localized prostate cancer for whom systemic therapy is not required).

2.1.1.11 Patient must be able to understand and willing to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who have had standard of care chemotherapy, large field radiotherapy, or major surgery must wait 2 weeks prior to entering the study. For recent experimental therapies a 28 day period of time must elapse before treatment.

2.1.2.2 Patients who have undergone prior liver transplantation are ineligible.

2.1.2.3 Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

2.1.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active systemic infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia (excluding insignificant sinus bradycardia and sinus tachycardia) or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.2.5 History of chronic autoimmune disease (e.g., Addison's disease, multiple sclerosis, Graves' disease, Hashimoto's thyroiditis, rheumatoid arthritis, hypophysitis, etc.) with symptomatic disease within the 3 years before randomization. Note: Active vitiligo or a history of vitiligo will not be a basis for exclusion.

2.1.2.6 Dementia or significantly altered mental status that would prohibit the understanding or rendering of Information and Consent and compliance with the requirements of the protocol.

2.1.2.7 Diverticulitis (either active or history of) within the past 2 years. Note that diverticulosis is permitted.

2.1.2.8 Active or history of inflammatory bowel disease (colitis, Crohn's), irritable bowel disease, celiac disease, or other serious, chronic, gastrointestinal conditions associated with diarrhea. Active or history of systemic lupus erythematosus or Wegener's granulomatosis.

2.1.2.9 Currently receiving immunosuppressive doses of steroids or other immunosuppressive medications (inhaled and topical steroids are permitted)

2.1.2.10 History of sarcoidosis syndrome

2.1.2.11 Patients should not be vaccinated with live attenuated vaccines within 1 month of starting Tremelimumab treatment.

2.1.2.12 Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies)

2.1.2.13 HIV-positive patients receiving anti-retroviral therapy are excluded from this study due to the possibility of pharmacokinetic interactions between antiretroviral medications and Tremelimumab. HIV positive patients not receiving antiretroviral therapy are excluded due to the possibility that Tremelimumab may worsen their condition and the likelihood that the underlying condition may obscure the attribution of adverse events.

2.1.2.14 History of hypersensitivity reaction to human or mouse antibody products.

2.1.2.15 Pregnancy and breast feeding are exclusion factors. The effects of Tremelimumab on the developing human fetus are unknown. Enrolled patients must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, the duration of study participation and 3 months after the end of the treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.2.16 Patients with unhealed surgical wounds for more than 30 days.

2.1.3 Re-Induction Criteria (if applicable)

2.1.3.1 Subjects must have entered the Maintenance Phase and are under ongoing maintenance treatment

or Subjects who stopped maintenance treatment because of a CR
or Subjects with an initial PR or CR or at least 3 months of SD on tumor assessment
and who subsequently have a confirmed and documented disease progression (per immune-related RECIST criteria).

2.1.3.2 Patients are excluded from re-induction if they have experienced any related Dose-limiting toxicities as described in Section **3.6**, delayed dosing beyond 35 days due to Tremelimumab-related AEs, or have been taken off treatment due to toxicity as described in Section **3.8.3**.

2.1.3.3 Patients who have progressed on initial therapy will not be considered for re-induction treatment.

2.1.3.4 Tremelimumab Dose: Patients will be treated at the same dose of Tremelimumab as they previously received. For patients on Dose Level 1 who had already satisfied criteria for escalation to 10mg/kg they will be re-treated at 10mg/kg.

2.1.3.5 The patient must – at the time of re-induction – satisfy all the eligibility criteria as set out in Sections **2.1.1** & **2.1.2**.

2.1.3.6 The patient must be discussed at GI Tumor Board, NCI and suitability for the interventional procedure (TACE or RFA) re-affirmed.

Eligible patients will follow the same treatment criteria, evaluation plan and schedule as was previously described for the main part of the study, and doses may be delayed or modified as described in Section **3.8**. There will be no limit to the number of additional re-inductions a patient may receive if the same efficacy and safety criteria described above are met, until intolerable toxicity or progressive disease necessitates ending the re-induction phase.

2.1.4 Inclusion of Women and Minorities

Men and women of all races and ethnic groups are eligible for this trial.

2.1.5 Recruitment Strategies

The study will be posted on the CCR website and on clinicaltrials.gov.

2.2 SCREENING EVALUATION

2.2.1 History and Physical Examination

Complete history (including prior hormone use) and physical examination (including height, weight, vital signs, EKG, and performance status) will be conducted prior to starting study drug.

2.2.2 Laboratory Evaluation

- Hematological profile: CBC with differential and platelet count, PT, INR, aPTT, fibrinogen.
- Biochemical profile: electrolytes, BUN, creatinine, AST, ALT, total bilirubin (including direct and indirect), calcium, phosphorus, albumin, magnesium, uric acid, amylase.
- Serum or urine pregnancy test for female participants of childbearing age (in the absence of prior hysterectomy). Test to be performed within 72 hours prior to initiating treatment.

2.2.3 Histologic confirmation (at any time point prior to commencement)

A block or unstained slides of primary or metastatic tumor tissue will be required from each participant to confirm diagnosis with analysis being performed by the Laboratory of Pathology, NIH. As stated in the inclusion criteria above, histopathological confirmation of carcinoma may be sufficient. Fibrolammelar variant is also allowed.

2.3 BASELINE STUDIES

2.3.1 Imaging studies (obtained within 28 days prior to first dose)

- CT scan of chest, abdomen and pelvis

  And/or

- MRI liver

2.3.2 Laboratory evaluation (obtained within 72 hours prior to first dose)

If laboratory tests are done within 72 hours of Cycle 1 Day 1 they do not need to be repeated on Day 1.

- Hematological profile: CBC with differential and platelet count, PT, INR, aPTT, fibrinogen.
- Biochemical profile: electrolytes, BUN, creatinine, AST, ALT, total bilirubin (including direct and indirect), calcium, phosphorus, albumin, magnesium, uric acid, amylase.
- Tumor marker profile: aFP or Ca19.9 (obtained within 7 days prior to first dose)

2.3.3 Laboratory evaluation (obtained within 28 days prior to first dose)

- Thyroid function tests (TSH, T3, T4)
- Hepatitis B and/or C viral load and serology
- HLA-A2 phenotype

2.3.4 History and physical exam with vital signs (obtained within 1 week prior to first dose).

2.3.5 Electrocardiogram (obtained within 28 days prior to first dose)

2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and f sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

- The proposed study is a pilot study of Tremelimumab - at a planned dose 10mg/kg/IV q 4 weekly - in patients with advanced HCC treated with either TACE or RFA.

The initial study population is Cohort A as described below and these patients will be treated with either RFA or TACE. The decision on which treatment to administer (i.e. RFA vs. TACE) will be made in consultation with the interventional radiologists. The reason for this is that there are technical considerations (size and location of lesion, main portal vein occlusion etc.) which would make one procedure preferable over the other.

Cohort	Population	Immune stimulative procedure	Dose level (Trem)	Planned N
A	Advanced HCC, BCLC Stage C	RFA/TACE	3.5mg/kg	3-6
			10mg/kg	20
B	Intermediate HCC, BCLC B	TACE	10mg/kg	20

- Transarterial chemoembolization is percutaneous procedure performed under general anesthesia or conscious sedation. (See **3.9.1** for details)
- Patients undergoing trans-arterial chemoembolization, RFAor cryoablation are pre-medicated with analgesics, antibiotics and anti-emetics per clinical standards. For details regarding each procedure please see **3.9**.
- We will also enroll an additional cohort of N=20 patients (Cohort B) with liver-confined disease for whom TACE is indicated as standard of care. These patients will receive TACE in combination with Tremelimumab using the same schema, although for these patients TACE may be repeated (as per standard of care). They will not receive RFA.
- With protocol amendment F, it was proposed that once cohort A has demonstrated safety and feasibility for the combination of ablative treatment and tremelimumab in HCC, we will add 3 additional cohorts (C-E) to the study. However, with amendment I we have revised the study design to remove Cohort C, so only 2 cohorts, D and E, remain. For these cohorts, the schedule of administration for tremelimumab and the timing of the immune-stimulative procedure is identical to cohorts A and B, as summarized below.

Cohort	Population	Immune stimulative procedure	Dose level (Trem)	Planned N
D	Advanced HCC, BCLC Stage C	Cryoablation	10mg/kg	10

E	Intrahepatic cholangiocarcinoma	RFA	10mg/kg	20

3.2 SCHEDULE

- Day 1: Tremelimumab IV every 4 weeks x 6 doses and then every 12 weeks for 2 years or until disease progression whichever comes first.
- Day 36 [+/- 96 hours]: RFA or TACE.
- Restaging CT /MRI scan every 8 weeks to evaluate TTP in target lesion. (Both standard RECIST and Immune-related Response Criteria [irRC] will be employed).
- Dose: (Cohort A only): Initially three to six patients will be treated at 3.5mg/kg (with subsequent dosing also at 3.5mg/kg) prior to enrolling to the10mg/kg dose level. If a single patient experiences dose-limiting toxicity (DLT) related to the combination this cohort will be expanded to N=6 as per a standard 3+3 design. If 3.5mg/kg is established to be safe we will proceed to the 10mg/kg dose level. N=20 patients in cohort A will be treated at 10mg/kg.
- For Cohort A (BCLC Stage C patients): TACE or radiofrequency ablation (RFA) will be performed once only.
- For Cohort B (BCLC Stage B patients): TACE may be repeated (as per standard of care) on months 3, 7, and 13, and q6 months thereafter (if indicated).
- Cohort B will only be recruited following completion of the dose-escalation portion of Cohort A.

For Cohort E (BTC patients, status post prior chemotherapy with intrahepatic disease) RFA will be performed once only, also on Day 36 (+/- 96hrs).

3.3 COHORT A

3.3.1 Definition

The definition of Cohort A is as follows:

Cohort A (N=23-26):

- Advanced stage HCC, BCLC group C
- Patients must have progressed on, been intolerant of prior sorafenib therapy.
- Child Pugh A/B7
- No available surgical option (i.e. transplantation or hepatic resection) or curative ablative option.
- Disease amenable to either TACE or RFA

3.3.2 Treatment

Three patients will be treated at 3.5mg/kg (with subsequent dosing also at 3.5mg/kg i.e. no intrapatient dose escalation) prior to enrolling to the10 mg/kg dose level. If a single patient experiences dose-limiting toxicity (DLT) related to the combination of TACE/RFA and

Tremelimumab this cohort will be expanded to N=6 as per a standard 3+3 design. If 3.5mg/kg is established to be safe we will proceed to the 10 mg/kg dose level. N=20 patients will be treated at 10 mg/kg to evaluate the feasibility endpoint, provided no more than 2 of 3-6 patients have a DLT at this level.

Dose level	Tremelimumab dose (mg/kg)	N
1	3.5	3-6
2	10	20

- The 3.5 mg/kg cohort will have at least 3 patients to evaluate for toxicity to establish MTD for the combination of Tremelimumab with either TACE or RFA. Three patients will be treated at this dose level. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. If none of the three patients at the 3.5 mg/kg dose level experiences dose-limiting toxicity (DLT), accrual to DL2 will proceed to the 10mg/kg cohort. If one of three patients treated at a dose level experiences DLT that is considered to be at least possibly related to the combination of Tremelimumab with either TACE or RFA, then three more patients will be enrolled at that same level. If the incidence of DLT among those six patients is one in six, then we will proceed to 10 mg/kg. If two or more of the six patients experience DLT, then the MTD is considered to have been exceeded.
- Evaluation period for Dose-limiting Toxicities (DLT) will extend for the first 8 weeks of the study.
- Following completion of the dose escalation portion of the study Tremelimumab will be administered at the MTD at the same schedule. A total of 20 patients will be treated in cohort A at the MTD, not including those patients treated at the lower doses.
- For patients on Dose Level 1 who have completed at least 16 weeks of protocol therapy, we will consider dose escalation to 10mg/kg if criteria for continuance of therapy are met and the patient does not have >Grade 2 toxicity attributable to tremelimumab.

3.3.3 Protocol Stopping Rules

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for grading systemic toxicity. For safety reasons, the protocol will be temporarily halted pending discussions with the NCI IRB and Sponsor regarding necessary amendment for either of the following events:

- One occurrence of grade 5 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- Two occurrences of grade 4 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.

3.3.4 Re-Induction

Patients who entered the maintenance phase and have confirmed PD (per immune-related RECIST criteria) during the maintenance treatment may be eligible to receive re-induction therapy using Tremelimumab at the assigned dose and schedule: i.e. 10mg/kg monthly for 6 months followed by maintenance 3-monthly Tremelimumab. Patients who stopped maintenance treatment because of a CR and subsequently had confirmed PD (per immune-related RECIST criteria) are eligible to receive re-induction as well. Subjects with an initial PR or CR or SD for at least 3 months and subsequently have PD are also eligible for re-induction. As part of re-induction they will also undergo the repeat ablative procedure on Day 36 (+/- 96hrs). This may be either an RFA or TACE depending on the opinion of the interventional radiologist. To be eligible for re-induction patients must satisfy the criteria stated in section **2.1.3**.

3.3.4.1 Tumor Biopsies

If a patient opts to undergo re-induction therapy we would like to perform pre- and post-re-induction tumor biopsies to better understand the role of emerging resistance to anti-CTLA4 therapy. These would occur prior to re-induction (≤ 2 weeks prior to starting) and then during the interventional radiologic procedure.

3.4 COHORT B

3.4.1 Definition

The definition of Cohort B is as follows:

Cohort B (N=20):

- HCC, Barcelona Clinic Liver Cancer (BCLC) Stage B;
- Child Pugh A/B7;
- No available surgical (i.e. transplantation or hepatic resection) or curative ablative option.
- Disease amenable to TACE

3.4.2 Treatment

The treatment schema for both cohorts is identical with the exception of the fact that in cohort B TACE may be repeated (as per standard of care) on months 3, 7, and 13, and q6 months thereafter (if indicated).

Patients in Cohort B will only be recruited following completion of the dose-escalation portion of Cohort A and will be treated at the dose level which has been determined to be safe in that cohort.

3.5 COHORT C

This cohort, designed to evaluate tremelimumab in combination with external beam radiation was removed with amendment I.

3.6 COHORTS D-E

Once safety is established for the initial concept of combining anti-CTLA4 with ablative procedures (RFA and TACE), we will similarly explore feasibility of tremelimumab at the same dose and schedule in 2 additional cohorts (denoted D-E) evaluating tremelimumab in

combination with cryoablation in HCC (cohort D) and with RFA in a cohort of patients with biliary tract carcinoma (cohort E).

- For these additional cohorts, the dose (10mg/kg) and schedule of tremelimumab will be as per cohorts A and B. The timing of the interventional procedure will also be identical.
- Cohort Definition: Cohort D will have the same definition as Cohort A above:

  Advanced stage HCC, BCLC group C

  Patients must have progressed on, been intolerant of prior sorafenib therapy.

  Child Pugh A/B7

  No available surgical option (i.e. transplantation or hepatic resection) or curative ablative option.

  Disease amenable to cryoablation
- Cohort E will contain BTC patients as specified in the eligibility criteria.
- For cohorts D-E no dose escalation will occur. All patients will receive tremelimumab at 10mg/kg at the same schedule established as safe and feasible in Cohort A of the study. However, protocol stopping rules will apply as outlined in Section **3.3**.

3.7 DOSE LIMITING TOXICITIES

3.7.1 Definition of Dose-limiting Toxicities (DLTs):

A DLT is defined as a ≥ Grade 3 adverse drug reaction (ADR) according to the NCI-CTCAE v4.0, that is possibly, probably, or definitely related to the combination of Tremelimumab with either TACE or RFA, occurring during the DLT evaluation period except for any of the following outlined in section **3.6.2**. ADRs are defined in this trial as any AEs suspected to be related to Tremelimumab by the investigator. Evaluation period for Dose-limiting Toxicities (DLT) will extend for the first 8 weeks of the study.

Treatment-related SAE will be ascribed as related to drug except where a clear relationship to the underlying disease or recognized co-morbidities is evident. For this trial, the MTD is defined as the highest dose where < 2 of 6 subjects experience a DLT.

3.7.2 Exclusions to Dose Limiting Toxicities

- Grade 3 infusion-related reaction resolving within 6 hours and controlled with medical management.
- Any Grade 3/4 non-hem toxicity that occurs in the first 10 days after RFA or TACE and which is an expected toxicity of the procedure in the opinion of the PI. These symptoms include, but are not limited to, fevers/rigors, pain, fatigue and nausea.
- Any grade 3 immune-mediated adverse event (including but not limited to Grade 3 diarrhea, Grade 3 skin toxicity, or Grade 3 liver enzyme elevation) that improves to ≤ Grade 2 within 1 week of onset with supportive care (which may include systemic corticosteroids).
- Nausea and vomiting Grade 3 will only be considered dose-limiting if it is refractory to anti-emetic therapy and unable to be corrected to Grade 1 or less within 48 hours.

- Grade 3 rise in creatinine, not corrected to Grade 1 or less after 2 liters of intravenous fluids within 24 hours, will be considered dose limiting.
- Single laboratory values out of normal range that are unlikely related to trial treatment according to the investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management.
- Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to ≤ Grade 1.
- Grade 4 elevation in transaminase elevation that improves to ≤ Grade 2 within 10 days of onset with supportive care.
- Grade 3 endocrinopathy that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the following criteria are met:
  - The subject's hormone levels are within normal limits
  - The subject is asymptomatic
- Grade 3 inflammatory reaction attributed to a local antitumor response (eg, inflammatory reaction at sites of metastatic disease, lymph nodes, etc.)

3.8 DRUG ADMINISTRATION

3.8.1 Tremelimumab Drug Administration

The first day of dosing is considered Day 1. Each dose of investigational product should be administered using the following guidelines:

1) Investigational product must be administered at room temperature (25°C) by controlled infusion at a rate of 250 mL/hr via an infusion pump into a peripheral vein. Prior to the start of the infusion, ensure that the bag contents are at room temperature to avoid an infusion reaction due to the administration of the solution at low temperatures.
2) Investigational product must **not** be administered via IV push or bolus but as a slow IV infusion. The entire content of each IV bag will be infused using an infusion pump.
3) The infusion lines should be attached only at time of use. Lines used for infusion during dose administration will need to be equipped with 0.22 or 0.2 μm in-line filters.
4) If there are no requirements to slow, interrupt, or permanently stop the infusion, the anticipated infusion time to deliver each dose (250 mL) is anticipated to be approximately 60 minutes.

The duration of the investigational product administration will be recorded.

3.8.2 Monitoring of Dose Administration

Vital signs will be collected before investigational product infusion and at the completion of the infusion.

As with any antibody, allergic reactions to dose administration are possible. Therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis, as per local institutional guidelines.

3.9 DOSING DELAYS

The following broad guidelines for dose delays apply to both of the planned dosages of Tremelimumab and are dependent on the clinical and laboratory assessment on the day of dosing. No dose reductions will be employed. Patients will either proceed or forgo treatment. Dosing may be delayed up to 14 days to allow recovery from treatment-related toxicity. For specific immune-mediated adverse reactions see Section **3.8.3** which will take precedence over this broad management guideline.

Condition	Management
Onset of any toxicity	Rule out alternative etiology In case of doubt, investigator should consult with study medical monitor promptly
NCI CTCAE Grade 1	Provide symptomatic treatment Possible topical steroids if applicable
NCI CTCAE Grade 2	Provide symptomatic treatment In the case of an immune-mediated adverse event do not give scheduled dose; dosing may be resumed once symptoms are resolved. Consider oral or IV steroids at the onset of symptoms. Taper steroid if symptoms improve.
NCI CTCAE Grade 3	Start high-dose IV steroids at the onset of the symptoms Provide symptomatic treatment
NCI CTCAE Grade 4	Start high-dose IV steroids at the onset of the symptoms Provide symptomatic treatment Permanent discontinuation [a] of Tremelimumab for all NCI CTCAE Grade 4 events (unless specific exemption stated elsewhere in protocol)
Steroid refractory toxicity (no improvement after 5 days on high-dose IV steroids) or relapse after reducing high-dose steroids	Continue symptomatic treatment and steroids Possible infliximab [b] 5 mg/kg IV for GI toxicities unless contraindicated [consult with GI specialist]. Caution: rule out bowel perforation and refer to label before using infliximab For subjects with increased AST/ALT, or total bilirubin levels, consider mycophenolate mofetil

ALT = alanine transaminase; AST = aspartate transaminase; GI = gastrointestinal; IV = intravenous; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

a Subjects will not receive any subsequent dose, but will remain on study and follow the other procedures required from the study (e.g., follow-up procedures, disease assessment scans, blood sample collections).

b Use will result in discontinuation of treatment.

During the study, subjects may require immunosuppressive medications such as steroids for management of underlying disease, treatment-related toxicity, or unrelated conditions. If symptoms resolved to NCI CTCAE Grade ≤ 1, Tremelimumab dosing may be resumed during steroid taper. Subjects with adrenal insufficiency may take daily prednisone or equivalent therapy for their endocrinopathy while receiving Tremelimumab treatment. Topical and inhaled steroids in standard doses are allowed.

3.9.1 Dosing Delays/Dose Modifications: General Guidelines

3.9.1.1 Grade 1 or 2 Toxicity

Treatment with Tremelimumab need not be interrupted, although for chronic low grade toxicity causing significant detrimental effect in patient's well-being the PI may elect to delay/omit dosing at his discretion, in particular in the case of a suspected immune-mediated adverse event.

3.9.1.2 Grade 3 Hematologic or Non-Hematologic Toxicity

Hold Tremelimumab and re-evaluate until toxicity improves to ≤ grade 1 or pre-treatment baseline. Treatment will be discontinued in patients who experience grade 3 non-hematologic toxicities felt to be drug-related.

3.9.1.3 Grade 4 Non-Hematologic Toxicity

Patients with clinical treatment-related grade 4 non-hematologic toxicity (except pulmonary embolism without significant hypoxia and hemodynamic instability) will be taken off treatment permanently. Unacceptable toxicities that have not resolved at time of "off treatment" must be followed until stabilization or resolution, at which time they will continue in follow up for survival.

3.9.2 Toxicities Mandating Permanent Discontinuation of Tremelimumab

As per the Investigator's brochure the following criteria will result in permanent discontinuation of tremelimumab.

Condition	Action
CTCAE Grade ≥3 related diarrhea or colitis	**Permanent discontinuation of tremelimumab for all listed conditions**
AST or ALT >8 x ULN or total bilirubin > 5 x ULN	
CTCAE Grade ≥3 hypersensitivity reaction or infusion reaction; Recurrent/persistent CTCAE Grade 2 hypersensitivity	
CTCAE Grade ≥3 endocrine disorders, if symptomatic and not controlled with hormone replacement therapy. (Tremelimumab may de dosed for CTCAE Grade ≤3 endocrine disorders, if asymptomatic and controlled with hormone replacement therapy.)	
CTCAE Grade 4 rash or other skin disorders (with the exception of vitiligo, which may be dosed regardless of severity)	
Any other CTCAE Grade ≥3 events thought to be drug-related	
Any CTCAE Grade ≥3 laboratory abnormalities thought to be drug-related	
Patient receives infliximab or any other TNF-α inhibitor	
2 consecutive doses missed due to on-going related toxicities	
Patient begins new investigational therapy, chemotherapy, cytokine therapy, or immunotherapy (including vaccines)	
Patient becomes pregnant	

ALT Alanine transaminase; AST Aspartate aminotransferase; CTCAE Common Terminology Criteria for Adverse Events; TNF Tumor necrosis factor; ULN Upper limit of the normal range.

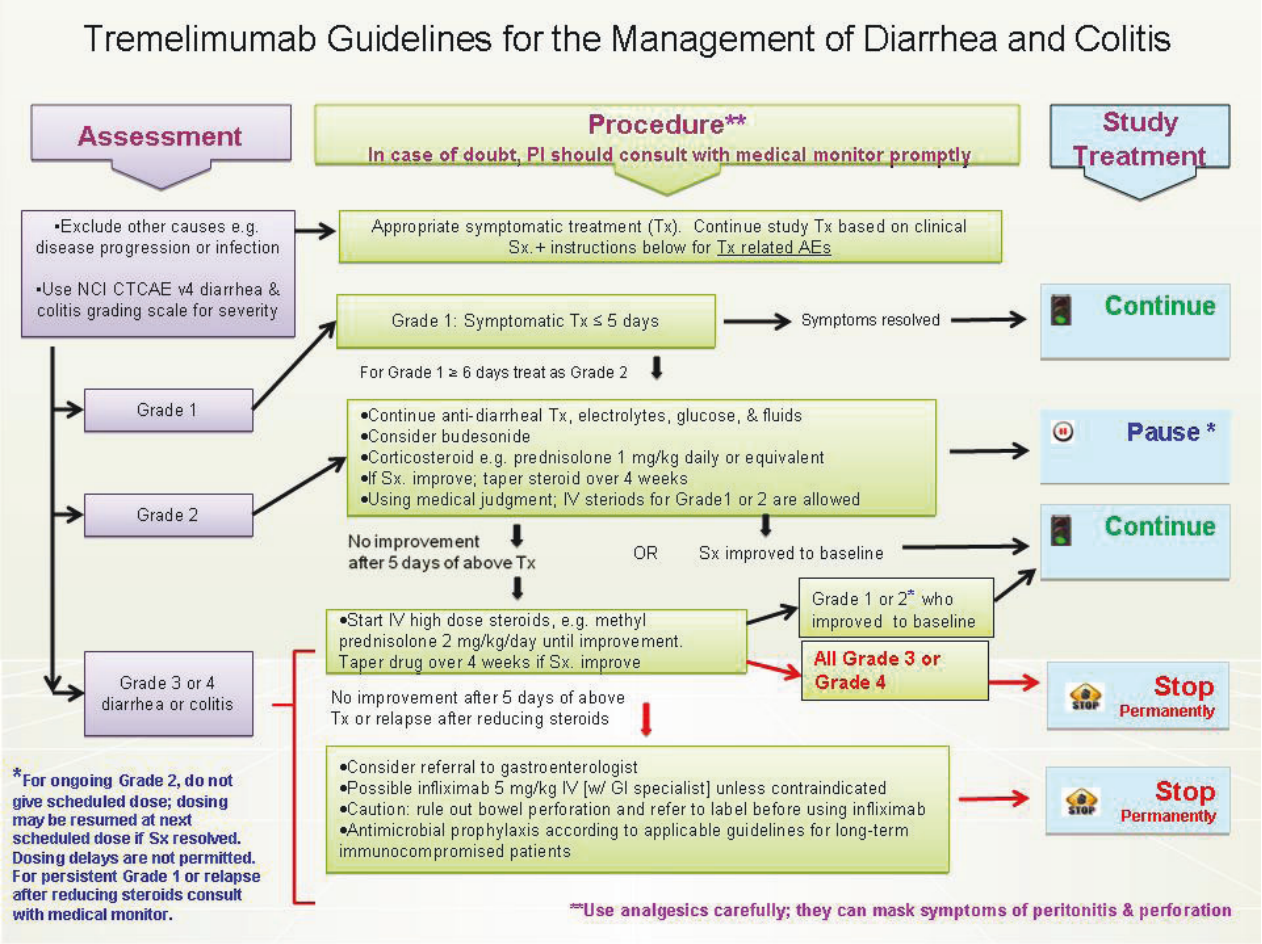
3.9.3 Specific immune-mediated adverse reactions

Tremelimumab can result in severe and fatal immune-mediated adverse reactions due to T-cell activation and proliferation. These immune-mediated reactions may involve any organ system, however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifest during treatment, however, a minority may occur weeks to months after discontinuation of Tremelimumab. Tremelimumab will be discontinued and systemic high-dose corticosteroid therapy initiated for severe immune-mediated reactions.

Patients will be assessed for signs and symptoms of enterocolitis, dermatitis, neuropathy and endocrinopathy and evaluate clinical chemistries including liver function tests and thyroid function tests at baseline and before each dose.

The following are anticipated immune-mediated adverse reactions for which specific guidance and management is outlined below.

3.9.3.1 Gastrointestinal



3.9.3.2 Liver (Immune-mediated hepatitis)

Tremelimumab can result in severe and fatal inflammation of the liver most commonly manifested as elevation of transaminases and hyperbilirubinemia. Liver enzymes and liver function tests (hepatic transaminase and bilirubin levels) will be evaluated and patients assessed for signs and symptoms of hepatitis before each dose of Tremelimumab.

In patients who experience AST or ALT > 5.0 ULN and/or baseline or total bilirubin > 3.0 ULN liver biopsy should be considered as per standard of care investigation for automimmune hepatitis.

Symptoms	Immediate action	Subsequent management
Moderate • AST or ALT >2.5 to 5.0 ULN and/or baseline (if this was abnormal). • Total bilirubin >1.5 to 3.0 ULN	Withhold Tremelimumab • Rule out infectious or malignant causes • Increase frequency of liver function test monitoring until resolution	**Symptoms resolve:** Resume Tremelimumab if liver function tests are 2.5 ULN or return to baseline and bilirubin level is 1.5 ULN or returns to baseline **IF AST OR ALT CONTINUES TO**

		ELEVATE TO >5.0 ULN OR TOTAL BILIRUBIN >3.0 ULN, See management for 'severe'

Symptoms	Immediate action	Subsequent management
Severe • AST or ALT > 5.0 ULN and/or • Total bilirubin > 3.0 ULN	Hold Tremelimumab and re-evaluate until toxicity improves to ≤ grade 1 or pre-treatment baseline. Treatment will be discontinued in patients who experience grade 3 non-hematologic toxicities that do not resolve to grade 1 or baseline within 4 weeks. • Increase frequency of liver function test monitoring until resolution • Administer systemic corticosteroid of 1 to 2 mg/kg/day of prednisone or equivalent. Consider liver biopsy as per standard of care investigation for autoimmune hepatitis	**Symptoms resolve:** When liver function tests show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month Consider alternative immunosuppressive therapy **Symptoms Ongoing >1 week:** Consider alternative immunosuppressive therapy

3.9.3.3 Skin: Immune-mediated dermatitis

Symptoms	Immediate action	Subsequent management
Moderate Non-localized rash (diffuse, <50% of skin surface)	**Withhold** Tremelimumab Administer topical or systemic corticosteroids if there is no improvement of symptoms within 1 week	**Symptoms resolve:** Resume Tremelimumab if dermatitis resolves or improves to mild (localized) symptoms and systemic steroid dose is 10 mg prednisone equivalent or less **If symptoms worsen see management for 'severe'**

Symptoms	Immediate action	Subsequent management
Severe Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations	Permanently d/c Tremelimumab Administer systemic corticosteroid therapy of 1 to 2 mg/kg/day of prednisone or equivalent	**Symptoms resolve:** When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month

3.9.3.4 Central/Peripheral nervous system

Symptoms	Immediate action	Subsequent management
Moderate Moderate symptoms, clinically detectable with no impact on activities of daily living (ADLs)	**Withhold** Tremelimumab Introduce appropriate medical intervention	**Symptoms resolve:** Resume Tremelimumab when symptoms resolve or return to baseline **If symptoms worsen see management for 'severe'**

Symptoms	Immediate action	Subsequent management
Severe Severe symptoms (impact on ADLs) or life threatening	Permanently d/c Tremelimumab Institute appropriate medical intervention • Consider the use of systemic corticosteroid of 1 to 2 mg/kg/day of prednisone or equivalent	Permanently d/c Tremelimumab Continue appropriate medical intervention

3.9.3.5 Endocrine system

Symptoms	Immediate action	Subsequent management
Moderate Signs and/or symptoms of dysfunction • Endocrinopathies requiring hormone	**Withhold** Tremelimumab Evaluate endocrine function • Consider radiographic pituitary gland imaging	**Symptoms resolve:** Resume Tremelimumab when Patient is stable and symptoms are resolved or return to baseline

replacement or medical intervention • Adverse reactions requiring hospitalization, urgent medical intervention, or interfering with activities of daily living (including adrenal crisis)	• Continue to assess as indicated • Withhold Tremelimumab in symptomatic patients • Administer systemic corticosteroid therapy of 1 to 2 mg/kg/day of prednisone or equivalent • Initiate appropriate hormone-replacement therapy	• Patient is stable on hormone-replacement therapy (as indicated) • Patient is receiving "7.5 mg prednisone or equivalent per day

3.10 SPECIFIC PROCEDURES FOR TACE, RFA AND CRYOABLATION

3.10.1 Transarterial chemoembolization

Is percutaneous procedure performed under general anesthesia or conscious sedation. Access is obtained from the common femoral artery and selective catheterization of the superior mesenteric and celiac arteries is performed to define anatomy and hepatic arterial supply. Superselective catheterization of hepatic artery branches feeding the tumors is then performed followed by infusion of a chemotherapeutic mixture.

Trans-arterial chemoembolization will be performed with drug eluding beads (DEB). In this case, 100mg of doxorubicin are loaded into 100-250 micron beads. The DEB are mixed with contrast and infused into the hepatic artery branches supplying the tumor. Patients undergoing trans-arterial chemoembolization are pre-medicated with analgesics, antibiotics and anti-emetics per clinical standards.

3.10.2 Radiofrequency ablation

Thermal ablation is a minimally invasive image guided procedure performed under general anesthesia or conscious sedation. Radiofrequency or microwave ablations are performed; the ablation zone includes the tumor and a safety margin of surrounding tissue. Thermal ablation therapy will be administered according to the manufacturer's instructions of the device. The probe chosen varies depending on the type of thermal ablation, size of the tumor. The ablation probe will be inserted into the lesion, under image guidance. After confirmation of appropriate positioning, the lesion will be heated typically for 10-15 minutes for RFA and 5-10 minutes for microwave, according to manufacturer's guidelines. The time of ablation will depend on thermal ablation technique (RF vs. microwave) as well as the size of the ablation zone desired. The ablation time might also vary depending on proximity of critical structures, patient-specific anatomy or issues. Depending on ablation zone size, several probes might be required with several heating times to achieve complete coverage of the desired ablation zone. Balloon catheter may be used in cases where the tumor is adjacent to a hepatic vein to avoid "heat sync" where the ablation zone is cooled by the blood inflow. This catheter is typically inserted from the neck vein and is then placed into a hepatic vein. Hydrodissection may also be used in cases where the tumor is adjacent to a critical structure such as the heart or colon. These organs can

sustain damage from heating, therefore a catheter is inserted into the space separating them and the tumor and fluid (dextrose 5%) is administered during the ablation.

3.10.3 Cryoablation

The freeze zone of cryoablation effects depend upon the probe size and configuration as well as the nadir of the freeze cycle (dependent upon the specific cryogen). Two cryoablation systems are commercially available and FDA cleared for human use (Endocare / Healthtronics and Galil) Both use helium and argon gases to induce lethal temperatures locally.

The systems require argon and helium gases which produce low temperatures upon phase change from gas to liquid. The cryotherapy equipment will be an argon-helium gas system using 1.6mm or 2.4 mm cryoprobes (Endocare, Inc., Irvine, CA) or 0.049 inches = 18 gauge = 1.3 mm cryoprobes (Galil). The ice-ball diameters for these probes may vary according to the tissue and blood supply, but their freeze lengths should be comparable to the RFA system (10-30 mm). Ice-ball diameters refer to the outer 0°C margin, but cytotoxic temperatures (e.g., < -20 to -40 °C) are known to occur 3-5 mm behind visualized ice margins. A 10- 20 minute freeze, followed by a 5-10 minute thaw, and a 10-20 minute re-freeze produce visible ice and necrosis by rapid and slow mechanisms (dehydration and vascular stasis). The specific times depend upon the temperatures and potentially the temperatures of any remote thermometry used to asse4ss temperature at the margin of a freeze. Rapid intracellular ice formation causes irreversible cell death. Cell membrane dysfunction occurs below -10 degrees C, leading to intracellular ice formation, disrupting organelles. During thawing, osmolar shifts cause cellular swelling and rupture. Apoptosis and vascular stasis may also play a role in cell death by freezing.

The probes are placed into the tumor(s) by ultrasound, CT or MRI guidance. Up to 8 simultaneous probes may be placed sequentially for simultaneous treatment (different from current monopolar RFA). 1 % lidocaine is applied to the subcutaneous tissues down to the organ of interest. The helium and argon gases are hooked up to the system, and the cryotherapy machine and software are relatively automatic at this point with the freeze, thaw, refreeze cycles as described above. The process is monitored with ultrasound and CT scans as clinically required for standard operating procedures described by the manufacturers.

3.11 STUDY CALENDAR

	Pre-study	Cycle 1				Cycle 2				Cycle 3				Cycle 4				Cycle 5				Cycle 6				Every 12 weeks for 2 years or until PD	Off Treatment [l]	FU[n]
Day		1	8	15	22	1	8 (day 36)	15	22	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22			
Tremelimumab[a]		X				X				X				X				X				X				X		
RFA/TACE/ /Cryoablation [b]							X																					
Informed consent	X																											
Demographics	X																											
Medical history and concomitant meds	X	X				X				X				X				X				X				X	X	
Adverse event evaluation		X				X				X				X				X				X				X	X	
Physical exam	X	X				X				X				X				X				X				X	X	
Vital signs[i]	X	X				X				X				X				X				X				X	X	
Height	X																											
Weight	X	X				X				X				X				X				X				X		
Performance Status	X	X				X				X				X				X				X				X	X	
CBC w/differential,[c] Platelets	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

PT, INR, PTT,[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Thyroid Panel[c]	X					X				X				X				X				X				X		
Serum chemistry[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Serum aFP or Ca 19.9, hepatitis serology[d]	X					X				X				X				X				X				X	
HLA-A2 Phenotyping	X																										
ECG	X																										
Restaging radiologic Evaluation[e]	X									X								X								X	
Tumor biopsy[f,m]	X						X																			X[m]	
Immune monitoring[j]		X				X	X			X				X												X	
Pharmacokinetic studies[g]		X	X	X		X				X																	X	
Immunogenicity[h]		X				X				X				X												X	X	
Liver autoantibody panel (autoimmune hepatitis)	X													X														
Tumor-specific αFP responses		X				X				X				X												X		
MDSC functional assay		X				X				X				X												X		
Hepatitis serology and viral load	X					X				X				X				X				X				X		
T cell activation/ICOS expression (PBMC)	X			X		X				X				X												X		

Plasma-based assays for circulating receptors/ligands e.g. PDL1	X	X				X	X			X				X											X		
NIH Advance Directive Form[k]	X																										
Annual telephone contact																											X

a: Tremelimumab: Dose as assigned

b: RFA/TACE/cryoablation on day 35 +/- 96 hrs.

c: Acute, hepatic and mineral panels, uric acid and amylase. If laboratory tests done within 72 hours of Cycle 1 Day 1 they do not need to be repeated on Day 1.Thyroid Panel and Hepatitis Serologies do not need to be repeated on Day 1 if done within the last 28 days. For all labs a '+/- 48hrs' window applies, with the exception of those needed to determine proceeding with treatment. Labs may be performed outside of NIH. Once patients are in the every 12 week phase, weekly labs only need to be completed after their dose of tremelimumab for the 28 day period during the 12 weeks.

d: For Hepatitis B: HBV viral load; Anti-HBc Antibody, Anti-HBe Antibody, HBeAg and quantitative HepB Ag, HBV specific immune response.
For Hepatitis C: HCV viral load, anti-HCV Antibody titer.

e: Restaging CT /MRI scan every 8 weeks to evaluate TTP in target lesion. Post cycle 6 restaging will occur every 8 weeks.

f: An optional tumor biopsy may be performed at baseline and at the time of the ablative therapy for analysis of immune infiltration.

g: pre-infusion and immediately post infusion. Day 8 and 15 for local patients only (optional research blood).

h: pre-infusion.

i: Vitals may be done +/- 30 minutes at required timepoints. If patients are local or still post op procedure.

j: Immune monitoring +/- 7 days.

k: As indicated in section **9.3** , all subjects will be offered the opportunity to complete an NIH Advance Directive form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

1: The off treatment visits/labs will occur if patient is agreeable to coming back and physically able to return for the visit. **This visit should occur around day 30 post the last dose of study drug**. If toxicities cause discontinuation of therapy, patients will be followed until resolution of toxicity to at least Grade 1. If the patient cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs from a local physician or laboratory. If this is not possible, patients may be assessed by telephone for symptoms

m: An optional tumor biopsy may be obtained ≤2 weeks prior starting re-induction treatment.

n: Follow-up will be annual telephone contact to assess survival status. Every attempt will be made to contact patient/subject including: contacting referring physician, contacting emergency contact patient identified on admission, checking SSDI (Social Security Death Index)

3.12 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.12.1 Criteria for removal from protocol therapy

Prior to removal from the study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression (per immune-related response criteria)
- Completion of 2 years' protocol therapy with tremelimumab
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test
- Delayed recovery from toxicity that prevents re-treatment in ≤ 28 days of scheduled therapy
- Completed end of treatment safety visit

3.12.2 Criteria for Removal from Study

- Death
- Investigator discretion
- Participant requests to be withdrawn from study
- PI decision to close the study

3.12.3 Off protocol therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any AE experienced by the patient.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

The correlative studies which we wish to perform are outlined below and summarized in the table. A description of each test including a brief statement of rationale and processing information is made below.

Test/assay	**Volume blood (approx)**	**Type of tube**	**Collection point (+/- 48hrs)**	**Location of specimen analysis**
Immune-monitoring	120mls (for PBMC)	EDTA	C1D1, C2D1, C2D8 (D36) (or corresponding day+7 after TACE/RFA/ cryoablation **will be optional**) C3D1, C4D1, every 12 weeks until PD	Figg Lab
	5-10mls (for serum)	EDTA		
PK	3mls	SST	C1D1 (pre-infusion and immediately post-infusion*), C1D8 (local only), C1D15 (local only), C2D1 (pre-infusion and immediately post-infusion*), C3D1 (pre-infusion and immediately post-infusion*), and End-of-treatment (EOT) *Immediately post-infusion PK samples should be collected within approximately 15 minutes. **Dose 1:** pre-infusion, immediately post	MedImmune/Intertek Alta

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
			infusion, C1D8 (local only), and C1D15 (local only) **Dose 2 and thereafter:** pre-infusion, immediately post infusion **EOT**	
Immunogenicity	4mls	SST	C1D1 (pre-infusion), C2D1 (pre-infusion), C3D1 (pre-infusion), C4D1, Every 3 months, End-of-treatment (EOT)	MedImmune/PPD
ANA	4mls	SST	Baseline and C4D1	CC Department of Laboratory Medicine (DLM)
AMA & Liver/kidney microsomal antibody	4 mL	SST	Baseline and C4D1	CC DLM will send to Mayo Labs
Tumor-specific responses (e.g. αFP)	20mls	EDTA	C1D1, C2D1, C3D1, C4D1, every 12 weeks until PD	Greten Lab
CFSE-proliferation T cells	5mls	EDTA	C1D1, C2D1, C3D1, C4D1, every 12 weeks until PD	Greten Lab
MDSC functional assay	20mls	EDTA	C1D1, C2D1, C3D1, C4D1, every 12 weeks until PD	Greten Lab
Hepatitis B & C viral load	8mls	SST	Baseline and q28 days.	CC Department of Transfusion Medicine
T cell activation/ICOS expression (PBMC)	10 mL	EDTA	Baseline (both screening and preinfusion C1D1 if possible), C1D15, C2D1,	MedImmune/Covance

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
			C3D1, C4D1, every 12 weeks until PD	
Plasma-based assays for circulating receptors/ligands e.g. PDL1	6mls	EDTA	Baseline, pre-infusion every cycle C1D1, C2D1, C2D8 (D36), C3D1, C3D8 (if seen in clinic), D85, every 12 weeks until PD and EOT.	MedImmune
Optional tumor biopsy	NA	NA	Baseline and at time of ablative procedure	Pathology

5.2 NCI CORRELATIVE STUDIES

5.2.1 Immune Monitoring (All cohorts)

We will analyze PBMC for quantitative and functional changes of effector cells as well as analyze sera for cytokines and chemokines. The effect on (i) CD4 T cell number and activity, (ii) CD8 T cell number and activity, (iii) NK cell number and activity, (iv) Treg number, (vi) MDSC: frequency + functional assay, (vii) selected cytokines in serum, and (viii) the detection of HCC-associated antigens using ELISPOT assay.

Patients will undergo blood sampling (c.120mls blood) on the time points outlined in the table (+/- 48 hrs). Blood will initially be sent to the Figg laboratory for barcoding and processing. On certain occasions the blood may also be brought to the Greten lab for processing and analysis.

For Dr. Figg's Blood Processing Core (BPC):

Please e-mail Julie Barnes at Julie.barnes@nih.gov and Paula Carter pcartera@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.. PBMC will be isolated by Ficoll density centrifugation. Aliquots of 1 x 10^7 PBMC/tube will be individually frozen – after initial handling and processing at the Figg laboratory.

5.2.2 Liver autoantibody panel for autoimmune hepatitis (AIH)

AIH is a chronic disorder characterized by progressive hepatocellular loss and cell-mediated immunologic attack. Histologic inflammation is present and is usually accompanied by fibrosis, which can progress to cirrhosis and liver failure. AIH accounts for 11% to 23% of chronic liver disease in North America and about 6% of liver transplants in the United States.

The AIH Diagnostic Panel includes tests for actin (smooth muscle) antibody, antinuclear antibodies (ANAs), and liver/kidney microsome antibody (LKM-1). ANAs and actin antibody are associated with type 1 AIH, the most common form in the United States, while LKM-1 antibody is associated with type 2 AIH, more commonly found in Europe and in some South American countries. The panel also includes mitochondrial antibody, which can help differentiate AIH from PBC. Given that autoimmune hepatitis is a potential complication of immune checkpoint inhibition we will perform this panel of autoimmune antibodies to investigate this. Samples will be collected at baseline and on D85 approximately. These antibody titers are exploratory and will not be used to guide therapy in the absence of clinical correlation.

5.2.3 Hepatitis serology and viral load

As mentioned in the background section the phase II study of Tremelimumab in HCC showed significant anti-HCV immune response with progressive decline in serum HCV viral load (median values: basal 3.78x10e5 copies/ml vs. day 120 3.02x10e4 copies/ml, p=0.02; vs. day 210 1.69x10e3 copies/ml, p=0.04). Therefore, for all viral hepatitis patients we will measure viral load and antibody titers at baseline and every 30 days. For patients with chronic HBV infection, in addition to the studies already described, we plan to:

- determine HBsAg concentrations in serum
- determine HBeAg (in HBeAg positive patients)
- determine HBsAg fractions (small, middle, large HBsAg)
- determine HBV specific Tcell responses

These studies will be performed on samples obtained at baseline and every 28 days (collection point +/-48 hours). Samples with be processed by CC Department of Laboratory medicine. For HBsAg and Hepatitis B specific immune response, samples will be processed by the Department of Gastroenterology, Hannover Medical School, Germany.

With Amendment E the NCI Thoracic and GI Oncology Branch will release coded serum samples collected in conventional “CryoTubes” in association with this protocol to:

Heiner Wedemeyer
Department of Gastroenterology
Hannover Medical School
Carl Neuberg Strasse 1
30625 Hannover
Germany

for HBsAg and Hepatitis B specific immune response, as will be specified in NCI Material Transfer Agreement #38376-14.

Specimens will be labeled with the study identifier only and will be shipped to the address above, either on dry ice or at ambient temperatures as required by the type of sample to be analyzed. Tumor-specific αFP responses

Frequency of AFP-specific $CD4^+$ T cells will be analyzed using the interferon (IFN)-[gamma] cytokine secretion assay as previously described[68]. In brief, PBMCs will be resuspended in medium cultured in duplicate with the following peptides: $AFP_{137-145}$ (PLFQVPEPV), $AFP_{249-258}$ (KVNFTEIQKL), and $AFP_{364-373}$ (QLAVSVILRV) or an irrelevant control peptide (SIINFEKL). Recombinant interleukin-2 (25 IU/mL) will be added on day 2 of culture. After 7 days, PBMCs will be re-stimulated with the same peptide and incubated for 5 hours at 37°C and IFN-[gamma] secretion by $CD4^+$ T cells analyzed by IFN-[gamma] capture assay. Live gating on $CD4^+$ T cells will be performed until up to 100,000 events were acquired. Only AFP responses >0.1% of $CD4^+$ T cells will be considered positive.

5.2.4 MDSC functional assay

Freshly isolated PBMC will be labeled with CSFE and stimulated with CD2/CD3/CD28 beads in the presence/absence of L-NMMA and NorNOHA. In control experiments CD14 depleted PBMC will be stimulated under the same conditions. Proliferative response of T cells will be assessed by FACS analysis (Serafini et al. Journal of Experimental Medicine (2006) 203: 2691).

5.2.5 Optional Tumor Biopsy

An optional tumor biopsy may be performed at baseline and at the time of the ablative therapy for analysis of immune infiltration. Tumor Tissue will be processed by the Department of Pathology, NCI.

NIH (Dr. David Kleiner). Two core biopsies will be attempted. For each specimen obtained the core will be divided in two parts for Surgical Pathology and frozen preservation. If for some reason only one core is able to be obtained, the core will be divided, with half submitted to Surgical Pathology and half used for PD studies.

1) <u>Formalin-fixed.</u>
    i. The half fixed in 10% formalin will be submitted to Surgical Pathology, CCR/NCI (Bldg 10, 2N212).
    ii. The specimens will have routine H&E stains made as well as 5 additional unstained sections.
2) <u>Frozen-preservation</u>
    i. Two 1.5 ml cryogenic vials (obtained from Greten lab) will be labeled with the patient's name, accession number (HP#) and date using a waterproof sharpie.
    ii. The isotherm flask (Greten lab) will be filled with liquid nitrogen on the morning of the procedure and will be available together with the cryogenic vials for pick up from there when radiology page the contact person to collect the specimens.
    iii. Once the biopsy is ready, the half-core to be cryopreserved will be transferred into an empty 1.5-mL cryogenic vial with the use of sterile, pre-chilled (in dry ice) disposable tweezers.

iv. The vial with specimen will be immediately dropped into liquid nitrogen contained in an isotherm flask.

v. The frozen half will be transferred in the isotherm flask to the protocol-specified location for that particular analysis.

5.2.6 TCR Beta Sequencing

With Amendment H the NCI Thoracic and GI Oncology Branch will release coded tumor and PBMC samples collected in association with this protocol to:

Lara Gruye
Adaptive Biotechnologies
1551 Eastlake Ave E #200,
Seattle, WA 98102
(855) 466-8667

For TCR (cell receptor) gene usage may be quantitated in samples using conventional sequencing techniques of the T cell receptor variable region of the beta chain as will be specified in NCI Material Transfer Agreement #**pending**. Fewer than 100 genes will be analyzed.

5.3 MEDIMMUNE PLANNED STUDIES

All samples will be processed and cryopreserved in the Figg laboratory. Transfer in batch to Medimmune/vendor facilities for further analysis will occur at a later date. Please also see **Appendix D: MedImmune Sample Preparation Procedures**.

5.3.1 Pharmacokinetics

The time points for PK sampling are described in the table in section 5 above. A validated enzyme-linked immunosorbent assay (ELISA) will be used for the quantitative determination of Tremelimumab in human serum.

5.3.2 Immunogenicity

The time points for the assessment of anti-Tremelimumab antibodies are described in the table in section **5** above. A validated electrochemiluminescence assay (ECLA) using a Meso Scale Discovery (MSD) platform will be used for the detection of anti-drug antibodies against Tremelimumab in human serum.

5.3.3 T CELL Activation/ICOS expression

The number and subsets of T cells as well as other immune cells will be evaluated in PBMC by flow cytometry. The activation status of T cells will also be assessed in the same study. Whole blood samples will be collected pre-infusion at baseline (screening and D1), D8, D29, D57 and D85. Samples will be processed to PBMC and stored frozen until time of analysis. Additionally, absolute lymphocyte count at baseline and in response to Tremelimumab treatment will be evaluated for any relationship with treatment outcome.

5.3.4 Plasma-based assays for circulating receptors/ligands e.g. PDL1

Plasma samples are to be collected at the time points listed directly into plastic 6 ml lavender vacutainer tube with EDTA as anticoagulant. 2-3 ml of obtained plasma are to be aliquoted (1

ml each) and frozen. Samples may be analyzed for circulating levels of soluble factors such as CRP, cytokines, and chemokines. They may include but are not limited to soluble CTLA-4, soluble PD-L1, soluble B7.1/B7.2, soluble IL-6R, vascular endothelial growth factor, fibroblast growth factor, IL-1 IL-2, IL-4, IL-6, IL-8, IL-10, cancer biomarkers (alpha fetoprotein, carcinoembryonic antigen, cancer antigen 125, prostate specific antigen, soluble mesothelin-related protein [SMRP]), granzyme B, IFN, C-X-C motif chemokine 10 (CXCL10), suppressor of cytokine signaling 3 (SOCS3), a proliferation inducing ligand, B-cell activating factor, insulin-like growth factor (IGF)-1, IGF-2, and autoantibodies to host and tumor antigens and explore their association with Tremelimumab treatment and clinical outcome.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. All samples will be sent to Dr. Figg's lab for processing and storage until they are distributed to Dr. Greten's lab, Dr. Altan-Bonnet's lab or MedImmune for sample analysis as described in the protocol. Samples will not be sent outside NIH without IRB notification and an executed MTA.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.4.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at

NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.4.3 Protocol Completion/Sample Destruction

- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA, and NIH Intramural Records Retention Schedule regulations as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 DATA SHARING PLANS

6.2.1 HUMAN DATA SHARING PLANS

Human data generated in this research will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository
- Identified data in BTRIS

Data will be shared through:

- An NIH-funded or approved public repository: clinicaltrials.gov
- BTRIS
- Publication and/or public presentations.

Data will be shared at the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4-8 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 Definitions

6.3.1.1 Evaluable for toxicity

All patients will be evaluable for toxicity from the time of their first treatment with Tremelimumab.

6.3.1.2 Evaluable for objective response

Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

6.3.2 Disease Parameters

6.3.2.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$20 mm by chest x-ray, as $\geq$10 mm with CT scan, or $\geq$10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

6.3.2.2 Malignant lymph nodes

To be considered pathologically enlarged and measurable, a lymph node must be $\geq$15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

6.3.2.3 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with $\geq$10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

6.3.2.4 Target lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

6.3.2.5 Non-target lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

6.3.3.1 Clinical lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

6.3.3.2 Chest x-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

6.3.3.3 Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the

minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

6.3.3.4 Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

6.3.3.5 Endoscopy, Laparoscopy

The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

6.3.3.6 Cytology, Histology

These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-	No	SD	Documented at least once ≥4 wks. from baseline**

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
	PD/not evaluated			
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.5 Duration of Response

6.3.5.1 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

6.3.5.2 Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.4 MODIFIED IMMUNE-RELATED RESPONSE CRITERIA (IRRC)

Modified immune-related response criteria (irRC) will also be employed in this study. This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC. Please refer to Appendix C section **12.3** for further details.

6.5 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site

(http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2**, **7.3**, and **7.4**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or,

if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Protocol Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Adverse Events , and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor using the mandatory MedWatch form 3500a any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the Tremelimumab should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to Tremelimumab which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 3 months after the last dose of Tremelimumab.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 3 months after the last dose of Tremelimumab should, if possible, be followed up and documented.

7.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

All safety reports must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the MedImmune at:

MedImmune or designee (PharmaNet/i3, LLC) contact information:

Patient Safety PharmaNet/i3, LLC
MedImmune
One MedImmune Way
Gaithersburg, MD 20878

Fax: +1 301 398 4205

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process

- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

8 STATISTICAL CONSIDERATIONS

The primary objective of this pilot study is to determine if it is feasible to administer three doses of Tremelimumab to patients with hepatocellular carcinoma (HCC) and to determine clinical efficacy in several cohorts of patients. Secondary objectives include examining a variety of immunologic parameters prior to treatment and after treatment to determine if there is evidence of patients exhibiting an immune response to treatment, to assess safety and toxicity and to determine the feasibility of administering 3 doses of tremelimumab in addition to RFA in patients with biliary tract cancer.

8.1 COHORTS A AND B

Relative to the feasibility endpoint, this will be decided in Cohorts A and B; all patients enrolled into Cohorts A and B at the 10 mg/kg dose level will be considered as one group for feasibility evaluation. Feasibility will be evaluated in the first 20 evaluable patients enrolled in in these two cohorts on this trial at the 10mg/kg dose level following a dose-escalation phase of 3-6 patients treated at 3.5mg/kg dose level. The safety of the 10 mg/kg dose level will be evaluated in the first 3-6 patients treated at that dose. If there are 2 or more DLTs among the first 3-6 patients treated at this level, then accrual would stop. Regarding feasibility, it would be desirable if the fraction of patients who could receive three doses of the monoclonal antibody were consistent with 80% or higher and greater than 50%. With 20 evaluable patients, if there are 14 or more patients who are able to receive three doses of the monoclonal antibody, then there is a 5.8% probability of this being true if the true probability of an individual patient being able to receive this much antibody were 50% and there is a 91.3% probability of this being true if the true probability for an individual were 80%. Thus, 14 or more out of 20 patients receiving three doses of antibody would provide strong evidence that it is feasible to administer this antibody in a substantial fraction of patients, consistent with 80% or more and this would be considered a successful outcome for the trial.

Prior to amendment G, administration of three doses has been determined to be feasible based on the first 20 total patients enrolled and the feasibility evaluation was considered to have ended at that point. Evaluations for clinical efficacy will be the primary objective from this point forward.

With amendment G, there will be 20 patients enrolled in cohort A at 10mg/kg (10 receiving RFA and 10 receiving TACE).

Each of these sub-cohorts will be evaluated for efficacy as follows. In each sub-cohort of 10 patients the trial will have 82% power to rule out 15% 6 month as the proportion stable at 6

months in favor of 50% being stable at 6 months, with each evaluation assuming that a one-sided 0.10 significance level exact binomial test would be performed. If there are 3 patients in 10 who are able to have stable disease at a 6 month evaluation, then the lower one-sided 90% confidence bound for 3/10 is 11.6% (the two sided 80% confidence interval extends from 11.6% to 55.2%). Similarly, with 4 in 10 with stable disease at 6 months, the lower one-sided 90% confidence bound for 4/10 is 18.8% (the two sided 80% confidence interval extends from 18.8% to 64.6%). Thus, in Cohort A, 3 or 4 patients out of 10 attaining stable disease at 6 months with either TACE or RFA would rule out 10-15% as the fraction who would do so, with 90% one sided confidence. The results from the two sub-cohorts of cohort A treated at 10 mg/kg may also have their results combined in an exploratory fashion to report their overall efficacy.

Also by Amendment G, cohort B will be expanded to allow 20 evaluable patients to be enrolled for efficacy. With this number of patients, this cohort will have 97% power to rule out 15% as the proportion stable at 6 months in favor of 50% being stable at 6 months, with the evaluation assuming that a one-sided 0.10 significance level exact binomial test would be performed. If there are 6 patients in 20 who are able to have stable disease at a 6 month evaluation, then the lower one-sided 90% confidence bound for 6/20 is 16.6% (the two sided 80% confidence interval extends from 16.6% to 46.7%). Thus, in cohort B, having 6 or greater patients attaining stable disease at 6 months would rule out 15% as the fraction who would do so, with 90% one-sided confidence.

8.2 COHORTS D- E

With amendment F, one additional cohort of N=10 patients will be added to explore the immune effects of combining tremelimumab with cryoablation (Cohorts D) and one cohort with RFA in a BTC population. Thus, there will be 2 new cohorts added at this time for a total of 20 additional patients. Results to date from cohorts A and B suggest that the overall procedure is very likely to meet its feasibility goal since 9/10 patients have done so as of the date this amendment was constructed. As a result, the goal of the two new cohorts is to determine in a preliminary fashion if the treatment proposed in each cohort is able to offer clinical benefit to patients. Data on similar patients suggests a median time to progression of approximately 2 months, and approximately 10-15% would have stable disease at 6 months. It would be a very meaningful improvement if the treatment proposed could be associated with results which were consistent with 50% of patients having stable disease at 6 months. With the goal of keeping the trial small, in each cohort of 10 patients the trial will have 82% power to rule out 15% as the proportion stable at 6 months in favor of 50% being stable at 6 months, with each evaluation assuming that a one-sided 0.10 significance level exact binomial test would be performed. As a practical matter, 10 patients will be enrolled in each of the two cohorts and if there are 3 patients in 10 who are able to have stable disease at a 6 month evaluation, then the lower one-sided 90% confidence bound for 3/10 is 11.6% (the two sided 80% confidence interval extends from 11.6% to 55.2%). Similarly, with 4 in 10 with stable disease at 6 months, the lower one-sided 90% confidence bound for 4/10 is 18.8% (the two sided 80% confidence interval extends from 18.8% to 64.6%). Thus, in any given cohort, 3 or 4 patients attaining stable disease at 6 months would rule out 10-15% as the fraction who would do so, with 90% one sided confidence.

With amendment G, cohort E (cholangiocarcinoma patients) will be expanded to a total of 20 patients. This cohort will have 97% power to rule out 15% as the proportion stable at 6 months in favor of 50% being stable at 6 months, with the evaluation assuming that a one-sided 0.10 significance level exact binomial test would be performed. If there are 6 patients in 20 who are

able to have stable disease at a 6 month evaluation, then the lower one-sided 90% confidence bound for 6/20 is 16.6% (the two sided 80% confidence interval extends from 16.6% to 46.7%). Thus, in cohort E, having 6 or greater patients attaining stable disease at 6 months would rule out 15% as the fraction who would do so, with 90% one-sided confidence.

In addition to evaluations of response and 6 month disease stabilization, Kaplan-Meier curves for progression free survival (PFS) will be constructed based on each of cohorts A, B, and E.

8.3 SECONDARY AND EXPLORATORY ANALYSES

In all cohorts, the actual levels of changes of immune parameters will also be determined, and the fractions that are noted to have a change in the parameter values which would be considered immune responses will be reported. These will be considered secondary and exploratory analyses. Exploratory analyses may also be done informally comparing results for those receiving one procedure with another, e.g. RFA vs. TACE. In cohort B, 20 patients are expected to be enrolled (following amendment G); the same types of analyses are anticipated, but will be considered very tentative and hypothesis generating in view of the small numbers of patients to be evaluated.

Safety and toxicity will also be evaluated and addressed by tabulating and monitoring the grades of toxicity experienced by patients in the study.

At the conclusion of the trial, the clinical results and the immune parameter results may be used to arrive at an overall judgment involving which cohort or cohorts may merit expansion or further evaluation in subsequent trials.

8.4 ACCRUAL

It is expected that up to 2 patients per month may be able to enroll onto this trial. Cohort A may theoretically enroll a maximum of 26 evaluable patients at both dose levels combined, and there also may be 20 evaluable patients in cohort B, 10 in cohort D, and 20 in cohort E. The accrual ceiling for the protocol will be set at 90 patients to allow for a small number of inevaluable patients. Thus, it is expected that 48-60 months may be needed to accrue all patients onto this trial.

9 COLLABORATIVE AGREEMENTS

9.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA (02853) is in place with MedImmune, Inc. for supply of the investigational agent.

9.2 MATERIAL TRANSFER AGREEMENTS (MTA)

MTA (38376-14) is in place with Hannover Medical School

MTA (40860-16) is in place with Adaptive Biotechnologies

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study, will be individuals with advanced hepatocellular carcinoma (HCC), which has recurred (or persisted) after appropriate standard treatment. Individuals of any race or ethnic group will be eligible for this study. Eligibility assessment will be based solely on the patient's medical status. Recruitment of patients onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

10.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because they are unlikely to have hepatocellular or biliary tract carcinoma, and because of unknown toxicities in pediatric patients.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **10.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

10.4.1 Risks of exposure to ionizing radiation

This research study involves three CT guided biopsies collected for research purposes only. Subjects undergoing three optional biopsy collection will be exposed to 2.3 rem. This amount of radiation is below the guideline of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee.

10.4.2 Risk of optional biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

10.4.3 Other risks/benefits

The potential benefit to a patient that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described.

10.5 RISKS/BENEFITS ANALYSIS

For patients with hepatocellular carcinoma and biliary cancer, median survival is in the range of 6 months. It is possible that treatment on this protocol may reduce tumor burden or lessen symptoms caused by the cancer. While treatment on this protocol may not individually benefit subjects. The knowledge gained from this study may help others in the future who have hepatocellular carcinoma. Potential risks include the possible occurrence of any of a range of side effects listed. If patients suffer any physical injury as a result of the biopsies, immediate

medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

10.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Patients will meet with an associate or principal investigator on the trial in the GI oncology Clinic, during the initial evaluation for this study. During that meeting, the investigator will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The investigator will then provide a copy of the IRB-approved informed consent document that is included in this protocol. The patient will be allowed to take as much time as he wishes, in deciding whether or not he wishes to participate. If a prolonged period of time expires during the decision making process (several weeks, as an example), it may be necessary to reassess the patient for protocol eligibility. The original signed consent goes to Medical Records; copy placed in research record (NIH policy).

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on the study.

10.6.1 Telephone re-consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

10.6.2 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2)). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 TREMELIMUMAB

Tremelimumab is a human IgG2 anti-CTLA-4 mAb that is being developed as an immunotherapeutic agent for various cancers. Tremelimumab will be supplied by MedImmune.

Investigational Product	Manufacturer	Concentration and Formulation as Supplied
Tremelimumab	MedImmune	Formulated at a nominal concentration of 20 mg/mL in 20 mM histidine/histidine hydrochloride, 222 mM trehalose dihydrate, 0.02% (w/v) polysorbate 80, and 0.27 mM disodium edetate dihydrate (EDTA), pH 5.5.

Tremelimumab is to be administered as an IV solution of 10 mg/kg at a rate of 250 mL/hr, followed by observation for 60 minutes.

11.1.1 Investigational Product Dose Preparation

Tremelimumab is supplied as a sterile IV solution, filled in 20 mL clear glass vials with a rubber stopper and aluminum seal. Each vial contains 20 mg/mL (with a nominal fill of 20 mL accounting to 400 mg/vial) of Tremelimumab, in an isotonic solution at pH 5.5. Vials containing Tremelimumab must be stored in the refrigerator at 2-8^{o}C. The 20 mg/mL solution will be diluted into a saline bag for IV infusion. Vials containing Tremelimumab may be gently inverted for mixing, but should not be shaken.

For dose preparation steps, the following ancillary items are required:

- IV infusion bags of 0.9% sodium chloride injection (250 mL size). Saline bags must be latex-free and can be made of polyvinyl chloride (PVC) or polyolefins (e.g. polyethylene), manufactured with bis (2-ethylhexyl) phthalate (DEHP) or DEHP-free.

- IV infusion lines made of PVC/DEHP or PVC/tri octyl trimellitate (TOTM) or polyethylene or polyurethane. All DEHP-containing or DEHP-free lines are acceptable. Lines should contain a 0.22 or 0.2 μm in-line filter. The in-line filter can be made of

polyethersulfone (PES) or polyvinylidene fluoride DRF (PVDF). Lines containing cellulose-based filters should not be used with Tremelimumab.

- Catheters/infusion sets made of polyurethane or fluoropolymer with silicone and stainless steel and/or PVC components.
- Syringes made of polypropylene and latex-free. Polycarbonate syringes should not be used with Tremelimumab.
- Needles made of stainless steel.

11.1.2 Dose Calculation

The subject's weight (in kilograms) must be measured prior to each dosing for dose calculation. Measurements can be taken in street clothes without shoes and a calibrated scale must be used for all measurements.

The dose will be calculated at each dosing visit using the following formula:

Dose (mL) = [subject weight (kg) × dose level (10 mg/kg)] drug concentration (20 mg/mL)

The corresponding volume of investigational product should be rounded to the nearest tenth of a mL (0.1 mL). Each vial contains a small amount of overage and the overage should be utilized as much as possible before using another vial.

The number of vials required for dose preparation is the next greatest whole number of vials from the following formula: Number of vials = Dose (mL) ÷ 20 (mL/vial)

11.1.3 Investigational Product Inspection

Each vial selected for dose preparation should be inspected.

If there are any defects noted with the investigational product, the investigator and site monitor should be notified immediately. Refer to the Product Complaint section **(10.1.6)** for further instructions.

During the inspection if the solution is not clear or any turbidity, discoloration or particulates are observed, notify your site monitor and store the vial(s) in QUARANTINE at refrigerated (2-8ºC) temperature for drug accountability and potential future inspection.

Notify the IXRS that the unusable vials are damaged. The IXRS will indicate the replacement vials. Select appropriate replacement vials for the preparation of the subject's dose, and perform the same inspection on the newly selected vials. For accountability, record the total number of vials removed from site inventory. Used vials should be held for accountability purposes at ambient storage temperature.

11.1.4 Dose Preparation Steps

Tremelimumab does not contain preservatives and any unused portion must be discarded. Preparation of Tremelimumab and preparation of the IV bag are to be performed aseptically. Total in-use storage time for the prepared final IV bag should not exceed 24 hours at 2-8°C or 4 hours at room temperature (25°C). However, it is recommended that the prepared final IV bag be stored in the dark at 2-8°C until needed. The refrigerated infusion solutions in the prepared final

IV bag should be equilibrated at room temperature for about 2 hours prior to administration. If storage time exceeds these limits, a new dose must be prepared from new vials.

The investigational product manager or qualified personnel will be responsible for preparing the IV doses using the following steps:

1) Select the IXRS-assigned number of vials of investigational product required to prepare the subject's dose.
2) All investigational product vials should be equilibrated to room temperature for 30 minutes prior to dose preparation.

3) To prepare the IV bag, first, calculate the dose volume of investigational product required. Second, remove the volume of 0.9% sodium chloride IV solution equivalent to the calculated dose volume of investigational product from the IV bag. Lastly, add the calculated dose volume of investigational product to the IV bag. Gently mix the solution in the bag by inverting up and down. Avoid shaking the IV bag to prevent foaming.

Example: A subject weighing 85 kg will require 42.5 mL (3 vials) of investigational product. Remove 42.5 mL of saline from the commercial IV bag. Add the 42.5 mL of investigational product to the IV bag and gently mix by inverting up and down.

Tremelimumab should be stored at refrigerated temperatures (2°C to 8°C), and should not be frozen.

Labels will be prepared in accordance with Good Manufacturing Practice (GMP).

11.1.5 Investigational Product Accountability

The investigator's or site's designated investigational product manager is required to maintain accurate investigational product accountability records. Upon completion of the study, copies of investigational product accountability records will be returned to MedImmune. All unused investigational product will be returned to a MedImmune-authorized depot or disposed of upon authorization by MedImmune.

11.1.6 Reporting Product Complaints

Any defects with the investigational product must be reported *immediately* to the MedImmune Product Complaint Department by the site with further notification to the site monitor. All defects will be communicated to MedImmune and investigated further with the Product Complaint Department. During the investigation of the product complaint, all investigational products must be stored at labeled conditions unless otherwise instructed.

Product defects may be related to component, product, or packaging and labeling issues. The list below includes, but is not limited to, descriptions of product complaints that should be reported.

Component Issue: Defect in container or dosing mechanism of the investigational product. The component defect may be damaged, missing, or broken. Component examples include vials, stoppers, caps, spray barrels, spray nozzles, or plungers.

Product Issue: Defect in the product itself. The product appearance has visual imperfections such as foreign particles, crystallization, discoloration, turbidity, insufficient volume, or anything that does not apply to the product description.

Packaging/Labeling Issue: Defect in the packaging or labeling of the product. The packaging or labeling defects may be damaged or unreadable, or the label may be missing.

When reporting a product complaint, site staff must be prepared to provide the following information:

1) Customer information: reporter name, address, contact number, and date of complaint

2) Product information: product name, packaging kit number or lot number, expiry date, and clinical protocol number

3) Complaint information: complaint issue category and description

MedImmune contact information for reporting product complaints:

Email: productcomplaints@medimmune.com

Phone: +1-301-398-2105 +1-877-MEDI-411 (+1-877-633-4411)

Fax: +1-301-398-8800

Mail: MedImmune, LLC Attn: Product Complaint Department One MedImmune Way, Gaithersburg, MD USA 20878

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13 APPENDICES

13.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

13.2 APPENDIX B: CHILD-PUGH CLASSIFICATION SYSTEM

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/liter)	2-3 mg/dL (34.2 to 51.3 micromol/liter)	>3 mg/dL (>51.3 micromol/liter)
Albumin	>3.5 g/dL (35 g/liter)	2.8-3.5 g/dL (28 to 35 g/liter)	<2.8 g/dL (<28 g/liter)
Prothrombin time			
Seconds over control	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Modified Child-Pugh classification of the severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy. A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival: grade A - 100 and 85 percent; grade B - 80 and 60 percent; and grade C - 45 and 35 percent.

13.3 APPENDIX C: MODIFIED IMMUNE-RELATED RESPONSE CRITERIA (IRRC)

This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC.

For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1 criteria, the modified irRC criteria (a) require confirmation of both progression and response by imaging at 6 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.
The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at the end of trial visit. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below.

Modified immune-related response criteria are defined as follows:

New measurable lesions: Incorporated into tumor burden.

New non-measurable lesions: Do not define progression but precludes (irCR).

Overall irCR:	Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm.
Overall irPR:	Sum of the longest diameters of target and new measurable lesions decreases ≥ 30%.
Overall irSD:	Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline) or irPD (compared to nadir).
Overall irPD:	Sum of the longest diameters of target and new measurable lesions increases ≥ 20% (compared to nadir), confirmed by a repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.

Overall Responses Derived from Changes in Index, Non-Index, and New Lesions

Measurable Response	Non-Measurable Response		Overall Response Using Modified irRC
Index and New, Measurable Lesions (Tumor Burden)[1]	**Non-Index Lesions**	**New, Non-Measurable Lesions**	
Decrease 100%	Absent	Absent	irCR[2]
Decrease 100%	Stable	Any	irPR[2]
Decrease 100%	Unequivocal progression	Any	irPR[2]
Decrease ≥ 30%	Absent / Stable	Any	irPR[2]
Decrease ≥ 30%	Unequivocal progression	Any	irPR[2]
Decrease < 30% to increase < 20%	Absent / Stable	Any	irSD
Decrease < 30% to increase < 20%	Unequivocal progression	Any	irSD
Increase ≥ 20%	Any	Any	irPD

[1] Decreases assessed relative to baseline
[2] Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart (normally it should be done 6 weeks apart).

13.4 APPENDIX D: MEDIMMUNE SAMPLE PREPARATION PROCEDURES

Serum- SST Tube

1. Draw maximum volume of blood into gold top SST tube (s). Record time of collection.
2. Gently invert 5 times.
3. Allow the blood to clot for 30 minutes at room temperature.
4. Centrifuge within 2 hours of collection at 1100 -1300 g for 15 minutes.
5. Transfer serum into labeled cryovials.
6. Immediately freeze the cryovials upright at -20ºC or colder.
7. Store at -20ºC or colder until shipment on dry ice.

Plasma- K_2 EDTA Tube

1. Draw maximum volume of blood into lavender top K_2 EDTA tube(s). Record time of collection
2. Gently invert 8 to 10 times.
3. Centrifuge within 2 hours of collection at 1100 -1300 g for 10 minutes.
4. Transfer plasma into labeled cryovials.
5. Immediately freeze the cryovials upright at -20ºC or colder.
6. Store at -20ºC or colder until shipment on dry ice.

Tube Label should include:

1. Patient Identifier
2. Sample ID (Serum 1, Cycle 1, Day 1etc.)
3. Sample type (plasma, serum)
4. Sample volume
5. Collection time
6. Barcode (if this system is available)