SPONSOR:

IKF Klinische Krebsforschung GmbH
am Krankenhaus Nordwest
- Represented in law by its General Manager –
Prof. Dr. med. Salah-Eddin Al-Batran
Steinbacher Hohl 2-26
60488 Frankfurt

TITLE:

IMMULAB – A phase II trial of **immu**notherapy with pembrolizumab in combination with **l**ocal **ab**lation for patients with early stage hepatocellular carcinoma (HCC)

IND NUMBER: 123,482

EudraCT NUMBER: 2018-001381-42

Sponsor's Protocol Code Number: IMMULAB

ClinicalTrials.gov ID: NCT03753659

Coordinating Investigator (LKP):

Prof. Dr. med. Arndt Vogel
Hannover Medical School
Department of Gastroenterology, Hepatology and Endocrinology
Carl-Neuberg-Str. 1
30625 Hannover

Study Management:

IKF Klinische Krebsforschung GmbH
am Krankenhaus Nordwest
Steinbacher Hohl 2-26
60488 Frankfurt

TABLE OF CONTENTS

CONTENT

1.0 TRIAL SUMMARY

Abbreviated Title	IMMULAB – A phase II trial of immunotherapy with pembrolizumab in combination with local ablation for patients with early stage hepatocellular carcinoma (HCC)
Trial Phase	Phase II
Clinical Indication	Early stage hepatocellular carcinoma (HCC)
Trial Type	Interventional, single-arm, open-label, multicenter
Type of control	No control arm
Route of administration	• Intravenous (IV) pembrolizumab infusions • Local ablation via RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy will be performed via ultrasound- or CT-guided placement of a needle electrode / probe penetrating into the lesion center
Trial Blinding	Unblinded open-label
Treatment	All patients will be treated as follows: • pembrolizumab 200mg IV Q3W on day 1 of cycle 1 and 2 • RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy will be performed on day 1 of cycle 3 • pembrolizumab 200mg IV administration 2 days after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy pembrolizumab 200mg IV Q3W for up to 12 months total treatment duration
Number of trial participants	30
Estimated enrollment period	12 months
Estimated duration of trial	max. 54 months from FPI to LPO (consisting of 24 months recruitment, 12 months treatment after LPI, and 18 months FU for OS after LPLT)
Duration of Participation	Each subject will participate in the trial from the time the subject signs the Informed Consent Form (ICF) through the final contact (last follow-up visit). Eligible subjects will receive pembrolizumab IV on day 1 of cycles 1 and 2 (200mg fix dose, three week cycles). A CT scan will be performed on day 1 of cycle 3 and ORR will be evaluated. Subsequently, RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy (according to Investigator's choice) will be performed via ultrasound- or CT-guided placement of a needle electrode / probe penetrating into the lesion center (using commercially available systems according to local practice). Patients (including high risk patients) will be allowed additional RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy treatment, if deemed necessary by the Investigator (e.g. in case of residual viable tumor tissue at the outer margin of ablated lesions). The third dose of pembrolizumab (200mg fix dose) will be applied 2 days after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy (day 3 of cycle 3). In case of unexpected side effects of RFA, MWA, brachytherapy or combination of TACE with RFA,

	MWA or brachytherapy treatment , pembrolizumab administration can be delayed up to day 21 after RFA, MWA, brachytherapy or TACE intervention. Thereafter, patients receive pembrolizumab IV (200mg fix dose) every three weeks (Q3W) on day 1 of each cycle for a total treatment duration of up to 12 months or until recurrence/progression, unacceptable adverse events (AEs), intercurrent illness that prevents further administration of treatment, Investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, administrative reasons requiring cessation of treatment. After treatment discontinuation (EOT), subjects who discontinue for reasons other than disease recurrence/progression will have post-treatment follow-up for disease status until recurrence/progression, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed for overall survival (OS) until death, withdrawal of consent, loss to follow-up, or the end of the study. After the last administration of study medication, each subject will be followed for 30 days for AE monitoring (pembrolizumab-associated AEs for 110 days). Serious adverse events (SAEs) and Events of Clinical Interest (ECIs) will be collected for 110 days after the last administration of study medication or for 30 days after the last administration of study medication if the subject initiates new anticancer therapy, whichever is earlier. ***Pembrolizumab-associated SAEs and ECIs must be collected for 110 days after the last administration of study medication independent of the start of a new anticancer therapy.***
Estimated average length of treatment per patient	10-12 months

2.0 TRIAL DESIGN

2.1 Trial Design

This is a multicenter, single arm, prospective, open-label phase II trial investigating the clinical activity of peri-interventional treatment with the anti-PD1 antibody pembrolizumab in HCC patients who are candidates for local ablation via either radiofrequency ablation (RFA) or microwave ablation (MWA) or brachytherapy, or - as recommended for tumor larger than 3cm - combination of TACE with RFA, MWA or brachytherapy. I.e. according to Investigator's assessment an R0 state can be obtained after a maximum of two RFA/MWA/brachytherapy or combination of TACE with RFA, MWA or brachytherapy interventions (initial ablation + one additional re-ablation at maximum).
Patients with Child-Pugh Classification score ≤ 6, including high risk candidates for local ablation (defined as patient is having ≤ 5 tumor nodules with diameters ≤ 7cm [longest axis] each OR patient with vascular infiltration) will be included in the study. Patients with extrahepatic disease are excluded *(please note: patients which are on the waiting list for liver transplantation are **not** excluded)*.
The primary objective of this phase II trial is to assess the objective response rate (ORR) before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy intervention according to RECIST 1.1 criteria. Secondary objectives are time to recurrence (TTR), recurrence free survival and overall survival (OS) along with safety and tolerability of the treatment.

Imaging assessments will be performed using RECIST 1.1 for determining assessment of response. On study imaging assessments will be performed on day 1 of cycle 3 (D1C3) before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA, brachytherapy is performed, 6 to 8 weeks after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy for control of successful ablation and afterwards every 12 weeks (Q12W ± 7 days) calculated from the date of control imaging independent of treatment delays. RECIST 1.1 will be used by the site for treatment decision before ablation. Afterwards, imaging focusses on the identification of residual viable tumor tissue for evaluation of ablation result (control imaging) and later on the recurrence of ablated lesions as well as appearance of new lesions as evidence of PD (disease monitoring). If radiologic PD is detected during imaging at D1C3 before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy, the subject should be discontinued from study treatment IF lesion sizes determined in imaging are exceeding the lesion sizes given in the inclusion criteria [i.e. ≥1 lesion with >7cm]. In this case, the patient will leave the active part of the study but will be followed-up for further anti-cancer treatment and OS until death, withdrawal of consent, loss to follow-up, or the end of the study – whichever comes first. An exception to continue treatment may be considered following consultation with the Coordinating Investigator and Sponsor.
Subjects will continue to be treated with pembrolizumab until PD, unacceptable AEs, intercurrent illness that prevents further administration of treatment, Investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance

with trial treatment or procedure requirements, administrative reasons, or the subject has received all trial treatments per protocol.
Subjects who discontinue treatment for reasons other than PD will have post-treatment follow-up for disease status until PD, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed for OS until death, withdrawal of consent, loss to follow-up, or the end of the study – whichever comes first.

Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. After the end of treatment, each subject will be followed for 30 days after last administration of study medication for AE monitoring (pembrolizumab-associated AEs for 110 days). SAEs and ECIs will be collected for 110 days after the last administration of study medication or 30 days after the last administration of study medication if the subject initiates new anticancer therapy, whichever is earlier. ***Pembrolizumab-associated SAEs and ECIs must be collected for 110 days after the last administration of study medication independent of the start of a new anticancer therapy.***

There will be a near real time monitoring of safety parameters (e.g. SAEs reported) by an independent data monitoring committee (IDMC) for the first 5 patients enrolled to immediately identify any risks for patient safety. In addition to these ad hoc scheduled meetings depending on SAE reporting, the IDMC will meet after the first 5 patients have finished the third treatment cycle including RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy (according to Investigator's choice) and the third pembrolizumab administration to re-evaluate the risk-benefit ratio of the trial and provide a recommendation on the continuation of the trial to Coordinating Investigator and Sponsor. Recruitment will be halted at the discretion of the IDMC.

There is no full interim analysis planned for this study, due to the small sample size and the relatively short recruitment period. However, single objectives may be analyzed as soon as sufficient events are available for analysis as detailed in the Statistical Analysis Plan (SAP).

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

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart – Section 6.0.

2.2 Trial Diagram

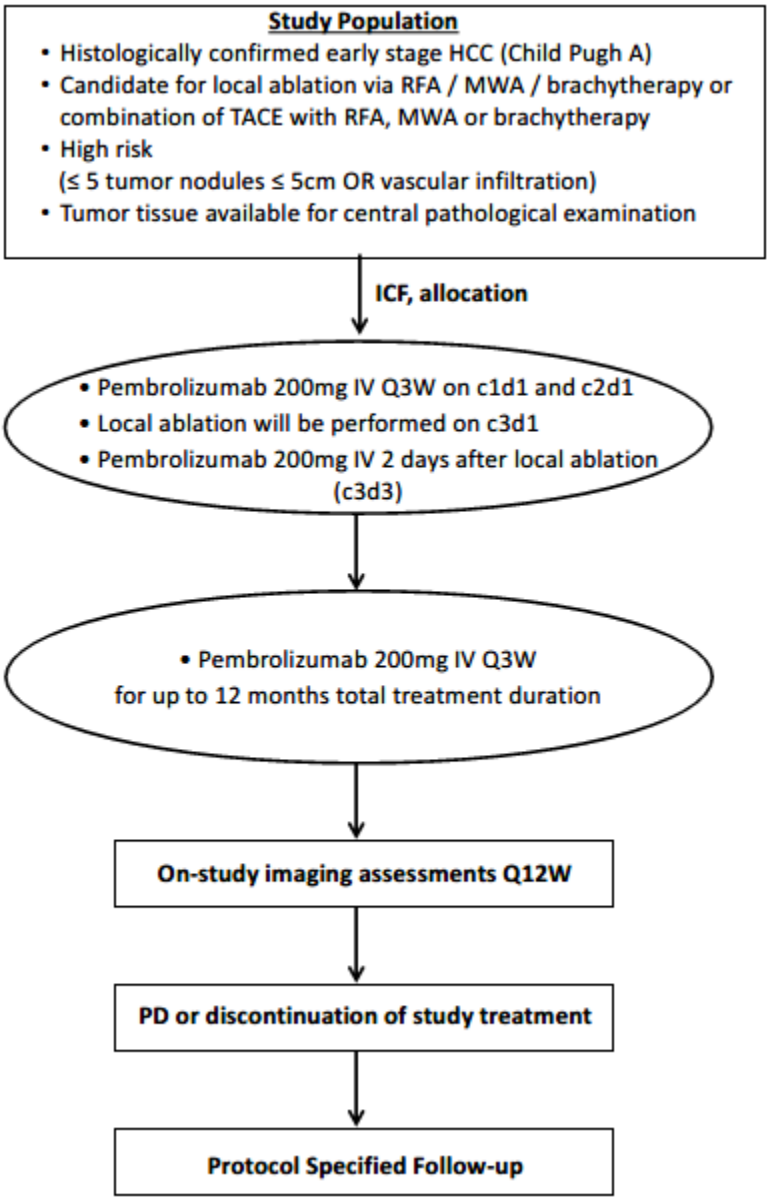

Figure 1: Trial diagram

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** Objective response rate (ORR) according to RECIST 1.1 criteria

Hypothesis: We hypothesize that treatment with pembrolizumab before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy will allow conversion / downstaging of borderline candidates for local ablation. This will be displayed by an ORR of 30% (measured before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy, compared to baseline).

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: Time to recurrence (TTR), recurrence free survival and overall survival (OS)

Hypothesis: We hypothesize that peri-interventional treatment with pembrolizumab will increase TTR, recurrence free survival and overall survival after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy.

(2) **Objective**: Safety and tolerability

Hypothesis: We hypothesize that combination of RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy with peri-interventional administration of pembrolizumab is safe and well tolerated.

3.3 Exploratory Objective(s)

(1) **Objective:** Identification of molecular biomarkers predictive for ORR, TTR, recurrence free survival and OS

4.0 BACKGROUND & RATIONALE

4.1 Hepatocellular Carcinoma (HCC) Incidence and Treatment Options for Early HCC

Hepatocellular carcinoma is one of the most lethal and prevalent cancers worldwide [1]. The prognosis of patients with HCC is dismal and the mortality rates are almost the same as the incidence rates. In the year 2008, 748,300 new HCC and 695,900 deaths have been registered (http://www.iarc.fr/). In most cases, HCC develops in cirrhotic livers, and cirrhosis is the strongest risk factor for the disease [2]. The variation in incidence and prevalence of HCC by geographical region is primarily a result of regional differences in exposure to causal factors for cirrhosis, such as hepatitis B virus (HBV) in Asia and sub-Saharan Africa and hepatitis C virus (HCV) in the West and Japan [2–4]. Dietary ingestion of fungal aflatoxins has also been recognized as a major risk factor in southern Asia and sub-Saharan Africa [5]. Although the incidence of HCC has historically been low in North America and Europe, there is evidence for a significant upward trend in the United States in recent years [6, 7], which has been

attributed to an increased prevalence of HCV infection [8]. The rise in obesity and diabetes worldwide, particularly in North America and Europe, is also leading to recognition of non-alcoholic fatty liver disease as a significant contributor to the aetiology of HCC [8].
Potentially curative treatments for HCC diagnosed in early stages include surgery (resection or transplant), radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI). Approximately 30–40% of HCC patients globally who are diagnosed with very early (BCLC Stage 0) or early (BCLC Stage A) disease are eligible for these procedures [3, 10-12].

The global HCC BRIDGE ('Bridge to Better Outcomes in HCC') study was the first multiregional, largescale, longitudinal cohort study to document the HCC patient experience from diagnosis to death, and aimed to include all patients, regardless of the treatment received [9]. Overall, data were available for a total of 18,031 patients treated for HCC.
In very early and early stage disease (Barcelona Clinic Liver Cancer [BCLC] stage 0 or stage A), percutaneous ethanol injection (PEI) / radiofrequency ablation (RFA) was used in approximately 20% to 30% of patients in Europe and North America as first-line treatment. RFA is designed to induce tumor destruction by delivering a high frequency alternating current through an active needle-electrode introduced into the neoplastic tissue (see Chapter 4.1.1).

4.1.1 Radio frequency ablation (RFA) / microwave ablation (MWA) / brachytherapy and combination with transcatheter arterial chemoembolization (TACE)

RFA is a promising and recently developed local ablation technique. It induces deep thermal injury in hepatic tissue while sparing the normal parenchyma. Its basic principle includes generation of high-frequency alternating current which causes ionic agitation and conversion to heat, with subsequent evaporation of intracellular water which leads to irreversible cellular changes, including intracellular protein denaturation, melting of membrane lipid bilayers, and coagulative necrosis of individual tumor cells [13]. RFA was recommended as the best treatment option for patients with early stage HCC who are not suitable for resection or transplantation in addition to PEI in the 2013 German S3 Guideline for the treatment of hepatocellular carcinoma [48] and the 2005 practice guidelines issued by the American Association for the Study of Liver Diseases [15]. Recently, a meta-analysis of randomized controlled trial (RCT) data showed that RFA is superior to PEI in the treatment of patients with relatively preserved liver function and early-stage non-surgical HCC with respect to survival and local control of the disease [16]. RFA has emerged as an accepted therapy for early HCC because of its effectiveness and safety. Nowadays, RFA is generally considered as an alternative treatment to partial hepatectomy for early HCC, especially for patients with impaired liver function and when liver transplantation is not indicated, although some authors believe RFA can be used as a first-line treatment for early HCC [17-20].

In contrast to RFA, MWA as another local ablation technique, uses microwave energy which induces an ultra-high speed, 915 MHz or 2.450 MHz (2.45 GHz), alternating electric field, which causes water molecule rotation and the creation of heat. This results in thermal coagulation and localized tissue necrosis. In MWA, a single microwave antenna or multiple antennas connected to a generator are inserted directly into the tumor or tissue to be ablated; energy from the antennas generates friction and heat. The local heat coagulates the tissue

adjacent to the probe, resulting in an elliptical area of tissue ablation. Meta-analysis of data obtained from several clinical trials indicates that MWA is an effective and safe technique for liver tumor ablation with low complication rates and survival rates comparable to RFA and PEI. No significant differences with regards to complication or survival rates were identified between MWA and RFA [44; 45; 46].

Data from the studies cited above and others indicate that associated morbidity and mortality as well as overall survival and disease-free survival rates are similar between RFA and MWA so that both techniques are considered appropriate local ablation procedures in patients with tumors not amenable to surgical resection.

Similar to the BRIDGE trial (see Chapter 4.1), around 18% (283 of 1,611 patients) were treated with RFA or MWA in first-line at the institution of the Coordinating Investigator of this trial (Medical School Hannover MHH, years 2000 to 2015, Kirstein / Vogel, manuscript submitted). Another 15% of all patients were treated with RFA / MWA in second-line after downstaging / neoadjvant treatment with TACE. According to several prospective studies and meta-analysis, recurrence-free survival rate at year one after RFA is approximately 65% [13, 14]. In the patient cohort at the MHH treated with RFA / MWA as best possible treatment, median overall survival (mOS) was 24 months. Hence, development of multimodal therapeutic approaches combining local ablation via RFA / MWA with systemic drug administration to improve patients' outcome after local ablation are urgently needed.

Patients with large HCC (> 5 cm) have poor prognosis and the use of TACE or RFA alone can only lead to poor local control. Ren et al. investigated safety and efficacy of TACE combined with RFA in treating Barcelona Clinic liver Cancer (BCLC) Stage A or B hepatocellular carcinoma (HCC) patients demonstrating an advantage of combined TACE/RFA over TACE alone in prolonging PFS and improving OS with a benefit for patients regardless of tumor size [49]. TACE combined with RFA revealed better survival than TACE alone for patients with stage B1 intermediate HCC according to Bolondi criteria [50]. Latest evidence suggests that combination of TACE + RFA offers outcomes comparable to surgical resection and with added benefit of lower morbidity [51]. As shown recently, simultaneous combination of TACE and RFA may improve therapeutic efficacy and survival for patients with large HCC [52]. However, the combination of TACE and RFA or MWA against HCC need more clinical data for a better strategy. The characters and advantages of TACE, RFA, MWA, and TACE combined with RFA or MWA are reviewed by Xu et al. [53]. In the current HCC S3 guidelines the use of TACE in combination with RFA and MWA is therefore recommended for tumor larger than 3cm. Very recently, Schnapauff and colleagues has additionally provided evidence that the combination of TACE is safe and effective [55]. To overcome the size limitation of thermal ablation techniques, CT-guided interstitial high-dose rate brachytherapy (CT-HDRBT) that uses high dose gamma radiation was developed for tumor ablation. In addition, since radiation is not subjected to the heat sink effect, CT- HDRBT is also applicable for lesions that are in direct contact to major vessels. Brachytherapy has been used in multiple centres in recent years and there is compelling evidence that brachytherapy is an effective treatment option in HCC as recommended in the current ESMO guideline (Vogel et al.). Morevoer. an explorative phase II trial showed a superior outcome of image-guided high-dose-

rate (HDR) brachytherapy (iBT) compared with conventional transarterial embolization (cTACE) in hepatocellular carcinoma and supports the use of CT-HDRBT in HCC [56].

4.1.2 Current Combinations of Local Ablation with Systemic Drug Administration

In order to increase the outcome of local ablation via RFA, several trials have analyzed the combination of RFA with the tyrosine kinase inhibitor sorafenib and the combination with TACE for larger tumors with a diameter of up to 7cm.

Sorafenib is a multikinase inhibitor against the vascular endothelial growth factor receptor (VEGF-R), the platelet-derived growth factor receptor (PDGF-R), and Raf with proven efficiency in advanced HCC [22]. However, the STORM study failed to improve median recurrence free survival as well as median overall survival for patients treated with a combination of RFA / resection and adjuvant treatment with sorafenib [21].

Sorafenib may not represent the ideal combination partner for local ablation via RFA / MWA as its efficacy as a monotherapy in patients with advanced HCC is only very modest ([22], (mOS 10.7 months in the sorafenib group and 7.9 months in the placebo group).
Therefore, there is clearly an unmet need for novel systemic therapies with more effective mechanisms for HCC to be combined with local ablation via RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy.

4.2 Immune Modulating Therapeutics in HCC

Monoclonal antibodies (mAbs) that target the immune checkpoints CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4) and PD-1 and its ligand PD-L1 are currently on the rise and are encouragingly active in a variety of malignancies, such as metastatic melanoma and non-small-cell lung cancer (NSCLC) [23].

Recently, PD-L1 expression has also been reported in HCC. As assessed in resected tumor specimen from 240 HCC patients and validated in further 124 patients, overexpression of PD-L1 was significantly associated with tumor recurrence [24]. In addition, combined PD-L1 low/ human leukocyte antigen class I (HLA class I) high expression in human HCC was confirmed to be prognostic for recurrence free and overall survival [25].

Together, these results suggest a prognostic role of PD-L1 as well as a rationale to target the PD-1 / PD-L1 axis in HCC. Furthermore, preliminary data from the CheckMate-040 trial strongly suggest that the PD-1-inhibitor nivolumab has clinical activity and is tolerable in patients with HCC, including those with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection [26]. Response rate was around 15% for patients that are sorafenib experienced and around 21% in sorafenib naïve patients. Most responses occurred within the first 6 weeks of treatment.

Currently, several phase II and III studies such as KEYNOTE-240 and KEYNOTE-224 are evaluating the efficacy of the PD-1 inhibitor pembrolizumab in patients with previously treated advanced HCC with results announced for 2018.

4.2.1 Background Pembrolizumab

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Pembrolizumab is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator's Brochure (IB).

4.2.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [27]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells/FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, pancreatic cancer, malignant melanoma, renal cell carcinoma as well as in hepatocellular carcinoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma [28, 29].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [30, 31].

The structure of murine PD-1 has been resolved [Zhang et al., 2004]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [31-35]]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [36, 37]. As a consequence, the

PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in HCC, as described in Chapter 4.2.

4.2.1.2 Preclinical and Clinical Trial Data

Please refer to the Investigator's Brochure for preclinical and clinical trial data.

4.3 Rationale

4.3.1 Rationale for Combining Local Ablation via RFA / MWA / brachytherapy or combination of TACE with RFA / MWA / brachytherapy and Immune Checkpoint Inhibition for Treatment of Early HCC

Several preclinical studies have shown that RFA induces local tumor destruction with subsequent antigen release to induce host adaptive immune responses against tumors [38]. Similarly, antigen-specific T-cell immune responses have also been observed in patients with hepatocellular carcinoma after RFA [39-41]. Although RFA augments systemic anti-tumor immune response in HCC patients, the frequent postoperative recurrence indicates that the RFA-induced tumor-specific immune response alone is not sufficient to prevent relapse completely. In preclinical mouse RFA models, tumors could quickly overcome the immune responses by inhibiting the function of CD8+ and CD4+ T cells by upregulating PD-L1/PD-1 expression, which was abolished by concomitant treatment with an anti-PD1 antibody suggesting that the PD-L1–PD-1 axis plays a critical role in dampening the RFA–induced antitumor immune response [42]. Emerging data also suggest that the anti-tumor effects of immunotherapy may be increased with the use of radiation therapy as used for brachytherapy. Radiation has not only direct cytotoxic effects on cancer cells, but is also capable of generating a robust anti-tumor immune response through effects on the tumor and the tumor microenvironment via a variety of mechanisms including enhanced tumor antigen presentation and upregulated major histocompatibility complex (MHC) class I expression [57]. An abundance of preclinical studies demonstrates that radiation combined with checkpoint blockade results in a synergistic effect [58].

These studies provide a strong rationale for combining RFA / MWA / brachytherapy and PD-L1/PD-1 blockade in the clinical setting aiming to improve the immunological effect of local ablation via RFA / MWA and to analyze if such effect can translate into an improved recurrence free interval and long-term survival of patients.
In current HCC guidelines (ESMO/ EASL) the use of TACE in combination with RFA and MWA is recommended for tumor larger than 3cm. Similarly, brachytherapy has been used in multiple centers in recent years and there is compelling evidence that brachytherapy is an effective treatment option in HCC as recommended in the current ESMO guideline (Vogel et al.). The multimodal approach of combining peri-interventional administration of an immune checkpoint inhibitor and local ablation via RFA / MWA / brachytherapy or combination of TACE with RFA, MWA or brachytherapy harbors the potential to satisfy the unmet need for an improvement in the outcomes of HCC patients treated with RFA / MWA / brachytherapy. Furthermore, early clinical data indicates that the combination of immune checkpoint

inhibition with RFA displays an acceptable safety profile [43], resulting in a positive benefit-risk ratio of this trial.

4.3.2 Justification for Dose

The planned dose of pembrolizumab (tradename: Keytruda®) for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2,262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg Q3W as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported

by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

4.3.3 Rationale for Endpoints

4.3.3.1 Efficacy Endpoints

Primary efficacy endpoint ORR:

Evaluation of ORR after two cycles of pembrolizumab and before performing RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy will allow testing of the hypothesis that pre-interventional treatment with pembrolizumab before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy will result in conversion / downstaging of borderline candidates for local ablation. Furthermore, ORR is generally accepted as a valid endpoint for efficacy evaluation in one-armed trials without control group.

Secondary efficacy endpoints time to recurrence (TTR), recurrence free survival and overall survival (OS):

Overall survival will be evaluated and is still considered the gold standard for efficacy evaluation in clinical trials. Its major drawback is the potentially long observation period necessary for OS evaluation – especially in the curative setting as represented by the treatment approach in this trial. Therefore, additional efficacy endpoints like the ones used here, TTR and recurrence free survival, have meanwhile been accepted by medicinal agencies in drug approval processes as they allow for quicker efficacy assessment. This is most important in explorative clinical trials in early phases (like this phase II trial) as it allows for more rapid evaluation of the potential of new treatment approaches. Furthermore, many of these parameters are not influenced by follow-up therapies.

Taken together, the selected endpoints are considered appropriate for this phase II trial to generate first efficacy data on the trial treatment as a basis for more comprehensive trials in case of promising results.

4.3.3.2 Biomarker Research

Correlation analysis will be performed between select molecular parameters and clinical data to identify molecular biomarkers predictive for ORR, TTR, recurrence free survival and OS. This approach is deemed appropriate to obtain hypothesis generating data for future research due to the explorative character of this trial.

5.0 METHODOLOGY

5.1 Study Population

5.1.1 Participant Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Histologically confirmed diagnosis of HCC
2. Has a Child-Pugh Classification score ≤ 6 for assessed liver function within 7 days before allocation
3. Candidate for local ablation (via either RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy [ablation technique according to Investigator's choice]), i.e.:

   According to Investigator's assessment an R0 state can be obtained after a maximum of two RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy interventions (initial ablation + one additional re-ablation at maximum).
4. Patients (including high risk patients) with:
   - Presence of ≤ 5 tumor nodules with diameters ≤ 7cm [longest axis] each OR
   - Vascular infiltration
5. Has received no prior systemic therapy for HCC

   NOTE: Patients who have received prior local therapy by transarterial chemoembolization (TACE) are not excluded if TACE has been performed >8 weeks before study allocation.
6. Have measurable disease based on RECIST 1.1. Lesions situated in a previously treated (e.g. irradiated or subject to TACE) area are considered measurable if vital tumor has been demonstrated by contrast enhanced imaging in such lesions.
7. Male/female participants who are at least 18 years of age on the day of signing informed consent will be enrolled in this study.
8. A female participant is eligible to participate if she is not pregnant (see Appendix 3), not breastfeeding, and at least one of the following conditions applies:
   a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix 3
   OR
   b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of study treatment.
   A male participant with female partner of childbearing potential is eligible to participate if he agrees to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of study treatment.
9. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.

10. Have provided archival tumor tissue sample or newly obtained biopsy of a tumor lesion not previously irradiated for mandatory pre-treatment evaluation (baseline).
    - Newly obtained biopsies are preferred to archived tissue (archived specimen ≤6 months may be acceptable).
    - Core or excisional biopsies mandatory (fine needle aspiration and bone metastasis samples are not acceptable).
    - Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides.
    - If submitting unstained cut slides, newly cut slides should be submitted to the central pathology lab within 14 days from the date slides are cut.
    - Availability of baseline tumor biopsy samples has to be ensured by site before first dose of study medication is administered.
    - Specimens have to be sent to central pathology lab for accompanying research project.
11. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Evaluation of ECOG is to be performed within 7 days prior to the date of allocation/randomization.
12. Have adequate organ function as defined in the following table (Table 1). Specimens must be collected within 7 days prior to the start of study treatment.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤2.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >2.5 × ULN
AST (SGOT) and ALT (SGPT)	≤5 × ULN
Coagulation	

International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal. [a] Transfusion are permitted to meet criteria. [b] Creatinine clearance (CrCl) should be calculated per institutional standard. Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.	

13. If patient has concurrent Hepatitis B virus (HBV) or Hepatitis C virus (HCV) infection, meets the following criteria:

    - Patients with HBV or HCV infection should be monitored for viral levels during study participation.
    - Patients with detectable hepatitis B surface antigen (HBsAg) or detectable HBV DNA should be managed per local treatment guidelines.

      Controlled (treated) hepatitis B subjects will be allowed if they started treatment at the time point of enrollment into the study by the latest and treatment is continued during study participation.
    - Patients with detectable HCV RNA are usually not treated for their HCV infection. However, patients treated for HCV are considered suitable for inclusion if antiviral therapy has been completed prior to first administration of study drug.

5.1.2 Participant Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Extrahepatic disease
2. Known fibrolamellar HCC, sarcomatoid HCC, or mixed cholangiocarcinoma and HCC
3. Presence of tumor thrombus involving main trunk of portal vein
4. Has at Screening and/or has had any prior history of Grade ≥ 2 hepatic encephalopathy
5. Has at Screening pericardial effusion, uncontrollable pleural effusion, or clinically significant ascites defined as meeting either of (a) detectable ascites on Screening physical examination OR (b) has at Screening ascites requiring paracentesis

6. A WOCBP who has a positive urine pregnancy test within 72 hours prior to allocation (see Appendix 3). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

   Note: in the event that 72 hours have elapsed between the screening pregnancy test and the first dose of study treatment, another pregnancy test (urine or serum) must be performed and must be negative in order for subject to start receiving study medication.

7. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX-40, CD137).

8. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks or at least 5 half-lives of the respective drug/IMP (whichever is longer) prior to allocation.

   Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible.

   Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.

9. Has received prior radiotherapy within 4 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.

10. Has received a live vaccine within 4 weeks or for a period of at least 5 half-lives of the respective drug/IMP (whichever is longer) before Screening and during Screening for this trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. FluMist®) are live attenuated vaccines and are not allowed.

11. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks or for a period of at least 5 half-lives of the respective drug/IMP (whichever is longer) before Screening and during Screening for this trial.
    Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks or at least 5 half-lives of the respective drug/IMP (whichever is longer) after the last dose of the previous investigational agent.

12. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.

13. Has a known additional malignancy that is progressing or has required active treatment within the past 3 years. Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.
14. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e. without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.
15. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
16. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
17. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.
18. Has an active infection requiring systemic therapy (exception: HBV infection – see inclusion criteria).
19. Has a history of Human Immunodeficiency Virus (HIV) (mandatory testing for HIV during screening is required).
20. Has a known history of active TB (Bacillus Tuberculosis).
21. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or is not in the best interest of the subject to participate, in the opinion of the treating Investigator.
22. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
23. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 120 days after the last dose of trial treatment.
24. Legal incapacity or limited legal capacity.

5.2 Trial Drug Treatment

The drug treatment to be used in this trial is outlined below in Table 2.

Table 2 Trial Drug Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle*	Experimental
*NOTE: In cycle 3, pembrolizumab administration is performed two days after RFA, MWA, brachytherapy or TACE combined with RFA, MWA or brachytherapy (RFA, MWA or brachytherapy should be performed between 1 and 2 weeks after TACE), i.e. Day 3 of cycle 3 (in case of unexpected side effects of RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy, pembrolizumab administration can be delayed up to Day 21 after RFA, MWA, brachytherapy or TACE session)					

Trial drug treatment should begin on the day of allocation or as close as possible to the date on which treatment is allocated.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Table 5 – Schedule of assessments

Trial Period:	**Screening Phase**	**Treatment Cycles (Q3W)**						**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Study Screening	D1**C1**	D1**C2**	D1**C3**	D3**C3**	D1**C4**	D1**C5-CX**	EOT	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):	-28 to -1					± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks ± 7 days	Every 12 weeks ± 7 days
Administrative Procedures											
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Demographics and Medical History	X										
Prior and Concomitant Medication Review	X	X	X		X	X	X				
Study Drug Administration		X	X		X[d]	X	X				
Local Ablation (RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy)[a]				X[d]							
Post-study anticancer therapy status								X	X	X	X
Survival Status											X
Clinical Procedures/Assessments											
Review Adverse Events	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X*							X			
Directed Physical Examination		X	X	X	X	X	X				
Vital Signs and Weight[e]	X*	X[p]	X[p]	X	X	X	X	X			

Trial Period:	**Screening Phase**	**Treatment Cycles (Q3W)**						**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Study Screening	D1**C1**	D1**C2**	D1**C3**	D3**C3**	D1**C4**	D1**C5-CX**	EOT	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):	-28 to -1					± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks ± 7 days	Every 12 weeks ± 7 days
ECOG Performance Status	X[*]	X	X	X	X	X	X	X			
Child-Pugh Score	X[§]	X	X	X	X	X	X				
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory											
HIV Testing	X[§]										
Pregnancy Test – Urine or Serum β-HCG[g]	X[§]	(X)	X	X		X	X	X	Monthly for 120 days after last dose of study drug		
PT/INR and aPTT[k]	X[§]	X	X	X	X	X	X				
CBC with Differential[h]	X[§]	X	X	X	X	X	X				
Comprehensive Serum Chemistry Panel[j]	X[§]	X	X	X	X	X	X				
Urinalysis[i]	X[§]	X	X	X	X	X	X				
T3, FT4 and TSH[l]	X[§]	X	X	X	X	X	X				
Efficacy Measurements											
Tumor Imaging	X[c]			X	control imaging 6 to 8 weeks after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy; afterwards **Q12W (± 7 days)** [f]						
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood											
Archival or Newly Obtained Tissue Collection / Biopsy	X[m]			X[n]							
Correlative Studies Blood Collection[o]	X[§]	X	X		X	X					

*: To be performed within 14 days prior to first administration of study medication.
§: To be performed within 7 days prior to first administration of study medication.
a: Local ablation by RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy (RFA, MWA or brachytherapy should be performed between 1 and 2 weeks after TACE) (ablation technique according to Investigator's choice) will be performed via ultrasound- or CT-guided placement of a needle electrode / probe penetrating into the lesion center (using commercially available systems according to local practice). In case of multiple lesions, it is left to the Investigator's decision if local ablation of all lesions is performed in a single intervention or in two successive interventions. After ablation of all lesions, control imaging must confirm absence of viable tumor tissue. If control imaging identifies residual viable tumor tissue, re-treatment via additional RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy is permitted. In case of re-treatment for residual tumor tissue, an additional control imaging has to be performed to confirm absence of viable tumor tissue.
b: Patients with EOT not due to disease progression will be followed-up for disease status by tumor imaging Q12W ± 7 days until the start of a new anticancer treatment, disease progression, death, withdrawal of consent, or the end of the study.
c: Baseline imaging to be performed between -28 and -1. In this protocol, RECIST 1.1 criteria are applied.
d: Local ablation by either RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy (RFA, MWA or brachytherapy should be performed between 1 and 2 weeks after TACE) (ablation technique according to Investigator's choice) will be performed on D1C3 according to local guidelines. Administration of study drug will be performed on D3C3. In case of unexpected side effects of RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy, pembrolizumab administration can be delayed up to day 21 after RFA, MWA, brachytherapy or TACE intervention.
e: Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.
f: Control imaging for confirmation of successful ablation (no residual viable tumor tissue) will be performed 6 to 8 weeks after RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy.
If control imaging displays residual viable tumor tissue and re-treatment by an additional RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy intervention is performed, a second control imaging will be performed 6 to 8 weeks after re-ablation.
Further on-study imaging for disease monitoring to be performed Q12W (84 days ±7 days).
g: WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test. If applicable, this test should be repeated a maximum of 24-hours before the first dose. Following initiation of treatment, pregnancy testing will be performed at each cycle or at least monthly (investigator decision according to operational workflow at study site) during treatment and until the end of relevant systemic exposure to the study drug (i.e. 120 days after the last dose), in accordance with the CTFG guidance on contraception.
h: White Blood Cell (WBC) count with differential & Absolute Neutrophil Count (ANC); Absolute Lymphocyte Count (ALC); Red Blood Cells (RBCs); Platelet count; Hemoglobin; Hematocrit; These assessments may be performed up to 1 day prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
i: Urinalysis by qualitative examination (stick) for: blood, glucose, proteins, nitrites, ketones, leucocytes, density, pH. If any of these parameters are positive with the urine multistick, a quantitative examination will be done before drug administration. These assessments may be performed up to 1 day prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
j: Albumin; Alkaline phosphatase; Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Lactate dehydrogenase (LDH); Carbon Dioxide (CO2 or biocarbonate); Uric Acid; Calcium; Chloride; Glucose; Phosphorus; Potassium; Sodium; Magnesium; Total Bilirubin; Direct Bilirubin (If total bilirubin is elevated above the upper limit of normal); Total protein; Blood Urea Nitrogen; C-reactive protein (CRP); Gamma-GT; Cholinesterase. These assessments may be performed up to 1 day prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
k: Quick's time [PT/INR], aPTT. These assessments may be performed up to 1 day prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
l: These assessments may be performed up to 1 day prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
m: Archival tumor tissue sample or newly obtained core or excisional biopsy of a tumor lesion not previously irradiated has to be sent to central pathology lab for mandatory accompanying research project (baseline sample). Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archived tissue (archived specimen ≤6 months may be acceptable). Core or excisional biopsies mandatory (fine needle aspiration and bone metastasis samples are not acceptable). Availability of baseline tumor biopsy samples has to be ensured by site before first dose of study medication is administered.
n: Biopsy of at least one lesion to be taken before RFA, MWA, brachytherapy or combination of TACE with RFA, MWA or brachytherapy. Same lesion as for baseline sample should be selected if feasible and considered safe for the patient. Core or excisional biopsy mandatory (fine needle aspiration and bone metastasis samples are not acceptable). FFPE tissue sample to be prepared and sent to central pathology lab.

o: EDTA blood sample (10 ml) for accompanying research project to be taken at baseline (up to 7 days before first administration of study medication) and on Day 1 of cycles 1 and 2, on Day 3 of cycle 3, and on Day 1 of cycle 4 (before study drug administration for each time point).
p: Refer to section for vital sign monitoring during IMP administration.

7.0 STATISTICAL ANALYSIS PLAN

7.1 Statistical Analysis Plan Summary

Justification of sample size:

Objective Response Rate towards the checkpoint inhibitor nivolumab was around 15% for patients who were sorafenib experienced and around 21% in sorafenib naïve patients according to the recently published results from the Checkmate-040 study, indicating that higher response rates may be achieved in treatment naïve patients. Considering that patients with less advanced and without prior local treatment are included in this study, an ORR of 30% can be rationally assumed.

This is an explorative phase II study. There is no formal sample size calculation. The primary endpoint is ORR and the number of 30 patients will allow to observe the expected ORR of 30% (0.3) with 90% confidence interval (CI) extending from 0.18 to 0.45 and 95% confidence interval extending from 0.16 to 0.48. Within the frame of an early phase II study, these CI are considered acceptable, since the lower bounds of CI are in the range of the ORR observed in sorafenib treated or sorafenib naïve patients, respectively.

There is no full interim analysis planned for this study, due to the small sample size and the relatively short recruitment period. However, single objectives may be analyzed as soon as sufficient events are available for analysis as detailed in the Statistical Analysis Plan (SAP).

7.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be drafted to provide details of the methods of analysis to address all study objectives. The SAP may be amended during the course of the study, but will be finalized before the cut-off date for any analysis. Due to the explorative nature of this trial and the small number of patients only descriptive statistics will be performed (e.g. describing the distribution of the baseline demographic data with predefined subgroups).