CLINICAL STUDY PROTOCOL

SPOTLIGHT-203

Protocol Title:	Phase 2 single arm clinical study to evaluate the efficacy and safety of intratumoral administration of BO-112 in combination with pembrolizumab in subjects that have progressed on anti-PD-1-based therapy in refractory unresectable malignant melanoma stage III or IV
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SPONSOR APPROVAL

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2. SYNOPSIS

Title	Phase 2 single arm clinical study to evaluate the efficacy and safety of intratumoral administration of BO-112 in combination with pembrolizumab in subjects that have progressed on anti-PD-1-based therapy in refractory unresectable malignant melanoma stage III or IV
Sponsor	Highlight Therapeutics
Development Phase	Phase 2
Rationale	Tumor resistance to immune checkpoint blockade remains a major obstacle to improving patient outcomes in melanoma. In a phase 1b study, the combination of intratumoral (IT) BO-112 and anti- programmed cell death 1 (PD-1) agents had a manageable safety profile and showed efficacy in the anti-PD-1-refractory setting with 2 out of 10 melanoma patients achieving a partial response (PR). Furthermore, this was confirmed by evidence of immunobiological activity in the tumor microenvironment, with an increase in interferon related activity and infiltration by active CD8+ T cells. Preclinical work has shown that resistance to PD-1 inhibition is associated with a lack of major histocompatibility complex (MHC) class I expression as a result of deficient tumor-interferon signaling. Activation of the double-strand RNA (dsRNA)–sensors by BO-112 induces MHC class I expression through an NF-κB mediated pathway and restores the anti-tumor efficacy of tumor-specific $CD8^+$ T cells. Overall, IT BO-112 has the potential to overcome resistance to anti-PD-1 therapy.
Study Design	This is a phase 2, single arm, open label, adaptive design study to determine the preliminary anti-tumor activity and confirm the safety of IT BO-112 in combination with intravenous (IV) pembrolizumab. The study will enroll patients with advanced and/or metastatic melanoma that have progressed on anti-PD-1-containing treatment. The study design is illustrated in Figure 1. Patients will be treated with the combination of BO-112 and pembrolizumab. IT administration of BO-112 will be performed once weekly (QW) for the first 7 weeks and then once every three weeks (Q3W); pembrolizumab Q3W will be administered IV. After enrolment of 40 patients, the sponsor and investigators will review the overall response rate (ORR), durability of response (DOR), disease control rate (DCR) and safety profile, and will

	decide whether to initiate a subsequent randomized phase of the trial. The randomized phase will be further specified in a substantial amendment to the protocol. The order of administration should be pembrolizumab then IT BO-112. BO-112 will be administered IT at a total dose of 1-2 mg at each administration to 1-8 tumor lesions using *tuberculin* (TB) syringes (or equivalent) with 20- to 25-gauge needles. The needle type can also be per the investigator's discretion, to best distribute the study drug within the lesion. Repeat IT dosing may occur if the lesion(s) remain(s) palpable or detectable by ultrasound, after which, only pembrolizumab infusion will continue. BO-112 can be administered IT into multiple accessible lesions amenable to repeat administration using an appropriate syringe and needle type for the location of the lesion(s). The rationale for dosing multiple lesions is to produce an immune response against a wider range of genetic mutations and antigenic diversity than may occur within a single lesion, which in turn is expected to reduce the chance of immune escape by the tumor. Administration is permitted only directly into the tumor or accessible lymph node. Avoid injecting lesions near large blood vessels. Injection of lung metastases is NOT permitted. Other deep lesions, including liver metastases, may be injected less frequently than QW, as feasible, on condition that at least one accessible lesion is also being treated on a weekly basis. If the investigator is doubtful as to the injectability of a lesion, it should be discussed with the medical monitor. If multiple lesions are designated for injection, the lesions should be at sites as disparate as possible in the body. Once lesions are identified, at each treatment, the maximum volume to be administered is 3.4 mL per visit, distributed over different injectable tumors. The minimum number of lesions to be injected per study visit is 1 and the maximum is 8. The minimum dose to be injected per visit is 1 mg (unless solitary injected lesion -if applicable- becomes smaller than 1.5 cm during treatment). Distribution of volume of injection will be determined based on the size of lesion to be injected per the following table:

<table>
<tr><td></td><td>
<table>
<tr><th>Lesion size (longest dimension)</th><th>Maximum Injection Volume</th><th>Maximum Total Dose</th></tr>
<tr><td>≥1.5cm</td><td>1.7 mL</td><td>1 mg</td></tr>
<tr><td>>0.5 cm to <1.5 cm</td><td>0.5-1 mL</td><td>0.3-0.6 mg</td></tr>
<tr><td>≤ 0.5 cm</td><td>0.1 mL</td><td>0.06 mg</td></tr>
</table>
The treating physician has the discretion to identify and treat lesions, but each lesion should receive at least 3 doses of BO-112 over the course of the study, unless the lesion should disappear or become inaccessible for injection. If all identified lesions disappear or become inaccessible for injection, the patient will continue study treatment with pembrolizumab alone. Liver lesions can be injected as necessary with a maximum frequency of every three weeks.
</td></tr>
<tr><td>Primary Objectives</td><td>The primary objective of this study is:
to investigate the anti-tumor activity of IT BO-112 in combination with pembrolizumab (IV) by evaluating:
ORR using RECIST 1.1, defined as the percentage of patients achieving a complete response (CR) or partial response (PR) as best overall response, by independent radiological central review (IRCR).</td></tr>
<tr><td>Secondary Objectives</td><td>The secondary objectives of this study are:
to further characterize the clinical activity of IT BO-112 in combination with IV pembrolizumab by evaluating:
disease control rate (DCR) by IRCR
duration of response (DOR) by IRCR
progression-free survival (PFS) by IRCR
overall survival (OS) at 1 year
iRECIST ORR, DCR, DOR and PFS by IRCR
ORR, DOR, DCR and PFS as assessed by investigator
to evaluate the safety and tolerability of IT BO-112 in combination with IV pembrolizumab.
to characterize the pharmacokinetics (PK) of BO-112 in combination with pembrolizumab in a subset of patients.</td></tr>
<tr><td>Exploratory Objectives</td><td>The exploratory objectives of the study are:
evaluate preliminary efficacy as defined by tumor response rate of the injected lesion(s) and non-injected lesion(s).</td></tr>
</table>

	• evaluation of antitumoral and immunological effects in the tumor microenvironment (TME) of the injected lesion by analysis of: – immune cell profile of the TME in injected and noninjected lesions. – genetic (transcriptomic) analysis of the injected and non-injected lesion TME. • itRECIST ORR.
Total Patient Enrollment	A total of 40 patients are planned to be treated. Sample size for the subsequent randomized portion will be determined in a substantial amendment.
Enrollment Criteria	**Inclusion Criteria** 1. Be willing and able to give written informed consent for the study. 2. Be ≥ 18 years of age on day of signing informed consent. 3. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. 4. Histologically or cytologically confirmed diagnosis of cutaneous or mucosal melanoma. 5. Known BRAF status. 6. Have unresectable stage III or stage IV melanoma. Patients must have progressed on treatment with an anti-PD-1/L1 monoclonal antibody (mAb) administered either as monotherapy, or in combination with other checkpoint inhibitors or other therapies. PD-1 treatment progression is defined by meeting all of the following criteria: a. Has received at least 6 weeks of standard dosing of an approved anti-PD-1/L1 mAb. b. Has demonstrated disease progression (PD) on or after PD-1/L1 as defined by RECIST v1.1. The initial evidence of PD is to be confirmed by a second assessment no less than four weeks from the date of the first documented PD, in the absence of rapid clinical progression. c. Progressive disease has been documented within 12 weeks from the last dose of anti-PD-1/L1 mAb. i. This determination is made by the investigator. Once PD is confirmed, the initial date of PD documentation will be considered the date of disease progression.

	7. Anti-PD-1-based therapy should have been the last line of systemic therapy as part of first line treatment. Prior treatment either in neo or adjuvant setting is allowed (if patient did not develop progressive disease while on receiving it). In the case of patients who develop progressive disease during adjuvant therapy, that treatment will be counted as one prior line, and will be eligible if only that prior line has been administered. 8. At least one tumoral lesion that is RECIST 1.1 measurable and amenable for IT injection. 9. At least one accessible tumor lesion that is amenable to weekly injection. If liver is a site of injection, presence of at least one additional tumor lesion outside the liver amenable for injection. 10. Willingness to provide biological samples required for the duration of the study including a fresh tumor biopsy sample. NOTE: If possible, the biopsied lesion should be a non-target lesion. 11. Adequate hematologic and organ function defined by the following laboratory results obtained within 2 weeks prior to the first dose of study treatment: a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L b. Platelet count ≥ 100 x 10^9/L c. Hemoglobin (Hgb) ≥ 9 g/dL d. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5x upper limit of normal (ULN) (5 × ULN if presence of liver metastases) e. Serum total bilirubin ≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN f. Prothrombin time (PT) (or international normalized ratio [INR]) within normal limits and activated partial prothrombin time (aPTT) within normal limits g. Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 30 mL/min (calculated per institutional standard) 12. Female patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the injection of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required (please see Appendix A). 13. Female patients who are not 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 study treatment.

Female patients of childbearing potential should be willing to use highly effective contraceptive methods (as specified in Appendix A).

14. Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, they must adhere to the contraception requirement (described in Appendix A). If there is any question that a patient will not reliably comply with the requirements for contraception, that patient should not be entered into the study.
15. Male patients should agree to use an adequate method of contraception from the beginning of the study through 120 days after receiving the study medication.
16. In countries where human immunodeficiency virus (HIV) positive patients can be included, HIV infected participants must be on anti-retroviral therapy (ART) and have a well-controlled HIV infection/disease defined as:

    a. Participants on ART must have a CD4+ T-cell count >350 cells/mm^3 at time of screening;

    b. Participants on ART must have achieved and maintained virologic suppression defined as confirmed HIV RNA level below 50 copies/mL or the lower limit of qualification (below the limit of detection) using the locally available assay at the time of screening and for at least 12 weeks prior to screening;

    c. Participants on ART must have been on a stable regimen, without changes in drugs or dose modification, for at least 4 weeks prior to study entry (Day 1).

17. Able and willing to comply with study and follow-up procedures.

Exclusion Criteria

1. Uveal melanoma.
2. Prior grade 3-4 irAE due to immune checkpoint inhibitors requiring systemic steroids for more than 2 weeks.

	3. Prior intra-tumoral treatments. 4. If a liver lesion is the site of injection: a. macroscopic tumor infiltration by the lesion to be injected into the main portal vein, hepatic vein or vena cava; b. portal vein thrombosis; c. prior embolization of liver lesions; d. radiofrequency, cryotherapy or microwave ablation in the last 6 months; e. Child-Pugh B or C; f. All AST, ALT and bilirubin greater than >2.5 ULN. 5. Contraindications to tumor biopsy and injections of the metastasis(es), such as coagulopathy, therapeutic dose anticoagulant treatment and treatment with long-acting agents such as clopidogrel which cannot be safely stopped. 6. Chemotherapy or biological cancer therapy within 4 weeks prior to the first dose of study treatment. Note: Participants must have recovered from all adverse events (AEs) due to previous therapies to ≤ Grade 1 or baseline. Participants with ≤ Grade 2 neuropathy may be eligible. 7. Palliative radiotherapy within 1 week of start of study treatment. Subjects must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. 8. Clinically active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects 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. 9. History of a second malignancy, unless potentially curative treatment has been completed with no evidence of malignancy for 2 years. 10. Allergy to BO-112 and/or any of its excipients. 11. Allergy to pembrolizumab and/or any of its excipients. 12. Active infection requiring systemic therapy.

	13. History of (non-infectious) pneumonitis/interstitial lung disease that required steroids or current pneumonitis/ interstitial lung disease. 14. Active autoimmune disease that required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed. 15. Receiving systemic immunosuppressive therapy within 28 days before enrolment with the exceptions of intranasal, topical, and inhaled corticosteroids or oral corticosteroids at physiological doses not exceeding 10 mg/day of prednisone or equivalent. 16. HIV-infected participants with a history of Kaposi sarcoma and/or Multicentric Castleman Disease. 17. Known history of hepatitis B (defined as HbsAg reactive) or known active hepatitis C (defined as HCV RNA [qualitative] detected) virus infection. Patients who are hepatitis B surface antigen negative and HBV viral DNA negative are eligible. a. Patients who had HBV but have received an antiviral treatment and show non-detectable viral DNA for 6 months are eligible. b. Patients who are seropositive because of HBV vaccine are eligible. c. Patients who had HCV but have received an antiviral treatment and show no detectable HCV viral DNA for 6 months are eligible. 18. Has received a live vaccine within 28 days prior to the first dose of study drug. For COVID vaccines a 72 hour wash out period is necessary. 19. History of allogenic tissue or solid organ transplant. 20. Is currently participating or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment (patients who are in a follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent).

	21. Any clinically significant psychiatric, social, or medical condition that, in the opinion of the Investigator, could increase patient's risk, interfere with protocol adherence, or affect a patient's ability to give informed consent.
Dose and Route of Administration	**BO-112**: IT injections into a maximum of 8 lesions per administration, QW for the first 7 weeks, and then Q3W as long as there are lesions amenable to injection, at a dose per lesion of: • Up to 1 mg (1.7 mL volume) in lesions with longest measure ≥ 15 mm. Up to 2 lesions can receive this dose per administration. • Up to 0.3-0.6 mg (0.5-1 mL volume) in lesions with longest measure of 5 to < 15 mm. • Up to 0.06 mg (0.1 mL volume) in lesions with longest measure of ≤5 mm. • A total of 2 mg (3.4 mL volume) distributed in a maximum of 8 lesions may be used. **Pembrolizumab**: 200 mg administered as an IV infusion over 30 minutes Q3W (Days 1, 22, 43 etc.). Pembrolizumab must be administered before BO-112 administration if on same day.
Safety Assessments	The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), v5.0 will be used for grading toxicities. Safety assessments will include AEs, serious adverse events (SAEs), physical examinations (PEs), vital sign measurements, ECOG status, clinical safety laboratory evaluations (hematology, serum chemistry and hepatic panels, coagulation and urinalysis) and electrocardiograms (ECG). The AE reporting period (for AEs and events of clinical interest -ECI-) for a patient enrolled in the study begins when the patient receives first treatment and is continued through 90 days after last dose. If there is any adverse event during screening period for a study specific procedure, it will also be recorded as AE. All AEs that occur in enrolled patients during the AE reporting period specified in the protocol must be recorded, regardless of the relationship of the AE to study drug. Concomitant medications will be recorded throughout the AE reporting period.
Efficacy Assessments	RECIST 1.1 will be used in this study for assessment of tumor response. While either computed tomography (CT) or magnetic resonance imaging (MRI) or PET-CT, or photos with a ruler may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

	Imaging assessment will be performed at baseline and week 8, week 16, then every 12 weeks while on treatment. Responses will be confirmed per RECIST. A radiological assessment of a CR or PR requires confirmatory imaging 4-8 weeks after the initial assessment of response was observed. Those patients with only skin lesions will be evaluated taking into account not only tumor size but also pathologic response. If a pathological complete response is achieved, patient may undergo surgical resection in order to get the best benefit for the patient. For those patients who withdraw treatment without PD, an imaging assessment is needed at the end of treatment visit, and every 12 weeks until progressive disease up to one year after first study treatment. Because of the potential for pseudoprogression, patients who are clinically stable or improved with an initial radiologic progression only, are permitted to continue on study drugs with repeat imaging according to iRECIST guidelines (with a subsequent imaging assessment in the following 4-8 weeks when there has been a RECIST PD). The assessments for DCR, DOR and PFS will be based on RECIST and iRECIST measurements. The time of first study treatment administration to the first documented disease progression or death will determine PFS. The time of first observed response to the first documented disease progression or death will determine DOR. The OS will be estimated from the time of first study treatment administration. Patients will be followed every 3 months after end of study treatment and for up to 12 months after first received dose.
Pharmacokinetics	Updated obtained results for BO-112 show no systemic exposure. Supporting data from at least 6 patients included at this study will be collected. BO-112 PK in plasma will be assessed via ELISA in a subset of at least 6 patients (these patients will be selected sequentially in the order they are enrolled). Blood samples will be drawn pre-dose, 15 minutes (+/- 5 minutes), 30 minutes (+/- 5 minutes), 240 minutes (+/- 10 minutes), and 24 hours (+/- 30 minutes) post-dose on day 1 and pre-dose and + 3 hours (+/- 10 min) post-dose on day 1 for the following 6 cycles.
Tumor Tissue Assessment	Pre and post (if feasible) treatment tumor tissue biopsies will be used for correlative research.
Sample Size Determination	The number and percentage of patients screened, enrolled, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables, baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies

	will be summarized by treatment either by descriptive statistics or categorical tables. **Efficacy analyses:** The primary efficacy variable, ORR by RECIST 1.1 (IRCR assessed) will be reported. Secondary efficacy variables will be reported by standard methods and as described in the protocol body. **Safety analyses:** Safety and tolerability will be assessed by analysis of all relevant parameters including AEs, SAEs, laboratory tests, ECGs and vital signs. Count and grade percentage of AE will be provided. Clopper-Pearson 95% confidence interval for the proportion of AEs of clinical interest will be estimated using exact method based on binomial distribution. All safety parameters will be presented by overall and by relationship to study drug. Both study therapies will be analyzed separately. **Exploratory endpoints:** Due to the exploratory nature of the PK and pharmacodynamic (PD) endpoints, only descriptive statistics will be used. **Analysis populations** **Efficacy Population:** Primary efficacy analyses will utilize the modified intent to treat population (mITT). Intent to Treat Population: The intent to treat (ITT) set is defined as all patients who are enrolled into the study. Modified Intent to Treat Population: The mITT population is defined as all patients who are enrolled, had at least one dose of trial treatment and undergo at least one post-baseline tumor response assessment. Per Protocol Population: The per protocol (PP) population is defined as all patients who received trial treatment and fulfil the following criteria: • The absence of any protocol deviations that could affect the primary efficacy analysis. • The completion of a minimal exposure to the treatment of 1 cycle (at least three doses of BO-112) and one dose of pembrolizumab. • Availability of baseline and at least one on-treatment imaging assessment. Major deviations will lead to an exclusion of patients from the PP and will be reviewed at the data review meeting prior to database snapshot for the primary analysis. **Safety Population:** The safety population consists of all patients who receive at least one dose of the any of the study medications,

	pembrolizumab or BO-112. All safety and tolerability evaluations will be based on this analysis set. **PK/PD Population:** The PK/PD population includes all patients without protocol deviations affecting interpretability of PK and/or PD. PK/PD analysis will require those patients to have received at least one dose of respective study drug for its analysis who undergo these assessments (a minimum of six patients). **Sample size justification and power calculations:** The study is designed as a single arm study: • $pA = 25\%$; and $p0 = 10\%$ as low response rate that is expected to be rejected. In addition, a 1-sided alpha of 4.19% and power of 81.8% are used. The Khan one-stage design for smaller phase 1 trials was selected to calculate the sample size. A total of 40 patients will be enrolled. If less than 8 patients out of 40 have ORR, the study will not meet the statistical bar.

Table 1 Schedule of assessments

Visits	**Screening**	**Cycle 1**				**Cycle 2**			**Cycle 3+**	**Weeks 8, 16, then Q12W** +/- 5 days	**End of Treatment Visit** Day 22 after the last dose +/- 3 days	**Safety Follow-Up**		**Survival Follow-Up Q3M after 90-day Follow-Up** +/- 7 days **(up to 12 months after first dose)**
Assessment	**Day -28 to 0**	**Day 1**(up to 3 days window)	**Day 2**[18]	**Day 8** +/- 2 days	**Day 15** +/- 2 days	**Day 1** +/- 3 days	**Day 8** +/- 2 days	**Day 15** +/- 2 days	**Day 1** +/- 3 days			**28 days post last dose** +/- 5 days	**90 days post last dose** +/- 5 days	
	Administrative procedures													
Informed Consent	X													
Verify eligibility criteria	X													
Demographics	X													
Medical history	X													
	Safety													
Physical examination[1]	X	X				X			X		X	X	X	
Height; Weight; ECOG PS[2]	X	X				X			X		X	X	X	
Vital signs[3]	X	X		X	X	X	X	X	X		X	X	X	
12-lead ECG[4]	X	X				X			X			X		
Pregnancy test (females)[5]	X					X			X		X			
Biochemistry[6]	X	X				X			X		X	X	X	
Hematology and coagulation	X	X		X	X	X	X	X	X		X	X	X	
Thyroid function	X	X							X[7]		X			
Concomitant meds[8]	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	
AE Assessment[9]		X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	X→	
	Efficacy/ PK and PD													
Biopsy[10]		X				X			X[11]					
Tumor Assessments[12]	X									X				
Survival status[13]												X	X	X
BO-112 PK sampling[14]		X	X			X			X	X				
ADAs[15]		X				X			X	X				
	Study drug administration													
Pembrolizumab dosing[16]		X				X			X					
BO-112 dosing[17]		X		X	X	X	X	X	X					

1. A complete PE is required at the screening visit. An abbreviated (symptom-directed) PE is acceptable at all subsequent visits and should be completed in a targeted manner covering related body systems.
2. Height is only required at screening.
3. Vital sign measurements (blood pressure, heart rate, respiratory rate, and temperature) are to be obtained with the patient in a sitting position. Vital signs are to be measured pre-dose and 1-hour post-dose of each study drug (both BO-112 and pembrolizumab) for the first 2 cycles. Prior to discharge, the patients must have stable vital signs.
4. ECGs will be recorded at the following time points on day 1 of Cycles 1 and 2: pre-dose, and 1 hour after the dose of BO-112ECGs will then be collected before the BO-112 dose every 4 cycles starting at Cycle 3. ECGs will be read on-site. QTc should be calculated per institutional guidelines.
5. For females of child-bearing potential only. Urine or serum tests are acceptable. If testing is required during treatment or post-treatment by local standard of care these results should also be recorded in the case report form (CRF). Female patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the injection of study medication.
6. Serum biochemistry and liver function tests, (see Table 2) will be performed at the local lab on pre-dose samples (day 1 of every cycle). Lab results should be reviewed by the PI or sub-investigator within 24 hours of sampling.
7. Samples for T3, T4 and TSH (thyroid function) will be taken at C1D1, C3D1 and thereafter every 6 weeks approximately (every 2 cycles).
8. Concomitant medications should be reported starting 4 weeks before BO-112 administration until 90 days after the last dose of study drug (BO-112 or pembrolizumab). However, the concomitant medications taken from the 28-day to 90-day follow-up period need only be reported if given for an AE related to BO-112 or pembrolizumab.
9. All AEs are to be collected from the time of C1D1 through 90 days after last dose of study drug. Any adverse event related to any study specific procedure performed during screening will be also recorded as AE.
10. First biopsy to be obtained at C1D1, before BO-112 is injected (it could be obtained before, during screening period, if it is more suitable for the site). If there are not enough tumoral cells in that biopsy, an archival sample may be used. Repeat biopsies will be performed on treatment (if feasible) at Cycle 2 day 1 prior to BO-112 (or up to 72 hours in advance). NOTE: If possible, the biopsied lesion should be a non-target lesion.
11. Optional biopsy to be performed between C3-C4.
12. Assess per RECIST 1.1 and iRECIST, besides pathologic response for patients with only skin lesions. At screening, this must include at least thorax-abdomen-pelvis + brain imaging assessments (CT or MRI or PET-CT). Imaging assessments performed before ICF signature (per standard of care) are acceptable provided they are within the required time window. On-treatment tumor assessments are to be performed at week 8, counting from C1D1 (+/- 5 days), week 16 (+/- 5 days), then on a fixed schedule starting every 12 weeks (+/- 5 days) until PD. For those patients who withdraw treatment without PD, an imaging assessment is needed at the end of treatment visit, and every 12 weeks until progressive disease. The assessments will be performed independent of cycle schedule.
13. In-person or phone contact is acceptable.
14. Plasma PK sampling of BO-112 will be performed for a subset of at least six patients, pre-dose, 15 minutes (+/- 5 min), 30 minutes (+/- 5 min), 240 minutes (+/- 10 min), and 24 hours (+/- 30 min) post-dose on day 1 and pre-dose and + 3 hours (+/- 10 min) post-dose on day 1 for the following 6 cycles.
15. Serum samples will be taken pre-dose on cycles 1 to 7 to permit the analysis of anti-drug antibodies to BO-112, in a subset of at least six patients.
16. Pembrolizumab should be administered on the same day as BO-112. The patients should be monitored for a 4-hour observation period after the pembrolizumab dose during cycles 1 and 2. Prior to discharge, the patients must have stable vital signs.
17. All patients will receive weekly BO-112 injections for Cycles 1-2 then Q3W from Cycle 3 onwards. In the case of a complete response, BO-112 dosing will be discontinued and pembrolizumab may be continued per the USPI for a maximum of 35 cycles in the absence of progressive disease, unacceptable toxicity, or patient withdrawal. If the investigator believes no further benefit will be derived from continued therapy after a complete response, then both BO-112 and pembrolizumab should be discontinued.
18. C1D2 visit may be performed through phone call for those patients who do not have PK analyses.

Figure 1 Study design

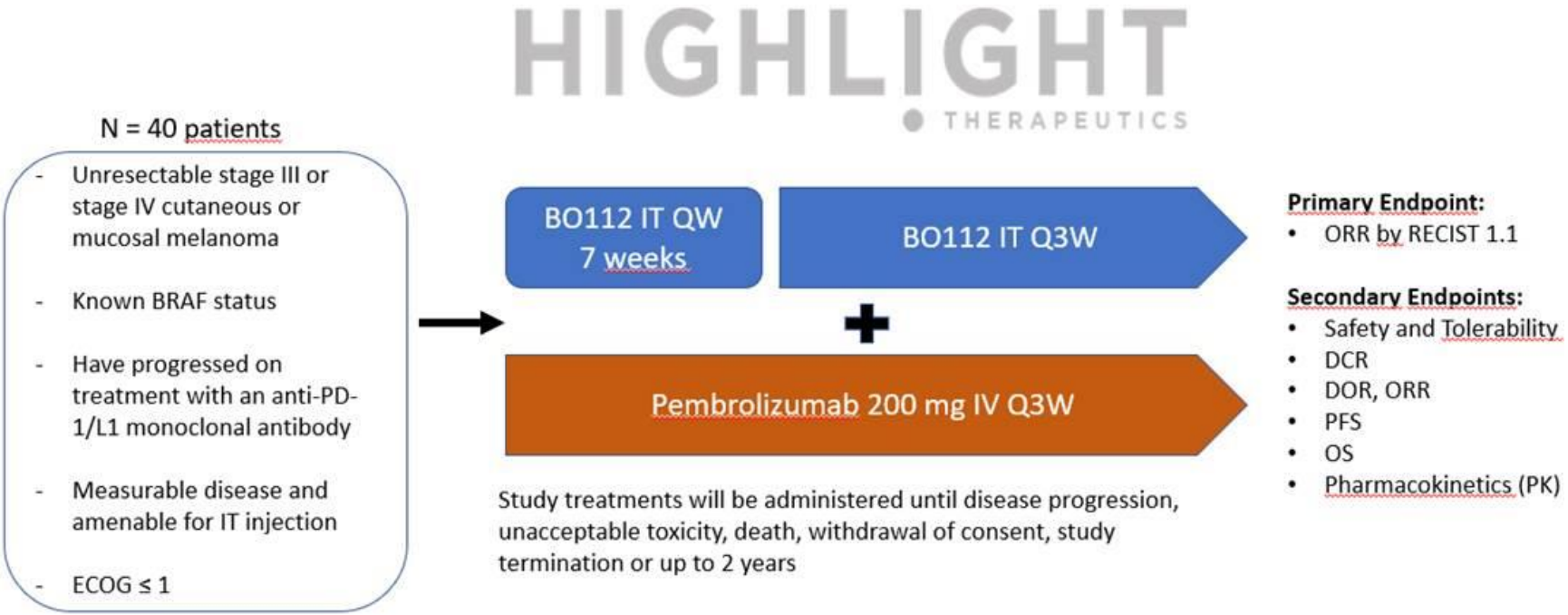

Table 2 Clinical Laboratory Panels

Hematology	**Serum Chemistry**
• WBC with differential (including neutrophils, basophils, eosinophils, lymphocytes, monocytes) • hemoglobin • hematocrit • platelet count • MCV • Reticulocytes	• albumin • amylase • alkaline phosphatase • ALT • AST • BUN or urea • calcium • creatinine • creatinine clearance calculated per institutional standar • GGT • glucose • potassium • sodium • total bilirubin^ • total protein • LDH
Coagulation	
• INR • APTT	
Hormones	
• Cortisol • βHGC (women of childbearing potential) • TSH • T3 and T4	

^ If total bilirubin elevated, conduct direct and indirect bilirubin on subsequent samples.

Please refer to the schedule of assessments for which samples should be drawn at which visit.

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4. LIST OF ABBREVIATIONS

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT (SGPT)	Alanine aminotransferase (serum glutamic pyruvic transaminase)
ANC	Absolute neutrophil count
APTT	Activated partial thromboplastin time
ART	Anti-retroviral therapy
AST (SGOT)	Aspartate aminotransferase (serum glutamic oxaloacetic transaminase)
BOR	Best overall response
BRAF	Proto-oncogene B-Raf
BUN	Blood urea nitrogen
CD	Cluster of differentiation
CFR	Code of Federal Regulations
CI	Confidence interval
CNS	Central nervous system
CRA	Clinical Research Associate
CRF	Case report form
CR	Complete remission
CRO	Contract research organization
CSR	Clinical study report
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA	Cytotoxic T-lymphocyte-associated

CV	Coefficient of variation
DCR	Disease control rate
DNA	Deoxyribonucleic acid
DMC	Data monitoring committee
DOR	Durability of response
dsRNA	Double-strand RNA
ECG	Electrocardiogram
ECI	Events of clinical interest
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FDA	Food and Drug Administration
FIH	First-in-Human
GCP	good clinical practice
GGT	γ-glutamyl transpeptidase
GLP	Good Laboratory Practice
GM-CSF	Granulocyte macrophage-colony stimulating factor
GMP	Good Manufacturing Practice
Gy	Gray, ionizing radiation dose
HbA1c	glycated hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus

Hgb	Hemoglobin
HIV	human immunodeficiency virus
IB	Investigator's Brochure
iBOR	Immune best overall response
ICH	International Conference on Harmonisation
icPD	Immune confirmed progressive disease
iCR	Immune complete response
IEC	independent ethics committee
IFN	Interferon
Ig	Immunoglobulin
IgG4	Immunoglobulin G4
INR	International normalized ratio
irAE	Immune-related adverse event
IRB	Institutional review board
IRCR	Independent Radiological Central Review
iRECIST	RECIST modified for immune-based therapies
IRR	Infusion related reactions
iSD	Immune stable disease
IT	Intratumoral
ITT	Intent to treat
itRECIST	RECIST modified for intratumoral therapies
iUPD	Immune unconfirmed progressive disease
IV	Intravenous
mAb	Monoclonal antibody

MAPK	Mitogen-activated protein kinase
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major histocompatibility complex
mITT	Modified intent to treat
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NE	Non-evaluable
NSAIDs	Nonsteroidal anti-inflammatory drugs
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
PBPK	Physiologically-based PK
PD	Progressive disease or pharmacodynamic(s)
PD-1	Programmed cell death 1
PD-L1	Programmed death-(Ligand)1, immune checkpoint
PD-L2	Programmed cell death ligand 2
PE	Physical exam
PEI	Polyethyleneimine
PET-CT	Positron emission tomography – computed tomography
PFS	Progression-free survival
PK	Pharmacokinetic(s)
PO	Oral
Poly I:C	Polyinosinic-polycytidylic acid
PP	Per protocol

PR	Partial response
PT	Prothrombin time
QP	Qualified Person
QRS	Complex on ECG
QT, QTcF	Interval between Q and T wave on ECG
QW	Once weekly
Q2W	Once every 2 weeks
Q3W	Once every 3 weeks
Q6W	Once every 6 weeks
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Stable disease
SoA	Schedule of Assessments
SOC	System organ class/standard of care
SOPs	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TB	Tuberculin
TEAE	Treatment-emergent adverse event
TLR3	Toll-like receptor 3
t_{max}	Time to maximum concentration
TME	Tumor microenvironment
TSH	Thyroid-stimulating hormone

ULN	Upper limit of normal
USA	United States of America
USPI	United States Prescribing Information
VOP	Verification of progression
WBC	White blood cell
WOCBP	Woman of childbearing potential
WHO	World Health Organization

5. BACKGROUND AND RATIONALE

5.1. BO-112

Highlight Therapeutics (Highlight) is developing the investigational drug BO-112, which is a noncoding dsRNA based on Poly I:C which is formulated with polyethyleneimine (PEI). BO-112 is an agonist to toll-like receptor 3 (TLR3) and targets the cytosolic helicase melanoma differentiation-associated gene 5 and retinoic acid-inducible gene I. By mimicking the effect of a viral infection, it is aimed at mobilizing the immune system to attack tumor cells. This includes activation of dendritic cells, CD8 T-cell infiltration, induction of interferons (IFNs), induction of apoptosis and enhancement of immunogenic cell death [Aznar 2019].

In vivo mechanism of action studies have shown a complex of mechanisms potentially involved in the antitumoral effect of IT administered BO 112 [Aznar 2019]. The role of the immune system appears to be predominant with various immune cells contributing. Conventional type-I dendritic cells, characterized by the expression of TLR3 in their endosomal compartment and their antigen cross-presenting capacity were shown to be a component of the antitumoral effect of BO-112. In addition, IFN α/β activity appears necessary to potentiate the antitumoral activity. This was demonstrated in tumor growth models using IFN alpha receptor knockout mice. Further, in vivo experiments highlighted the role of tumor-specific CD8 T-cells in halting both local (injected) tumor and distant tumor progression. Some additive effects of combining IT BO-112 with systemic anti-PDL1 inhibition have also been observed and tumor specificity of the T-cells was demonstrated using tumor-specific epitope staining.

5.1.1. BO-112 nonclinical pharmacology and toxicology

To investigate whether CD8, CD4 T-cells or IFNγ are involved in the BO-112 antitumor mediated effect, T-cell and IFNγ depletion experiments were performed in colon cancer and melanoma syngeneic tumor models. Tumor growth suppression remained in case of CD4 T-cell depletion but was lost upon cessation of BO-112 treatment in case of CD8 T-cell depletion, indicating the requirement of CD8 T-cells for maintenance of the achieved antitumoral effect, but not for the initial antitumoral effect. This was further verified by neutralizing IFNγ in the model, as this is a cytokine secreted by CD8 T-cells. Administration of anti-IFNγ antibodies resulted in loss of the tumor suppressive effect of BO-112.

BO-112 nonclinical toxicology has been characterized in multiple species using a variety of dosing regimens. More details on nonclinical data for BO-112 can be found in the Investigator's Brochure.

5.1.2. Clinical experience with BO-112

Clinical data for BO-112 are available from the first-in-human (FIH) phase 1 study (Protocol 112/2016-02, NCT02828098, 2016-000527-24**)** which evaluated single agent BO-112 (study Part 1; N = 16) and the combination of BO-112 with the checkpoint inhibitors pembrolizumab or

nivolumab (study Part 2; N = 28). This study evaluated single agent IT injections of BO-112 at 0.6 and 1 mg, and at a dose of 1 mg IT injections in combination with IV administered pembrolizumab or nivolumab.

Tumor biopsy results from the single agent portion of the study showed increased expression of gene signatures for IFNα, IFNγ, CD8 T-cell activation, cytotoxic T lymphocytes effector function and tumor inflammation at both doses evaluated. All patients had advanced, metastatic solid tumors including melanoma, leiomyosarcoma, breast cancer, neuroendocrine endometrial cancer, ovarian cancer, colorectal cancer, head and neck cancer, pleuro-peritoneal mesothelioma, and adenoid cystic carcinoma, with metastatic lesions suitable for IT injection at various sites, mainly lymph nodes and (sub)cutaneous lesions [Marquez-Rodas 2017, Marquez-Rodas 2018].

Preliminary data from Part 2 in 28 patients with inherently anti-PD-1 refractory tumors (i.e., determined to be radiologically progressing with no evidence of prior response) show the potential of 1 mg IT administered BO-112 in combination with PD-1 blockade to sensitize the immune system against the tumor with outcomes of disease stabilization and ORR of 11% (3 patients achieved PR, all had increased CD8 T-cells in the on-treatment tumor biopsy compared to baseline) and DCR of 46%. These patients had metastatic tumor burden from melanoma, Non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head and neck, or renal cell cancer which was non-responsive to the ongoing anti-PD-1 treatment and radiologically progressing at study entry. Upon study entry, 1 mg BO-112 was administered IT (skin/subcutaneous nodule, lymph node, soft tissue or visceral metastatic lesion), at intervals varying from 1 to 3 weeks, while treatment with pembrolizumab or nivolumab continued as an IV infusion according to the standard Q3W schedule at a dose of 200 mg for pembrolizumab and once every 2 weeks (Q2W) at a dose of 3 mg/kg or 240 mg for nivolumab.

A phase 2 clinical study of IT BO-112 in combination with the anti-PD-1 agent pembrolizumab in patients with liver metastases from colorectal or gastric/gastro-esophageal junction cancer patients is in temporary halt after including 17 patients (NCT04508140).

A phase 1 clinical study of IT BO-112 in combination with the anti-PD-1 nivolumab (BO-112-SARC) in patients with sarcoma is ongoing (NCT04420975).

A phase 1 clinical study of IT BO-112 in combination with the anti-PD-1 pembrolizumab (BO-112-HCC) in patients with hepatocellular carcinoma is ongoing (NCT04777708).

Ongoing phase 2 clinical study in melanoma (BOT112-03), has already included forty-two patients; 33 have discontinued the treatment while 9 are still in treatment. Final results for primary endpoint have already been presented at the 2022 American Association for Cancer Research Annual Meeting; April 8-13, 2022; New Orleans, LA. Abstract CT014. Primary endpoint was overall response rate (ORR) by RECIST 1.1 by independent reviewer. Recruitment was completed 24th August 2021 with 42 subjects; female 43%; median age 66 (range 27-88). With 40 evaluable for response subjects, 10 achieved response (25%): three CRs and seven PRs. Seventeen subjects

(44%) achieved a SD, meaning a disease control rate of 68% with 18 subjects still on treatment. The four subjects with a baseline LDH>3x upper limit of normal (ULN) developed PD no later than week 8. Responses per histology were: 66% mucosal, 28% cutaneous, 0% acral. Responses per BRAF/NRAS status were: BRAF mutant (Mut) 43%, NRAS Mut 31%, and BRAF/NRAS WT 17%. 33 pts (79%) had at least one BO-112 related AE being only in two cases grade>3 (grade 4 infusion reaction and grade 3 myalgia). Most common related AEs were asthenia, pyrexia, diarrhea, vomiting and chills. Study treatment was not discontinued in any subjects due to related AE. Conclusions: The primary efficacy endpoint has been met. Additionally, disease control (PR+CR+SD) is meaningful and durable in a population with no current standard treatment options. Very high LDH levels (LDH >3xULN) and acral melanoma could predict poor outcome. Safety profile was manageable without treatment discontinuation due to AEs. At this point, it was decided to amend the protocol in order to collect OS data up to 1 year, since primary and rest of secondary endpoints have already been met.

There has been no systemic exposure of BO-112 detected to date after IT administration. This has been recently confirmed in the phase 2 clinical study of IT BO-112 being injected directly in liver metastases in combination with iv pembrolizumab in patients with colorectal or gastric/gastro-esophageal junction cancer.

Hence, based on the confirmation of absence of detectable levels of BO-112 in plasma when lesions in the liver are injected (besides additional PK data from phase Ib study), it was considered that the addition of new PK time-points could result in an increase in the invasive procedures to be done to the patients in this trial with no additional benefit for the possibility to obtain clinically meaningful information. For this reason, PKs will be collected only in a reduced group of patients in the current study.

To date, a total of 115 subjects have been treated in the development program of BO-112. The treated population was 56.5% male, with a median age of 64.3 years and a variety of tumor types, including melanoma (56 subjects, 48.7%), NSCLC (13 subjects, 11.3%), CRC (11 subjects, 9.6%), GC (six subjects, 5.2%), sarcoma (11 subjects, 9.6%), HNSCC (4 subjects, 3.5%), leiomyosarcoma (four subjects, 3.5%), breast cancer (two subjects, 1.7%), hepatocellular carcinoma (one subject, 0.9%) and RCC (one subject, 0.9%).

One hundred percent of patients treated with BO-112 have experienced at least one treatment-emergent adverse event (TEAE), with the most frequent system organ class (SOC) being general disorders and administration site conditions, present in 103 (89.6%) subjects, gastrointestinal disorders in 77 (66.9%) subjects and musculoskeletal disorders in 57 (49.6%) subjects.

By preferred term, the most frequent TEAEs included asthenia (56 subjects, 48.7%), pyrexia (44 subjects, 38.3%), chills (41 subjects, 35.7%), and diarrhea (28 subjects, 24.3%). The TEAEs were mainly grade 1-2 events, with only eight (6.9%) subjects having a grade 3-5 toxicity related to BO-112. Although a total of 52 SAEs have been reported in 45 subjects (39.1%) most of them were related to disease progression, with only eight subjects (6.9%) having had a SAE related to BO-

112. Further detail of effects of BO-112 in humans can be found in Section 6 of the BO-112 Investigator's Brochure.

5.2. Pembrolizumab

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) 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 IV immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications. For more details on specific indications refer to the Investigator's Brochure.

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475 [Keytruda® USPI/SmPC 2020].

5.2.1. Pharmaceutical and therapeutic background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [Disis 2010]. 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 correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma [Dudley 2005, Hunder 2008].

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 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) [Greenwald 2005, Okazaki 2001].

The structure of murine PD-1 has been resolved [Zhang 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, protein kinase C-theta, and zeta-chain-associated protein kinase, which are involved in the CD3 T-cell signaling cascade [Okazaki 2001, Chemnitz 2004, Sheppard 2004, Riley 2009]. 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 [Parry 2005, Francisco 2010]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in malignant melanoma.

5.2.2. Pre-clinical data

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T cells and ultimately leads to tumor rejection, either as a monotherapy or in combination with other treatment modalities [Hirano 2005, Blank 2004, Weber 2010, Strome 2003, Spranger 2014, Curran 2010, Pilon 2010]. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute myeloid leukemia and colorectal carcinoma [Strome 2003, Curran 2010, Pilon 2010, Nomi 2007, Zhang 2004]. In such studies, tumor infiltration by CD8+ T cells and increased IFN-γ, granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T cell function in vivo [Curran 2010]. Experiments have confirmed the in vivo efficacy of anti-mouse PD-1 antibody as a monotherapy, as well as in combination with chemotherapy, in syngeneic mouse tumor models (see the Investigator's Brochure).

5.3. Rationale for conducting the study

Melanoma is a malignant tumor of melanocytes. Most melanomas arise in the skin, but they may also arise from mucosal surfaces or at other sites to which neural crest cells migrate, including the uveal tract. Uveal melanomas differ significantly from cutaneous melanoma in incidence, prognostic factors, molecular characteristics and treatment. The word melanoma, unless specified otherwise, will only refer to cutaneous and mucosal melanoma in this protocol.

Melanoma is the 19th most common malignancy worldwide with an estimated age-standardized incidence rate of 3.0 per 100,000. Approximately 287,700 new cases of melanoma and 60,700 deaths were estimated in 2018 [Ferlay 2019]. In North America, melanoma is the fifth most common cancer in males and sixth most common cancer in females [Siegel 2018]. In the US, approximately 100,350 patients will be diagnosed and there will be about 6,850 estimated deaths in 2020. The incidence has been increasing over the past 30 years. Invasive melanoma represents about 1% of skin cancers but results in most deaths [ACS 2020]. In Europe, melanoma is less common, being the seventh most common cancer [GLOBOCAN 2018]. Five-year survival for patients with early stage disease (i.e., localized) is achieved in approximately 99% of patients.

However, 5-year survival for patients with distant disease is approximately 27% [NCI 2019]. There is an increasing appreciation of the variations in specific genetic alterations among distinct clinical subtypes of melanoma, some of which have different therapeutic implications [NCCN 2019].

Advanced or metastatic melanoma (unresectable Stage III, Stage IV) remains a lethal disease with a high proportion of patients being resistant to approved therapies. Further, there are limited treatment options for patients who progress on targeted therapy or immunotherapy. Therefore, there is a high unmet medical need justifying the development of novel therapies for advanced melanoma patients who failed existing therapies.

There are two approaches for systemic treatment of unresectable Stage III/IV and recurrent melanoma. Either checkpoint inhibition or targeting the mitogen-activated protein kinase (MAPK) pathway. Both have demonstrated improvement in PFS and OS in randomized trials.

Immune checkpoint inhibitors targeting cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4; e.g., ipilimumab) and programmed T cell death 1 (PD-1; e.g., nivolumab and pembrolizumab) have been approved for the treatment of advanced or metastatic melanoma alone or in combination. In first-line therapy, nivolumab and ipilimumab combination therapy is associated with an improved ORR (57% vs. 19% vs. 44%) and median PFS (11.5 months vs. 2.9 months vs. 6.9 months) compared with single-agent ipilimumab or nivolumab, respectively. However, the combination is associated with substantial toxicity and the impact of combination therapy on OS is not yet fully established [Wolchok 2017]. Monotherapy treatment with PD-1 inhibitors (e.g., pembrolizumab or nivolumab) or CTLA-4 inhibitors (e.g., ipilimumab) is also an option for patients who are not candidates for combination therapy.

About half of patients with metastatic cutaneous melanoma harbor an activating mutation of proto-oncogene B-Raf (BRAF), an intracellular signaling kinase in the MAPK pathway. BRAF inhibitors (e.g., vemurafenib and dabrafenib) have shown clinical activity in melanomas with BRAF V600 mutations. BRAF inhibitors have monotherapy efficacy in patients with BRAF-mutated melanoma, but half of patients' relapse within approximately 6 months due to development of drug resistance. Combination therapy with BRAF and MEK inhibitors circumvents resistance and has better efficacy (i.e., improved ORR, DOR, PFS, and OS) than BRAF inhibitor monotherapy for patients with previously untreated unresectable or metastatic disease. Nevertheless, 50% of patients who respond to combination therapy still progress within the first 12 months [Mackiewicz 2018, Gellrich 2020]. Pembrolizumab and nivolumab are also approved for first line treatment of patients with BRAF mutations. For patients with BRAF V600-mutated tumors that do not progress very quickly, the currently recommended therapeutic sequence is immunotherapy (e.g., anti-PD-1 inhibitor) followed by targeted therapy with BRAF/MEK inhibitors [Michielin 2019].

Talimogene laherparepvec (T-vec, tradename Imlygic) is a genetically modified oncolytic viral therapy indicated for the local treatment of unresectable cutaneous, subcutaneous, and nodal

lesions in patients with melanoma recurrent after initial surgery. T-vec is a modified herpes simplex virus, type 1 that has undergone genetic modifications, e.g., insertion of two copies of the human cytokine granulocyte macrophage-colony stimulating factor (GM-CSF) gene to promote selective viral replication in tumor cells, while reducing viral pathogenicity and promoting immunogenicity. In a randomized phase 3 trial, IT T-vec compared to subcutaneous GM-CSF showed an ORR of 26% versus 5.7%. However, the difference seen in OS did not reach statistical significance and the response rate in visceral lesions was poor [Rehman 2016].

Treatment options for patients with advanced or metastatic melanoma who have progressed on targeted therapy or immunotherapy may include high-dose interleukin (IL)-2 or other cytotoxic therapies (e.g., dacarbazine, carboplatin/paclitaxel, albumin-bound paclitaxel). These agents have modest response rates of less than 20% in the first-line setting but no data exist in post PD-1 settings. Furthermore, little consensus exists regarding optimal standard chemotherapy [NCCN 2019]. First promising results were shown for a c-kit-inhibitor with response rates of 23.3% [Guo 2011] whereas the multi-kinase-inhibitor sorafenib targeting both the MAPK-cascade as well as the vascular endothelial growth factor and platelet-derived growth factor -cascade did not improve median PFS over placebo in a phase 3, randomized, double-blind, placebo-controlled trial in combination with carboplatin and paclitaxel [Hauschild 2009].

An important advantage of the local administration approach is that it often lessens toxicity through reduced systemic exposure [Marabelle 2019]. Although randomized clinical trial results are pending, early studies with different IT treatments, from cytokines and oncolytic viruses [Ribas 2017] to synthetic nucleotides [Rodriguez-Ruiz 2018] or analogs of bacterial products [Bhatia 2018], suggest potential synergistic effects in combination with anti-PD-1 therapy with no additional or unexpected toxicities.

This trial is designed to address the high unmet medical need of patients with unresectable stage III or IV melanoma who failed anti-PD-1 therapy. Due to the mechanisms of actions of BO-112 and pembrolizumab, it is expected that the combination of the two agents will have a synergistic anti-tumor effect in patients that have failed previous anti-PD-1 therapy. In the FIH trial of BO-112 in patients who had radiological progression of their cancer while on treatment with nivolumab or pembrolizumab at the time of study entry, 2/10 patients with advanced melanoma (one cutaneous and one mucosal) melanoma experienced durable partial responses. The first was a female patient, 48 years old, diagnosed with a wild-type BRAF cutaneous melanoma refractory to nivolumab and the second was a male patient, 29 years old, with maxillary mucosal melanoma, wild-type BRAF, refractory to a combination of ipilimumab and nivolumab. The two patients have completed the one year per protocol combination of BO-112 and nivolumab. One of these patients (cutaneous) has been evaluated by PET-CT scan. Imaging showed ongoing major metabolic response for more than 80 weeks, which persists. Furthermore, a biopsy of one of the injected lesions (skin lesion in the gluteal region), taken when injections of BO-112 were no longer feasible due to tumor shrinkage, revealed an absence of tumor cells. The mucosal melanoma patient

remains in PR after more than 60 weeks. Three more of the 10 patients with melanoma experienced stable disease lasting more than 16 weeks.

These data suggest the potential of BO-112 to sensitize the immune system against the tumor, including enhanced sensitivity to anti-PD-1 treatment, resulting in halting or reversal of the disease progression.

5.4. Justification for dose

5.4.1. BO-112

BO-112 is a suspension for injection, intended only for IT injection and has a concentration of 0.6 mg/mL. The recommended dose for BO-112 per lesion is up to 1 mg administered in 1.7 mL volume as an IT injection (lower doses are recommended based on tumor lesion size). This dose was shown to have single agent biological activity and to have both biological and clinical activity when given in combination with pembrolizumab or nivolumab in a phase 1 study of patients with advanced/metastatic solid tumors (see Section 5.1.2).

5.4.2. Pembrolizumab

The planned dose of pembrolizumab for this study is 200 mg Q3W. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is an appropriate dose of pembrolizumab for adults across all indications. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W) representing an approximate 5 to 7.5 fold exposure range (refer to the IB).
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W.
- 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 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 2262 participants were enrolled with melanoma and 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 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.

5.5. Benefit/Risk aspects

The poor prognosis of patients with unresectable stage III or IV melanoma who failed anti-PD-1 therapy represents a high unmet medical need for which investigational agents and/or combination of therapeutic agents may provide benefit. In this advanced disease setting, control or reduction of local or distant metastases may provide a meaningful clinical benefit. Data from the FIH phase 1 study of BO-112 in combination with either pembrolizumab or nivolumab in patients with anti-PD1 refractory tumors, suggest the potential of BO-112 to sensitize the immune system against the tumor, including enhanced sensitivity to the anti-PD1 treatment, resulting in halting or reversal of the disease progression. In the FIH study 2/10 patients with advanced melanoma experienced durable partial responses. Safety data from that study (N=44) indicate that IT injections of BO-112 are well tolerated and have an acceptable safety profile, also when given in the combination with pembrolizumab or nivolumab (N=28). The majority of the BO-112 related toxicities were mild to moderate and there was no amplification of the safety profile for combination versus monotherapy. The risk/benefit assessment is therefore supportive of further clinical studies.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of BO-112 and pembrolizumab may be found in the respective Investigator's Brochures.

6. STUDY OBJECTIVES

6.1. Primary objectives

The primary objective of this study is:

- to investigate the anti-tumor activity of IT BO-112 in combination with pembrolizumab (IV) by evaluating:
  - ORR using RECIST 1.1, defined as the percentage of patients achieving a CR or PR as best overall response, by IRCR.

6.2. Secondary objectives

The secondary objectives of this study are:

- to further characterize the clinical activity of IT BO-112 in combination with IV pembrolizumab by evaluating:
  - DCR by IRCR
  - DOR by IRCR
  - PFS by IRCR
  - OS at 1 year
  - iRECIST ORR, DCR, DOR and PFS by IRCR
  - ORR, DOR, DCR and PFS as assessed by investigator
- to evaluate the safety and tolerability of IT BO-112 in combination with IV pembrolizumab;
- to characterize the PK of BO-112 in combination with pembrolizumab in a subset of patients.

6.3. Exploratory objectives

The exploratory objectives of the study are:

- evaluate preliminary efficacy as defined by tumor response rate of the injected lesion(s) and non-injected lesion(s).
- evaluation of antitumoral and immunological effects in the TME of the injected lesion by analysis of:
  - immune cell profile of the TME in injected and noninjected lesions.
  - genetic (transcriptomic) analysis of the injected and non-injected lesion TME
- itRECIST ORR.

7. INVESTIGATIONAL PLAN

This investigational plan describes the study design, methods and the time course of the examinations in the planned study.

7.1 Description of overall study design and plan

This is a phase 2, single arm, open label, adaptive design study designed to determine the preliminary anti-tumor activity and confirm the safety of IT BO-112 in combination with intravenous (IV) pembrolizumab. The study will enroll patients with advanced and/or metastatic unresectable stage III or stage IV melanoma that has progressed on anti-PD-1-containing treatment, either as monotherapy, or in combination with other checkpoint inhibitors or other therapies. The definition of PD-1 treatment progression is provided in the study Inclusion Criteria in Section 8.

The study is expected to enroll 40 patients in one year counting from first patient first dose.

The study design is illustrated in

Figure 1.

Patients are required to have at least 1 measurable lesion per RECIST 1.1 that is amenable to weekly BO-112 injections. Patients will also be required to provide pre and on-treatment biopsies. Where possible, the biopsied lesion should be a non-target lesion.

Patients will be treated with the combination of BO-112 and pembrolizumab. IT administration of BO-112 will be performed QW for the first 7 weeks and then Q3W thereafter; pembrolizumab Q3W will be administered IV. After enrolment of 40 patients, the sponsor and investigators will review the overall response rate (ORR), durability of response (DOR) and safety profile, and will decide whether to initiate the randomized phase of the trial. A randomized phase of the study will be further specified in a substantial amendment to the protocol.

The order of administration should be pembrolizumab then IT BO-112. BO-112 will be administered IT at a total dose of 1-2 mg at each administration to 1-8 tumor lesions using *tuberculin* (TB) syringes (or equivalent) with 20- to 25-gauge needles. The needle type can also be per the investigator's discretion, to best distribute the study drug within the lesion. Repeat IT dosing may occur if the lesion(s) remain(s) palpable or detectable by ultrasound, after which, only pembrolizumab infusion will continue.

BO-112 can be administered IT into multiple accessible lesions amenable to repeat administration using an appropriate syringe and needle type for the location of the lesion(s). The rationale for dosing multiple lesions is to produce an immune response against a wider range of genetic

mutations and antigenic diversity than may occur within a single lesion, which in turn is expected to reduce the chance of immune escape by the tumor.

Administration is permitted only directly into the tumor or accessible lymph node. Injection of lesions near large blood vessels should be avoided. Injection of lung metastases is NOT permitted. Other deep lesions, including liver metastases, may be injected less frequently than QW, as feasible, on condition that at least one accessible lesion is also being treated on a weekly basis. If the investigator is doubtful as to the injectability of a lesion, it should be discussed with the Medical Monitor. If multiple lesions are designated for injection, the lesions should be at sites as disparate as possible in the body.

Once lesions are identified, at each treatment, the maximum volume to be injected is 3.4 mL per visit, distributed over different injectable tumors. The minimum number of lesions to be injected per study visit is 1 and the maximum is 8. The minimum dose to be injected per visit is 1 mg (unless the injected lesion, if solitary, becomes smaller than 1.5 cm). Distribution of volume of injection will be determined based on the size of lesion to be injected per the details provided in Table 5.

The treating physician has the discretion to identify and treat lesions, but each lesion should receive at least 3 doses of BO-112 over the course of the study, unless the lesion should disappear or become inaccessible for injection. If all identified lesions disappear or become inaccessible for injection, the patient will continue study treatment with pembrolizumab alone. Liver lesions can be injected as necessary with a maximum frequency of every three weeks.

7.2 Data monitoring committee

A DMC will be established to review and discuss study safety and efficacy data as patients are enrolled and followed. The DMC will meet at regular intervals throughout the course of the study as specified in the DMC Charter. The discussions of the DMC will include a review of key safety data (i.e., AEs, vital signs, and laboratory assessments) and tumor response data. Written records of the DMC meetings, the materials reviewed, and the decisions made will be maintained.

Details on the roles and responsibilities of the DMC and guidelines for monitoring study safety data will be described further in the DMC Charter.

7.3 Communication plan

Investigators and study staff who do not attend DMC meetings will be provided with an email update of all DMC decisions within 24 hours of each meeting.

7.4 Patient withdrawal, replacement and study discontinuation

7.4.1 Patient withdrawal from study

Stopping study drug for documented PD or lack of clinical benefit is not considered premature withdrawal but is considered study completion.

A patient has the right to voluntarily withdraw from the study at any time, for any reason, and without repercussion.

The Investigator and Sponsor have the right to withdraw a patient from the study at any time.

The Sponsor also has the right to discontinue the study for any reason.

The reason for any discontinuation from the study will be documented in the patient's medical records and recorded on the appropriate eCRF.

All patients who discontinue study treatment for any reason will have an End of Treatment and Safety Follow-up visits performed as described in the Schedule of Assessments (Table 1) before discontinuation, if possible.

7.4.2 Replacement of patients in study

If a patient does not undergo both a screening and post-treatment tumor assessment, or if a protocol violation occurs that substantially impairs evaluation of safety and/or activity in a patient, another patient may be enrolled to complete accrual of the planned number of evaluable patients. The decision to accrue replacement patient will be made by the Sponsor.

7.4.3 Lost to follow-up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The study center must attempt to contact the patient and reschedule the missed visit as soon as possible and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's medical records.

Should the patient continue to be unreachable, he/she will be considered to have withdrawn from the study.

7.4.4 Duration of study

The end of entire study is defined as the last visit of the last patient at any site.

For a given patient, the end of their participation is the last survival follow-up visit.

8. STUDY POPULATION

8.1.1. Study population

Only patients who meet all of the inclusion criteria and none of the exclusion criteria will be eligible to receive drug.

8.2. Inclusion criteria

Patients meeting all of the following inclusion criteria at screening/day 1 of first dosing will be eligible for participation in the study.

1. Be willing and able to give written informed consent for the study.
2. Be ≥ 18 years of age on day of signing informed consent.
3. ECOG performance status of 0 or 1.
4. Histologically or cytologically confirmed diagnosis of cutaneous or mucosal melanoma.
5. Known BRAF status.
6. Have unresectable stage III or stage IV melanoma. Patients must have progressed on treatment with an anti-PD-1/L1 mAb administered either as monotherapy, or in combination with other checkpoint inhibitors or other therapies. PD-1 treatment progression is defined by meeting all of the following criteria:
   a. Has received at least 6 weeks of standard dosing of an approved anti-PD-1/L1 mAb.
   b. Has demonstrated disease progression (PD) on or after PD-1/L1 as defined by RECIST v1.1. The initial evidence of PD is to be confirmed by a second assessment no less than four weeks from the date of the first documented PD, in the absence of rapid clinical progression.
   c. Progressive disease has been documented within 12 weeks from the last dose of anti-PD-1/L1 mAb.
      i. This determination is made by the investigator. Once PD is confirmed, the initial date of PD documentation will be considered the date of disease progression.
7. Anti-PD-1-based therapy should have been the last line of systemic therapy as part of the first line treatment. Prior treatment either in neo or adjuvant setting is allowed. (if patient did not develop progressive disease while on receiving it). In the case of patients who develop progressive disease during adjuvant therapy, that treatment will be counted as one prior line, and will be eligible if only that prior line has been administered.
8. At least one tumoral lesion that is RECIST 1.1 measurable and amenable for IT injection.
9. At least one accessible tumor lesion that is amenable to weekly injection. If liver is a site of injection, presence of at least one additional tumor lesion outside the liver amenable for injection.

10. Willingness to provide biological samples required for the duration of the study including a fresh tumor biopsy sample. NOTE: If possible, the biopsied lesion should be a non-target lesion.
11. Adequate hematologic and organ function defined by the following laboratory results obtained within 2 weeks prior to the first dose of study treatment:
    a. ANC ≥ 1.5 x 10^9/L
    b. Platelet count ≥ 100 x 10^9/L
    c. Hgb ≥ 9 g/dL
    d. AST and ALT ≤ 2.5x ULN (5 × ULN if presence of liver metastases)
    e. Serum total bilirubin ≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
    f. PT (or INR) within normal limits and aPTT within normal limits
    g. Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 30 mL/min (calculated per institutional standard)
12. Female patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the injection of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required (please see Appendix A).
13. Female patients who are not 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 study treatment.
    Female patients of childbearing potential should be willing to use highly effective contraceptive methods (as specified in Appendix A).
14. Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, they must adhere to the contraception requirement (described above). If there is any question that a patient will not reliably comply with the requirements for contraception, that patient should not be entered into the study.
15. Male patients should agree to use an adequate method of contraception from the beginning of the study through 120 days after receiving the study medication.
16. In countries where HIV positive patients can be included, HIV infected participants must be on ART and have a well-controlled HIV infection/disease defined as:

    a. Participants on ART must have a CD4+ T-cell count >350 cells/mm^3 at time of screening;
    b. Participants on ART must have achieved and maintained virologic suppression defined as confirmed HIV RNA level below 50 copies/mL or the lower limit of qualification (below the limit of detection) using the locally available assay at the time of screening and for at least 12 weeks prior to screening;

c. Participants on ART must have been on a stable regimen, without changes in drugs or dose modification, for at least 4 weeks prior to study entry (Day 1).

17. Able and willing to comply with study and follow-up procedures.

8.3. Exclusion criteria

Patients meeting any of the following exclusion criteria at screening/day -1 of first dosing will not be enrolled in the study:

1. Uveal melanoma.
2. Prior grade 3-4 irAE due to immune checkpoint inhibitors requiring systemic steroids for more than 2 weeks.
3. Prior intra-tumoral treatments.
4. If a liver lesion is the site of injection:
   a. macroscopic tumor infiltration by the lesion to be injected into the main portal vein, hepatic vein or vena cava;
   b. portal vein thrombosis;
   c. prior embolization of liver lesions;
   d. radiofrequency, cryotherapy or microwave ablation in the last 6 months;
   e. Child-Pugh B or C;
   f. All AST, ALT and bilirubin >2.5 x ULN.
5. Contraindications to tumor biopsy and injections of the metastasis(es), such as coagulopathy, therapeutic dose anticoagulant treatment and treatment with long-acting agents such as clopidogrel which cannot be safely stopped.
6. Chemotherapy, definitive (curative) radiation, or biological cancer therapy within 4 weeks prior to the first dose of study treatment. 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.
7. Palliative radiotherapy within 1 week of start of study treatment. Subjects must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis.
8. Clinically active CNS metastases and/or carcinomatous meningitis. Subjects 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.
9. History of a second malignancy, unless potentially curative treatment has been completed with no evidence of malignancy for 2 years.

10. Allergy to BO-112 and/or any of its excipients.
11. Allergy to pembrolizumab and/or any of its excipients.
12. Active infection requiring systemic therapy.
13. History of (non-infectious) pneumonitis/interstitial lung disease that required steroids or current pneumonitis /interstitial lung disease.
14. Active autoimmune disease that required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
15. Receiving systemic immunosuppressive therapy within 28 days before enrolment with the exceptions of intranasal, topical, and inhaled corticosteroids or oral corticosteroids at physiological doses not exceeding 10 mg/day of prednisone or equivalent.
16. HIV-infected participants with a history of Kaposi sarcoma and/or Multicentric Castleman Disease.
17. Known history of hepatitis B (defined as HbsAg reactive) or known active hepatitis C (defined as HCV RNA [qualitative] detected) virus infection. Patients who are hepatitis B surface antigen negative and HBV viral DNA negative are eligible.
    a. Patients who had HBV but have received an antiviral treatment and show non-detectable viral DNA for 6 months are eligible.
    b. Patients who are seropositive because of HBV vaccine are eligible.
    c. Patients who had HCV but have received an antiviral treatment and show no detectable HCV viral DNA for 6 months are eligible.
18. Has received a live vaccine within 28 days prior to the first dose of study drug. For COVID vaccines a 72 hour wash out period is necessary.
19. History of allogenic tissue or solid organ transplant.
20. Is currently participating or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment (patients who are in a follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent).
21. Any clinically significant psychiatric, social, or medical condition that, in the opinion of the Investigator, could increase patient's risk, interfere with protocol adherence, or affect a patient's ability to give informed consent.

9. TREATMENTS

9.1. Details of study treatment

9.1.1. BO-112

The investigational drug product, BO-112, will be provided by the sponsor. The description of the investigational product to be used in this clinical trial is presented below:

Table 3 BO-112 treatment details

Study Treatment Name:	Active ingredient: BO-112 is a noncoding dsRNA based on polyinosinic-polycytidylic acid (Poly I:C), formulated with polyethylenime (PEI).
Dosage Formulation:	Sterile isosmotic suspension. A clear to opalescent liquid, with a colorless to pale milky aspect and may contain functional visible particles.
Packaging and Labelling	BO-112 will be provided in a 12 mL glass vial containing 0.6 mg/mL on dry basis of active ingredient (Poly I:C) with 5% (w/v) anhydrous glucose and nominal 5.849 mM of PEI, as excipients. Each vial will be labelled as required per country requirement. The clear glass vials (20R size) are sealed using 20 mm grey bromobutyl stoppers and aluminum caps.
Unit Dose Strength(s)/Dosage Level(s):	At a dose of up to 1 mg in 1.7 mL volume per lesion. Injected dose depends on tumor lesion size.
Route of Administration	Intratumoral
Manufacturer	Highlight Therapeutics S.L.

The recommended storage condition for BO-112 is 2-8ºC protected from light. Before being administered, vials should be gently mixed a couple of times using a sideways motion for homogenization (avoid stirring, upside down mixing or vigorous mixing). BO-112 must be filtered prior to administration (within a time window of 8 hours). The type of filter to be used is a sterile and ready-to-use surfactant-free cellulose acetate (polyvinyl chloride-free) filter with 0.8 µm of pore size, 28 mm of diameter and 6.2 cm^2 of filtration area. The filter should have a bidirectional use and should be provided in a single blister pack.

Refer to Section 9.3.1 for dose administration information.

9.1.2. Pembrolizumab

Pembrolizumab will be provided by the sponsor. The description of the product to be used in this clinical trial is presented below:

Table 4 Pembrolizumab treatment details

Study Treatment Name:	Active ingredient: pembrolizumab (MK-3475) is a humanized IgG4 mAb.
Dosage Formulation:	Solution for infusion. A clear to slightly opalescent, colorless to slightly yellow solution.
Unit Dose Strength(s)/Dosage Level(s):	At a dose of 200 mg.
Route of Administration	IV infusion
Dosing Instructions:	Pembrolizumab will be administered using a 30-minute IV infusion on Day 1 of each 3-week treatment cycle prior to the BO-112 IT injection.
Packaging and Labelling	Pembrolizumab will be provided in a 10 mL clear glass vials containing 4 mL of liquid with 100 mg of pembrolizumab. Each vial will be labelled as required per country requirement.
Manufacturer	Merck Sharp & Dohme, LLC, Rahway, NJ, USA.

Pembrolizumab must be stored in the original carton at 2°C to 8°C to protect from light. Do not freeze and do not shake the vials.

After preparation of infusion: from a microbiological point of view, the product, once diluted, should be used immediately. The diluted solution must not be frozen. If not used immediately, chemical and physical in-use stability of pembrolizumab has been demonstrated for 24 hours at 2ºC to 8ºC. This 24-hour hold may include up to 6 hours at room temperature (at or below 25°C). If refrigerated, the vials and/or IV bags must be allowed to come to room temperature prior to use.

Refer to the Pharmacy Manual for dose administration information.

9.2. Manufacture of investigational medical product

All packaging and labeling operations will be performed according to Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP) guidelines, as well as the national regulatory requirements which apply to this clinical investigation.

Copies of the qualified person (QP) release certification for the packaging and labeling operations as well as certificates of analysis will be supplied to contract research organization (CRO)/sites by the sponsor, upon request.

9.2.1. Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

2. Only subjects enrolled in the study may receive study treatment and only authorized study center staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized study center staff.

3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

4. Further guidance and information for the final disposition of unused study treatment are provided in the study Pharmacy Manual.

The investigator, a member of the study center staff, or a hospital pharmacist must maintain an adequate record of the receipt and distribution of all study drugs using the Drug Accountability Form. These forms must be available for audit and/or inspection at any time.

9.3. Dosage schedule

Study treatment for this protocol consists of BO-112 IT injections QW for the first 7 weeks and then Q3W in combination with IV pembrolizumab infusions.

For each BO-112/pembrolizumab combination cycle, BO-112 IT injections will be administered after the pembrolizumab infusion, either on the same day 30-60 minutes post administration of pembrolizumab, or within a period of up to 36 hours after the pembrolizumab infusion (for organizational feasibility at the site).

9.3.1. BO-112 dose administration

In this study IT administration of BO-112 will be performed QW for the first 7 weeks and then Q3W in combination with pembrolizumab.

The BO-112 IT injections will be administered by appropriately trained surgical and/or interventional radiologist, sub-investigators or investigators, under, if needed, ultrasound or occasionally CT scan guidance. Preparatory and post-procedural measures such as those routinely used in institutional practice regarding sterility measures and local anesthetic use for percutaneous biopsy will be applied for each IT injection. BO-112 will be administered IT using TB syringes (or equivalent) with 20- to 25-gauge needles. The needle type can be per the investigator's

discretion, to best distribute the study drug within the lesion. The lesion should be punctured once and the volume to be injected should be distributed to the extent possible within the lesion either via deep penetration of the lesion and injection as the needle is withdrawn through the lesion, or via small volume injections at different points within the lesion in a 'fan-like' fashion without full removal of the needle from the lesion. Caution must be used to avoid injection of BO-112 outside of the intended lesion.

Administration is permitted only directly into the tumor or accessible lymph node. Avoid injecting lesions near large blood vessels. Injection of lung metastases is NOT permitted. Other deep lesions, including liver metastases, may be injected less frequently than QW, as feasible, on condition that at least one accessible lesion is also being treated on a weekly basis. If the investigator is doubtful as to the injectability of a lesion, it should be discussed with the Medical Monitor. If multiple lesions are designated for injection, the lesions should be at sites as disparate as possible in the body.

Once lesions are identified, at each treatment, the maximum volume to be injected is 3.4 mL per visit, distributed over different injectable tumors. The minimum number of lesions to be injected per study visit is 1 and the maximum is 8. The minimum dose to be injected per visit is 1 mg (unless the injected lesion, in case it is a solitary lesion, becomes smaller than 1,5 cm). Distribution of volume of injection will be determined based on the size of lesion to be injected per the following table.

Table 5 BO-112 administration dose and volume

Lesion size (longest dimension)	Maximum Injection Volume	Maximum Total Dose
≥1.5cm	1.7 mL	1 mg
>0.5 cm to <1.5 cm	0.5-1 mL	0.3-0.6 mg
≤ 0.5 cm	0.1 mL	0.06 mg

The treating physician has the discretion to identify and treat lesions, but each lesion should receive at least 3 doses of BO-112 over the course of the study, unless the lesion should disappear or become inaccessible for injection. If all identified lesions disappear or become inaccessible for injection, the patient will continue study treatment with pembrolizumab alone. Liver lesions can be injected as necessary with a maximum frequency of every three weeks.

Fever is the most common AE with BO-112, usually grade 1-2 and lasting no more than 24 hours. Therefore, it is recommended to administer, prophylactically, antipyretic therapy (e.g. NSAID or paracetamol or metamizole), starting 6 hours after treatment and during 24 hours, at investigator's discretion.

The Pharmacy Manual contains specific instructions on BO-112 dose preparation and administration instructions.

9.3.1.1. BO-112 temporary discontinuation/interruption

The role of BO-112 in inducing or potentiating the occurrence of anti-PD-1 associated immune-related AEs (irAEs) is currently unknown and therefore BO-112 treatment must also be interrupted in case of any irAE. Refer to Section 9.3.2.1 below for guidance on management for irAEs associated with pembrolizumab.

IT administration of BO-112 may be interrupted independently of pembrolizumab treatment (which continues as scheduled), in the following situations:

- Previously injected lesion(s) has decreased to a size for which it is not feasible to inject the dose.
- The lesion(s) has become non-injectable for other reasons such as density.

IT administration of BO-112 should be resumed as soon as feasible at subsequent scheduled administrations if the lesions(s) becomes amenable (even if this may be interpreted as a sign of progression) and there is no confirmed disease progression. There is no limit on the number of such interruptions as long as there is no confirmed disease progression. However, the case should be discussed with the Medical Monitor if 2 or more consecutive BO-112 administrations are missed. Pembrolizumab treatment should continue as scheduled.

In the case of a patient having pembrolizumab related AEs, or not being able to receive further pembrolizumab cycles, treatment with BO-112 can be continued, after discussion with medical monitor, while pembrolizumab is interrupted, delayed or withdrawn if applicable. A patient having BO-112 related AEs, or not being able to receive further BO-112 cycles, could continue receiving pembrolizumab, after discussion with medical monitor.

Both BO-112 and pembrolizumab may be interrupted in a patient who is clinically stable and has no confirmed disease progression for situations other than those specified in the preceding paragraphs. This may include delayed toxicity/diminishing tolerability over time, medical/surgical interventions or logistical reasons not related to study treatment. These interruptions should not exceed 2 cycles unless discussed with the Medical Monitor. Subjects should be placed back on study treatment within 3 weeks of the scheduled interruption, unless otherwise discussed with the Medical Monitor. The reason for interruption should be documented in the patient's medical and study record.

9.3.2. Pembrolizumab dose administration

Pembrolizumab will be administered using IV infusion on Day 1 of each Q3W treatment cycle starting on Cycle 1, after all study procedures and assessments have been completed.

Pembrolizumab will be administered at a dose of 200 mg Q3W.

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on anti-PD-1 associated infusion reaction are provided below.

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion and administration of infusion solution.

9.3.2.1. Dose modification and toxicity management for immune-related AEs associated with pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These irAEs may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care.

For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 6.

Table 6 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld, pembrolizumab may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	Monitor participants for signs and symptoms of enterocolitis (ie., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus) Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	Initiate insulin replacement therapy for participants with T1DM Administer an anti-hyperglycemic in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (e.g., propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3, 4	Continue	Initiate thyroid replacement hormones (e.g., levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 2, 3 or 4	Permanently discontinue	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 - 5.0 x ULN if baseline normal; >3.0 - 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 - 3.0 x ULN if baseline normal; >1.5 - 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 - 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 - 10.0 x ULN if baseline normal; >3.0 - 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (eg. vasculitis and sclerosing cholangitis).

9.3.2.2. Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 7.

Table 7 Pembrolizumab infusion reaction dose modification and treatment guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment.**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grades 3 or 4 **Grade 3:** Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) **Grade 4:** Life-threatening; pressor or ventilator support indicated	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

9.3.2.3. Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the medical monitor. The reason for interruption should be documented in the patient's study record.

9.3.3. Requirements for continuation of treatment post cycle 1

The decision to continue study treatment will be based on iRECIST guidelines [Seymour 2017]. Treatment will be discontinued if progressive disease is confirmed (iCPD) within 4 to 8 weeks of immune unconfirmed progressive disease (iUPD) or if iUPD is associated with clinical instability, (e.g., worsening of performance status, need for increased palliative intervention or more intensive management of disease-related symptoms).

9.4. Treatment assignment

Not applicable as this is an open-label study.

9.5. Treatment compliance

Treatment compliance will be monitored through the pharmacy records and clinical observations during study drug infusion. Actual dose administered vs planned dose will be used to assess compliance.

The drug dispensing logs and prescription records should contain the following information:

- identification of the patient to whom the study drug was administered
- date(s) and quantity of the study drug administered to the patient
- duration, interruptions or discontinuations of each injection/infusion and corresponding quantity of the study drug not administered to the patient.

9.6. Treatment duration and discontinuation of treatment

Patients will be treated with study drug until confirmed progressive disease, AE requiring discontinuation, withdrawal of consent or termination of the study.

Treatment with BO-112 and pembrolizumab will continue for up to a maximum of 35 cycles. Reasons for treatment discontinuation may include, but are not limited to:

- Patient withdrawal of consent at any time
- Investigator discretion
- Radiographic disease progression (unless clinically stable, meeting criteria noted in Section 9.6.1 below)
- Clinical disease progression

- Unacceptable toxicity
- Pregnancy
- Study termination
- Initiation of systemic steroid (prednisone >10 mg/day or equivalent) or other immunosuppressive treatment, excluding short courses to treat irAE, after discussion with medical monitor.
- Initiation of systemic anticancer therapy other than BO-112 or pembrolizumab

The reason for permanent discontinuation of BO-112 will be recorded in the CRF.

A patient who permanently discontinues study treatment should return for safety follow-up visits (Table 1).

9.6.1. Treatment beyond progression

Immunotherapeutic agents such as BO-112 may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

After the first evidence of disease progression determined by radiologic imaging, patients may receive study treatment at the discretion of the Investigator while waiting for confirmation of PD if they are clinically stable, defined as meeting all of the following criteria, and patient completing an informed consent form:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- Tolerance of BO-112 and pembrolizumab
- Stable ECOG PS
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (for example, central nervous system metastases).

If treatment continues beyond initial progression, tumor assessment will be repeated by the site 4-8 weeks later in order to re-assess for PD. If repeat imaging confirms PD, patients will be discontinued from study therapy. In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions as well as non-target lesions (Table 8).

Table 8 Treatment beyond progression

	Clinically Stable		Clinically Unstable	
	Next Imaging	**Treatment**	**Next Imaging**	**Treatment**
First radiologic evidence of progressive disease	Repeat imaging at approximately 4-8 weeks	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	No additional imaging required	Discontinue treatment
Repeat scan confirms progressive disease	No additional imaging required	Discontinue treatment	n/a	n/a
Repeat scan does not confirm progressive disease	Continue imaging assessments	Continue study treatment at the Investigator's discretion	n/a	n/a

If disease progression is due to brain metastasis, patients may continue study treatment after the local treatment of the brain lesions after consultation with the medical monitor and provided that:

- Brain metastases have been treated locally and are clinically stable for at least 2 weeks prior to re-initiation of study treatment
- There are no ongoing neurological symptoms that are related to the brain localization of the disease (sequelae that are a consequence of the treatment of the brain metastases are acceptable)
- Patients must be either off steroids or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent).
- Treatment interruption of BO-112 does not exceed 12 weeks.

If disease progression is mainly due to a metastatic lesion (nodal or visceral) which in the opinion of the Investigator may be surgically removed or treated with palliative radiation therapy, patients may continue study treatment after the local treatment of such a lesion after consultation with the medical monitor and provided that:

- It has been at least 2 weeks (post minor surgery) or 4 weeks (post major surgery) and the patient has fully recovered from the surgery.
- It has been at least 2 weeks since the patient's last dose of radiation therapy and any toxicity related to the radiation therapy is recovered to G < 2.
- Cumulative lung irradiation including lung irradiation within 26 weeks of the first dose of study drug <30 Gy.

Treatment interruption of BO-112 does not exceed 12 weeks.

9.6.2. Discontinuation of pembrolizumab

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

A participant must be discontinued from pembrolizumab treatment but continue to be monitored in the study for any of the following reasons:

- Recurrent Grade 2 pneumonitis.
- Discontinuation of treatment may be considered for participants who have attained a confirmed CR and have been treated for at least 8 cycles (at least 24 weeks), receiving 2 cycles of the combination including 2 doses of pembrolizumab beyond the date when the initial CR was declared.
- Completion of 35 administrations (approximately 2 years) with pembrolizumab.
  Note: The number of administrations is calculated starting with the first dose of pembrolizumab.

Side effects and/or concomitant medications required of treatment of HIV infection and/or its complications that are incompatible with continued study treatment (exceptions are permissible but should be discussed with the medical monitor).

9.7. Prior and concomitant illnesses and medications

9.7.1. Prior and concomitant illnesses

The investigator should document all prior significant illnesses that are relevant to patient safety or that the patient has experienced prior to screening. Additional illnesses present at the time of informed consent are to be regarded as concomitant illnesses. Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs on the eCRF.

9.7.2. Prior and concomitant medications

Any medication (including over-the-counter medications and supplements) used within 4 weeks prior to the first dose of investigational product until 90 days following the end of treatment will be recorded in the CRF, together with the main reason for its prescription.

COVID vaccines may be administered, but always allowing a wash out period of at least 72 hours between vaccine and BO-112 dose.

9.7.3. Prohibited treatment

Patients should not receive other investigational agents or participate in a device study within 3 weeks prior to study entry or receive systemic anti-cancer therapy within 4 weeks, and during the study, and will make best efforts not to start any other investigational product or device study within 30 days after last drug administration.

Listed below are specific restrictions for concomitant therapy or vaccination during the course of the study:

- Antineoplastic systemic chemotherapy or biological therapy.

- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Investigational agents other specified in this protocol.
- Live or live attenuated vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Note: Killed vaccines are allowed.
- Systemic glucocorticoids are permitted only for the following purposes:
    - To modulate symptoms of an AE that is suspected to have an immunologic etiology
    - As needed for the prevention of emesis
    - Premedication for IV contrast allergies
    - Short-term oral or IV use in doses >10mg/day prednisone equivalent for COPD exacerbations
    - For chronic systemic replacement not to exceed 10 mg/day prednisone equivalent
    - In addition, the following glucocorticoid use is allowed:
        - For topical use or ocular use
        - Intraarticular joint use
        - For inhalation in the management of asthma or chronic obstructive pulmonary disease.

  Note: Inhaled steroids are allowed for management of asthma.
- Radiation therapy of a predefined target lesion for evaluating efficacy
    - Radiation is allowed in patients after their Week 8 tumor assessment
- Surgery on a predefined target lesion for evaluating efficacy
    - Surgery is allowed in patients after their Week 8 tumor assessment
- Therapeutic anticoagulation for a thromboembolic event (prophylactic anticoagulation is allowed).

Systemic corticosteroids should be avoided if at all possible, during the study conduct, unless the patient's well-being is in question. If corticosteroids are required, a short course can be tolerated with the patient remaining on study. If continuation beyond 7 days is necessary, the patient should be removed from study treatment unless permitted to remain by the medical monitor after discussion with the Principal Investigator and Sponsor including an assessment of individual risk/benefit.

9.7.4. Other restrictions

None.

10. STUDY PROCEDURES AND DESCRIPTION OF ASSESSMENTS

10.1. Study procedures

The schedule of assessments (see Table 1) lists all assessments to be performed during the study. Unless otherwise specified, all assessments will be performed by the investigator or other study personnel. Please refer to the schedule of assessments to see which assessments need to be performed at each visit.

10.2. Description of assessments

10.2.1. Medical history

Medical history will be recorded during screening to ensure eligibility of the patients and will focus on relevant current or past abnormalities or diseases of the following systems: gynecologic, cardiovascular, respiratory, gastrointestinal, hepatic, biliary, renal, endocrine/metabolic, musculoskeletal, hematologic/lymphatic, neurologic/psychiatric, dermatologic, immunologic, infectious disease, bleeding tendency, and allergy/drug sensitivity.

10.2.2. Demographics

Information about date of birth, gender, detailed smoking history and alcohol history will be recorded during screening.

10.2.3. Physical examination

Complete physical examinations will include examination of general appearance, skin, head, ears, eyes, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, reproductive (if indicated based on symptomology and medical history) and nervous system and measurement of body weight.

Abbreviated (targeted) physical examinations should include a cardiorespiratory assessment and abdominal exam, but will focus on new symptoms and will include examination of relevant systems as identified by the investigator. An AE CRF should be completed for all changes identified as clinically significant.

Height and weight will be evaluated at screening and weight only thereafter.

An assessment of ECOG will be performed.

10.2.4. ECOG and vital signs

ECOG will be recorded per the investigator's assessment of patient performance status. Vital signs will include body temperature, respiratory rate, radial pulse rates, and systolic and diastolic blood pressures.

Blood pressure and heart rate will be measured after 5 minutes in a sitting or semi-recumbent position.

If systolic blood pressure is below 100 mmHg or above 150 mmHg and/or diastolic pressure is below 50 mmHg or above 90 mmHg, measurement will be repeated. The heart rate measurement will be repeated when below 50 beats per minutes (bpm) or above 100 bpm. If the measurement is still outside normal ranges, it is up to the investigator to judge if the measurement should be repeated.

Vital sign measurements outside normal ranges will be assessed as 'abnormal, not clinically significant', or 'abnormal, clinically significant' by the investigator. In the latter case, the abnormal vital sign measurement will be reported as an AE and further investigated as clinically indicated.

10.2.5. Electrocardiograms

A single standard 12-lead ECG will be conducted at screening, day 1 of Cycle 1 and 2: pre-dose, and 1 hour after BO-112. ECGs will then be collected before the BO-112 dose every 4 cycles starting at Cycle 3. ECGs will be read on-site. QTc should be calculated per institutional guidelines.

If any clinically-significant changes are found, then it must be performed pre- and post-dose at each subsequent dose, and dose modification rules should be adhered to.

The ECG parameters to be documented in the CRF are as follows: rhythm, heart rate, and QT interval.

ECGs will be recorded while the patient is resting in a supine or in a semi-recumbent position. ECGs will be read at site and categorized as 'normal', 'abnormal, not clinically significant', or 'abnormal, clinically significant'.

10.2.6. Pregnancy test

For females of child-bearing potential only. Urine or serum tests are acceptable. If testing is required during treatment or post-treatment by local standard of care these results should also be recorded in the CRF.

10.2.6.1. Contraception and pregnancy control measures

All female patients of childbearing potential must have a negative urine or serum pregnancy test at screening. In addition, all female patients of childbearing potential must use effective contraception with male partners who are not surgically sterile (i.e., who have not undergone vasectomy), as defined in Appendix A.

Effective contraception methods are defined in Appendix A. If a study subject inadvertently becomes pregnant while on treatment with BO-112 or pembrolizumab, the subject will be immediately discontinued from study treatment. The site will contact the study subject at least

monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is a serious adverse event (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the foetus or newborn to the Sponsor.

10.2.7. Laboratory parameters

The following laboratory tests are to be performed as indicated by the Schedule of Assessment and Clinical Laboratory Panel (see Table 1 and Table 2). All laboratory tests will be analyzed by the same local laboratory throughout the study, as designated by the principal investigator.

The Investigator is responsible for reviewing the results of all laboratory tests as they become available. Laboratory tests will be graded according to CTCAE v 5.0. Laboratory values that fall outside of clinically accepted reference ranges or values differing significantly from previous values must be evaluated by the Investigator. The Investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests.

Laboratories should not be reported as AEs unless they are considered clinically significant, which is defined as meeting any one of the following criteria:

- Any criterion for an SAE is fulfilled.
- The laboratory abnormality causes the patient to discontinue from the study treatment.
- The laboratory abnormality causes the patient to interrupt the study treatment.
- The laboratory abnormality causes the patient to modify the dose of study treatment.
- The laboratory abnormality requires intervention.
- The investigator considers the abnormality clinically significant based on clinical symptoms.

10.2.8. Serology/virology

Serology and virology measurements are not required to be performed at screening but any previously-reported results should be used for eligibility purposes. Investigators may test per their discretion.

- Hepatitis B virus surface-antigen
- Hepatitis C virus RNA
- HIV-1/HIV-2 antibodies.

10.3. Assessments per visit

Screening Visit

The screening visit procedures will be obtained within 28 days before first treatment dose.

- Sign Informed Consent Form.
- Assess eligibility based on the inclusion and exclusion criteria.
- Record patient's demographics.
- Collect medical history.
- Perform a complete physical examination.
- Record patient height, weight and ECOG performance status (height is only required at screening).
- Measure vital signs (blood pressure, heart rate, respiratory rate, and temperature) with the patient in a sitting position.
- Perform a 12-lead electrocardiogram (ECG). The ECG will be read on-site. QTc should be calculated per institutional guidelines.
- Collect sample for pregnancy test (females of child-bearing potential only). Urine or serum test are acceptable. Female patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the injection of study medication.
- Collect samples for hematology, serum biochemistry, coagulation and liver function tests (see Table 2) at the local laboratory.
- Collect samples for T3, T4 and TSH (thyroid function).
- Collect prior and concomitant medications.
- Perform tumor imaging and RECIST 1.1 and iRECIST assessment, including brain CT/MRI.

Treatment Phase

The following procedures will be performed on Day 1 of every cycle (prior to treatment administration unless otherwise indicated) during the treatment phase (± 3 days for all procedures, except tumor imaging ± 5 days):

- Perform a complete physical examination. An abbreviated (symptom-directed) physical examination is acceptable after the screening visit and should be completed in a targeted manner covering related body systems.
- Record patient weight and ECOG performance status.
- Measure vital signs (blood pressure, heart rate, respiratory rate, and temperature) with the patient in a sitting position. Vital signs are to be measured pre-dose and 1-hour post-dose of each study drug (both BO-112 and pembrolizumab) for the first 2 cycles. Prior to discharge, the patient must have stable vital signs.
- Perform a 12-lead electrocardiogram (ECG) at the following time points on day 1 of Cycles 1 and 2: pre-dose, and 1 hour after BO-112. ECGs will then be collected before the BO-112 dose every 4 cycles starting at Cycle 3. Perform a 12-lead electrocardiogram (ECG). The ECGs will be read on-site. QTc should be calculated per institutional guidelines.
- Collect sample for pregnancy test (females of child-bearing potential only). Urine or serum test are acceptable. Female patient of childbearing potential should have a negative urine

or serum pregnancy within 72 hours prior to receiving the injection of study medication. In cycle 1 day 1 pregnancy test does not need to be repeated if the test in the screening period was performed within 72 hours prior to first study dose.

- Collect samples for hematology, serum biochemistry, coagulation and liver function tests (see Table 2) at the local laboratory on pre-dose samples (day 1 of every cycle), which should be reviewed by the PI or sub-investigator within 24 hours of sampling. Hematology and coagulation to be obtained before each BO-112 dose.
- Collect samples for T3, T4 and TSH (thyroid function) every 6 weeks approximately (at C1D1, C3D1 and every two cycles thereafter).
- Collect prior and concomitant medications. Concomitant medications should be reported starting 4 weeks before BO-112 administration until 90 days after the last dose of study drug (BO-112 or pembrolizumab). However, the concomitant medication taken from 28-day to 90-day follow-up period need to be reported only if given for an AE related to BO-112 or pembrolizumab.
- Record adverse events (AEs). All AEs are to be collected from the time of first treatment is received, or any study related procedure is performed, through 90 days after last dose of study drug.
- For a subgroup of at least six patients, collect plasma samples for PK analysis of BO-112 pre-dose, 15 minutes (+/- 5 min), 30 minutes (+/- 5 min), 240 minutes (+/- 10 min), and 24 hours (+/- 30 min) post-dose on day 1 and pre-dose and + 3 hours (+/- 10 min) post-dose on day 1 for the following 6 cycles.
- For a subgroup of at least six patients, collect serum samples pre-dose on cycles 1 to 7 to permit the analysis of anti-drug antibodies (ADAs) to BO-112.
- Collect baseline biopsy at C1D1, before BO-112 injection. Any adverse event related to biopsy procedure will be recorded as AE. If there are not enough tumoral cells in that biopsy, an archival sample may be used.
- Collect biopsy, if feasible, at Cycle 2 Day 1, prior to BO-112 (or up to 72 hours in advance). An optional biopsy will also be collected between Cycle 3 and the end of Cycle 4 (NOTE: If possible, the biopsied lesion should be a non-target lesion).
- Administer pembrolizumab and BO-112.

On Day 8 and Day 15 (± 2 days) of Cycles 1 and 2 the following procedures will be performed:

- Collect samples for hematology and coagulation (see Table 2) at the local laboratory on pre-dose samples, which should be reviewed by the PI or sub-investigator within 24 hours of sampling.
- Collect prior and concomitant medications.
- Record adverse events (AEs).
- Administer BO-112.

On Week 8, Week 16 and every 12 weeks thereafter (± 5 days), counting from C1D1, until progressive disease the following procedures will be performed:

- Perform tumor imaging and RECIST and iRECIST assessments. For those patients who withdraw treatment without PD, an imaging assessment is needed at the end of treatment visit, and every 12 weeks until progressive disease. The assessments will be performed independent of cycle schedule.
- Collect prior and concomitant medications.
- Record adverse events (AEs). All AEs are to be collected from the time of first treatment dose through 90 days after last dose of study drug; serious AEs should be collected until 90 days after last dose of either BO-112 or pembrolizumab.

End of Treatment Visit

The End of Treatment Visit will be performed within 22 days (±3 days) after the last study dose. The following procedures will be performed at the End of Treatment Visit:

- Perform a complete physical examination. An abbreviated (symptom-directed) physical examination is acceptable after the screening visit and should be completed in a targeted manner covering related body systems.
- ECOG performance status.
- Measure vital signs (blood pressure, heart rate, respiratory rate, and temperature) with the patient in a sitting position.
- Collect sample for pregnancy test (females of child-bearing potential only). Urine or serum test are acceptable. Not needed if performed previously within 7 days.
- Collect samples for hematology, serum biochemistry, coagulation and liver function tests (see Table 2) at the local laboratory. Not needed if performed previously within 7 days.
- Collect samples for T3, T4 and TSH (thyroid function). Not needed if performed previously within 7 days.
- Collect prior and concomitant medications.
- Record adverse events (AEs).
- Perform an imaging assessment is needed at the end of treatment visit for those patients who withdraw treatment without progressive disease.

Safety Follow-Up Visit

The Safety Follow-Up Visits will take place at 28 days and 90 days (±5 days) after the last study dose. The following procedures will be performed:

- Perform a complete physical examination. An abbreviated (symptom-directed) physical examination is acceptable after the screening visit and should be completed in a targeted manner covering related body systems.

- Record patient weight and ECOG performance status.
- Measure vital signs (blood pressure, heart rate, respiratory rate, and temperature) with the patient in a sitting position.
- Perform a 12-lead electrocardiogram (ECG) at first Safety Follow-Up Visit only. The ECG will be read on-site. QTc should be calculated per institutional guidelines.
- Collect samples for hematology, serum biochemistry, coagulation and liver function tests (see Table 2) at the local laboratory.
- Collect prior and concomitant medications. Concomitant medications should be reported until 90 days after the last dose of study drug (BO-112 or pembrolizumab). However, the concomitant medication taken from 28-day to 90-day follow-up period need to be reported only if given for an AE related to BO-112 or pembrolizumab.
- Record adverse events (AEs). All AEs are to be collected through 90 days after last dose of study drug; serious AEs should be collected until 90 days after last dose of either BO-112 or pembrolizumab.
- Collect survival status (In-person or phone contact is acceptable).

Survival Follow-Up Visits

Survival data will be collected every 3 months (±7 days) after the 90-day Safety Follow-Up Visit up. Survival information may be obtained via telephone contact with the patient, patient´s family or by contact with the patient´s current physician. Public information sources (e.g. country records) may also be used to obtain information about survival status only in case the patient withdrew from the study.

10.4. Concomitant medication assessment

Information about concomitant medication will be recorded during screening, throughout treatment and for 90 days after discontinuation of study medication.

10.4.1. Safety assessments and time period and frequency for collecting AE, SAE, ECI, pregnancy and exposure during breastfeeding information

Safety assessments will be performed at the intervals indicated in the Schedule of Assessments (see Table 1) and at any time deemed necessary by the investigator.

AEs and SAEs (including clinically significant abnormal laboratory test results) will be collected from the time of treatment (or during screening for those AEs which are related to a study specific procedure) until 90 days after the last dose.

- All AEs or ECIs (Events of Clinical Interest) from the time of treatment through 90 days following cessation of study treatment must be reported by the investigator.

- All AEs meeting serious criteria, from the time of treatment through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.
- All pregnancies and exposure occurring during breastfeeding, from the time of treatment/ allocation through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.
- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to the Sponsor if the event is considered to be drug-related.

Only AEs/abnormalities occurring after the first dose will be considered treatment-emergent.

10.4.1.1. Events of clinical interest (ECI) for pembrolizumab

Selected non-serious and serious adverse events are also known as ECI and must be reported to the Sponsor by the investigator from the time of treatment through 90 days following cessation of study treatment.

Events of clinical interest for this trial include:

1. an overdose of Sponsor's product, as defined below, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be made available. It may also be appropriate to conduct additional evaluation for an underlying etiology in the setting of abnormalities of liver blood tests including AST, ALT, bilirubin, and alkaline phosphatase that do not meet the criteria noted above. In these cases, the decision to proceed with additional evaluation will be made through consultation between the study investigators and the medical monitor. However, abnormalities of liver blood tests that do not meet the criteria noted above are not ECIs for this trial.

10.4.1.2. Treatment of overdose

For this study, an overdose of pembrolizumab will be defined as any dose of 1000 mg or ≥ 5 times the indicated dose. For BO-112 the definition of overdose is difficult since administration is via IT injections and to date there are no detectable levels of BO-112 systemically. The potential risk of overdose will likely come from leakage from the injected tumor into circulation and this may be volume related. An overdose is therefore defined for this protocol as an IT injection volume in mL which exceeds the approximate volume of the tumor and this would become applicable for injected lesions that have a diameter of < 15 mm since the corresponding volume of a sphere with this diameter is approximately 1.8 mL. In addition, any dose (inadvertently) administered outside of the intended tumoral lesion into normal tissue will be considered an overdose.

No specific information is available on the treatment of overdose of pembrolizumab or BO-112. In the event of overdose, the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator/treating physician should:

1. Contact the Medical Monitor immediately.
2. Document the quantity/description of the excess dose as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the patient.

10.4.1.3. Grading toxicity

The CTCAE Version 5.0 will be used for grading toxicities. Safety assessments will include AEs, SAEs, PEs, vital sign measurements, clinical safety laboratory evaluations (hematology, serum chemistry, liver function tests, coagulation and urinalysis), IRRs and ECGs. Markers of study drug immunogenicity will also be monitored (ADAs). See Table 2 for details of all safety labs. Safety labs will be performed locally and PD assessments centrally.

10.4.1.4. AE reporting period

All AEs from the time of treatment (or if related to any study specific procedure during screening) through 90 days following cessation of study treatment must be reported by the investigator.

All AEs that occur in enrolled patients during the AE reporting period specified in the protocol must be recorded, regardless of the relationship of the AE to study drug. Any serious known untoward event that occurs beyond the AE reporting period that the investigator assesses as at least possibly related to drug should also be reported to the Sponsor.

10.4.2. Adverse events

An AE is any symptom, physical sign, syndrome, or disease that either emerges during the study or, if present at screening, worsens during the study, regardless of the suspected cause of the event. All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented on the Medical History CRF page. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF page during the rest of the study. Laboratory, vital signs and ECG abnormalities should also be recorded as AEs when considered clinically significant.

Laboratory, vital signs and ECG assessments will be carried out locally and evaluated by the Investigator. The Investigator is responsible for reviewing the results of all laboratory tests as they become available. Laboratory tests will be graded according to CTCAE v 5.0. Laboratory values that fall outside of clinically accepted reference ranges or values differing significantly from previous values must be evaluated by the Investigator. The Investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests.

Laboratories/ECG and vital signs should not be reported as AEs unless they are considered clinically significant, which is defined as meeting any one of the following criteria:

- Any criterion for an SAE is fulfilled.
- The laboratory/vital signs/ECG abnormality causes the patient to discontinue from the study treatment.
- The laboratory/vital signs/ECG abnormality causes the patient to interrupt the study treatment.
- The laboratory/vital signs/ECG abnormality causes the patient to modify the dose of study treatment.
- The laboratory/vital signs/ECG abnormality requires intervention.
- The investigator considers the abnormality clinically significant based on clinical symptoms.

AEs may be volunteered spontaneously by the patient, discovered as a result of general questioning by the study staff, or determined by physical examination. During each visit to the study clinic, the patient will be asked, “Have you experienced any problems since your last visit?” All AEs will be recorded on the eCRF. For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess if it meets the criteria for classification as an SAE requiring immediate notification. Follow-up of the AE, even after the date of therapy discontinuation, is required if the AE persists until the event resolves or stabilizes at a level acceptable to the investigator.

In order to avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than the patient’s own words. Each AE will also be described

in terms of duration, frequency, intensity, association with the study medication, assessment of possible causes, actions taken, and outcome, using choices given on the eCRF. Whenever possible, intensity will be classified according to the criteria provided by the CTCAE v5.0). If the AE is not listed in the CTCAE v5.0, then the highest intensity level reached according to the scale in Table 9 will be assigned.

Table 9 Classification of adverse events by intensity

Grade 1	Mild: An AE that is easily tolerated by the patient. It incurs only a minimum of discomfort, and does not influence ordinary daily tasks.
Grade 2	Moderate: An AE that is of sufficient severity to have a negative influence on ordinary daily tasks.
Grade 3	Severe: An AE that effectively hinders ordinary daily tasks, often requiring intervention.
Grade 4	Life-threatening or disabling: An AE that puts the patient's life at risk.
Grade 5	Death related to an AE.

Specific guidelines for classifying AEs by relationship to study medication are given in Table 10.

Table 10 Classification of adverse events by relationship to study medication

Probably related	There are facts, evidence and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
Possibly related	The association of the AE with the study treatment is unknown; however, the AE is not reasonably supported by other conditions.
Unlikely related	Only a remote connection exists between the study treatment and the AE. Other conditions, including chronic illness, progression or expression of the disease-state or reaction to concomitant therapy, appear to explain the reported AE.
Unrelated	There is not a reasonable possibility that the study treatment caused the AE.
Unknown	There is no possibility to assess a relationship between study treatment and AE.

Consider the following when assessing causality:

- temporal associations between the agent and the event
- effect of de-challenge and/or re-challenge
- pre-existing risk factors
- a plausible mechanism
- concurrent illnesses.

10.4.3. Serious adverse events

An SAE is defined as any AE that meets one or more of the following criteria:

- The event is fatal or life-threatening.
- The event is permanently disabling (incapacitating or interfering with the ability to resume usual life patterns).
- The event results in unplanned in-patient hospitalization or prolongation of an existing hospitalization.
- Is or results in a congenital abnormality or birth defect.
- The event requires medical intervention of any kind in order to prevent any of the aforementioned outcomes.

An AE does not need to be severe in order to be classified as an SAE. For example, an overnight hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: for example, nausea of several hours' duration may be rated as severe but may not be considered serious.

10.4.3.1. SAE reporting instructions

All AEs meeting serious criteria, from the time of treatment through 90 days following cessation of study treatment, or until the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator (contact details in Section 1). Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to the Sponsor if the event is considered to be drug-related.

Any such SAE due to any cause, whether or not related to the study medication, must be reported within 24 hours of occurrence or when the investigator becomes aware of the event. The investigator must send a preliminary report of any such SAE within 24 hours using an SAE Report Form.

The event must be also recorded on the electronic SAE CRF page. Preliminary reports of SAEs must be followed by detailed descriptions later on, including clear pseudonymized photocopies of hospital case reports, consultant reports, autopsy reports, and other documents when requested and applicable. All photocopies should be redacted to remove patients' personal details and annotated with the patient's unique study identifiers. SAE reports must be made whether or not the investigator considers the event to be related to the investigational drug.

Appropriate remedial measures should be taken to treat the SAE, and the response to treatment should be recorded. Patients must be closely followed until sufficient information is obtained to indicate a return to normal status or until the event stabilizes at a level acceptable to the investigator. Clinical, laboratory, and diagnostic measures should be employed as needed in order to determine the etiology of the problem. The results will be reported promptly to the sponsor.

10.4.4. Other significant adverse events and SUSARs

To ensure patient safety, the investigator should also notify the study medical monitor should any AE occur that is considered significant but does not meet criteria for an SAE, or that is considered unexpected. An unexpected AE is an AE that is not identified in nature, intensity, or frequency in the IB or subsequent safety regulatory reporting [Investigator's Brochure]. Any suspected, unexpected serious adverse reaction (SUSAR) must be reported to the sponsor, the Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) and the regulatory authorities within the required timeframes.

10.4.5. Tumor samples

First biopsy should be obtained at C1D1 although, if more suitable for the site, it could be performed during screening within the 28 days prior to starting the study drug, after informed consent, after the last dose of any prior anti-cancer treatment, and only if the PI is reasonably sure that the eligibility criteria will be met. If there are not enough tumoral cells in that biopsy, an archival sample may be used.

Repeat biopsies will be performed on treatment (if feasible) at Cycle 2 day 1 prior to BO-112 (or up to 72 hours in advance). Biopsies must be taken from an injected lesion. If possible, the biopsied lesion should be a non-target lesion.

When feasible, an optional biopsy will be performed between cycle 3 and the end of cycle 4.

10.4.6. Efficacy assessments

Efficacy will be assessed according to RECIST 1.1 [Eisenhauer 2009] (see Appendix D) and iRECIST. Tumor assessments will be conducted at time points stipulated in Table 1.

Tumor imaging is strongly preferred to be acquired by CT scan. For the abdomen and pelvis, contrast-enhanced MRI may be used when CT with iodinated contrast is contraindicated, or when mandated by local practice. MRI scan is the strongly preferred modality for imaging the brain. The same imaging technique regarding modality, ideally the same scanner, and the use of contrast should be used throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging. Imaging should include the chest, abdomen, and pelvis. PET-CT may be used if considered as necessary by investigator.

Initial tumor imaging at screening must be performed within 28 days prior to the date of treatment. The site study team must review screening images to confirm the patient has measurable disease per RECIST 1.1.

For patients with only skin lesions, the investigator's assessment will be the primary assessment of response. Pathological response will be taken into account and, if a pathological response is achieved, surgical resection will be allowed and the patient will continue to be considered

evaluable for response. In the event of suspected complete response, any residual cutaneous pigmented areas or other residual masses will be documented as not containing tumor by a representative biopsy. In addition, investigators will be encouraged to take biopsies of residual pigmented areas or masses suspected of no longer containing the tumor at any time point during the study. The DMC will evaluate patients with a best response per investigator and/or IRCR of CR or PR, by reviewing photographs of all visible lesions, other imaging assessments, and biopsy results.

The first on-study imaging assessment should be performed at 8 weeks from the date of start of treatment. Subsequent tumor imaging should be performed at week 16 and every 12 weeks until PD (or more frequently if clinically indicated). Imaging timing should follow calendar days and should not be adjusted for delays in cycle starts. Imaging should continue to be performed until disease progression is identified and confirmed by the investigator (iRECIST), the start of new anticancer treatment, withdrawal of consent, or death, whichever occurs first. If a patient withdraw treatment without PD, an imaging assessment will be needed at End of Treatment visit and every 12 weeks until PD.

In case of iUPD, a confirmatory CT scan should be done between 4 to 8 weeks after the iUPD scan depending on the clinical status of the patient and extent of progression. If this interval is 6 weeks after iUPD, this coincides with the next scheduled assessment in the first 24 weeks of the study. If disease progression is not confirmed and treatment continues, tumor imaging should resume at the next scheduled timepoint ensuring an interval between imaging of at least 6 weeks.

For patients who show evidence of radiological PD by RECIST 1.1 as determined by the investigator, the investigator will decide whether to continue a patient on study treatment until repeat imaging is obtained (using iRECIST for patient management; see Table 11).

Table 11 Imaging and treatment after first radiologic evidence of progressive disease

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of PD by RECIST 1.1	Repeat imaging at 4 to 8 weeks to confirm PD.	May continue study treatment at the investigator's discretion while awaiting confirmatory tumor imaging by site by iRECIST.	Repeat imaging at 4 to 8 weeks to confirm PD per the investigator's discretion only.	Discontinue treatment

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
Repeat tumor imaging confirms PD (iCPD) by iRECIST the per investigator's assessment	No additional imaging required.	Discontinue treatment (exception is possible upon consultation with the Sponsor).	No additional imaging required.	Not applicable
Repeat tumor imaging shows iUPD by iRECIST per the investigator's assessment	Repeat imaging at 4 to 8 weeks to confirm PD. May occur at next regularly scheduled imaging visit.	Continue study treatment at the investigator's discretion.	Repeat imaging at 4 to 8 weeks to confirm PD per investigator's discretion only.	Discontinue treatment
Repeat tumor imaging shows iSD, iPR, or iCR by iRECIST per the investigator's assessment.	Continue regularly scheduled imaging assessments.	Continue study treatment at the investigator's discretion.	Continue regularly scheduled imaging assessments.	May restart study treatment if condition has improved and/or clinically stable per the investigator's discretion. Next tumor imaging should occur according to the regular imaging schedule.

Objective response rate based on best overall response (all time points) using RECIST 1.1 will be the primary efficacy endpoint. RECIST 1.1 will be used as the primary measure for assessment of tumor response, date of disease progression, and as a basis for all protocol guidelines related to disease status except for discontinuation of study treatment.

iRECIST is based on the RECIST 1.1 but adapted to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by the investigator to make treatment decisions. When clinically stable, patients should not be discontinued until progression is confirmed by the investigator, according to the rules outlined in APPENDIX E. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some patients can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response. This data will be captured in the clinical database.

10.4.6.1. Independent Radiological Central review

Radiological disease assessments will be sent to a third-party radiology service for independent radiological central review. Sites will be requested to archive imaging in standard DICOM format or other accepted formats in readiness for data transfer.

10.4.7. Pharmacokinetic assessments

In a subgroup of at least six patients, plasma samples of approximately 3 mL will be collected to evaluate if BO-112 is detectable in plasma as specified in the SoA (Table 1).

BO-112 PK in plasma will be assessed via ELISA. Blood samples will be drawn pre-dose, 15 minutes (+/- 5 min), 30 minutes (+/- 5 min), 240 minutes (+/- 10 min), and 24 hours (+/-30 min) post-dose on day 1 and pre-dose and + 3 hours (+/- 10 min) post-dose on day 1 for the following 6 cycles.

Samples will be used to evaluate the PK of BO-112 after first IT injection for systemic exposure. Samples collected for analyses of BO-112 plasma concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

The Laboratory Manual will provide greater detail on PK sampling, preparation, processing and storage at the study sites.

10.4.8. ADAs

In a subgroup of at least six patients, BO-112 anti-drug antibodies will be assayed pre-dose on cycles 1 to 7 to permit the analysis. Details of sample processing and shipping will be provided in the laboratory manual.

10.4.9. Drug administration

BO-112 will be administered IT per the schedule in Table 1. BO-112 will be administered by appropriately trained surgical and interventional radiology sub-investigators or investigators. Refer to Section 9.3.1.

Pembrolizumab will be administered as a 30-minute IV infusion on a Q3W regimen per the schedule in Table 1. Refer to Section 9.3.2.

11. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Electronic CRFs will allow all clinical parameters to be entered into a database within 3 business days of each study visit. The statistical analysis of the clinical data will be performed by the sponsor's representative. Analysis of the PK and PD data may be performed separately and entered

into a separate database. Data from the clinical, PK and PD databases will be integrated in the clinical study report.

All data obtained either from the eCRFs or from an external laboratory will be listed, and summary tables will be provided.

A separate statistical analysis plan (SAP) detailing statistical assessment (except for PK/PD) for this protocol will be prepared. Pharmacokinetic and pharmacodynamic analysis plans will be described in separate documents.

Any changes in the statistical methods described herein compared to the final SAP will be documented in the integrated clinical study report.

11.1. Sample size justification

The study is designed as a single arm study:

- $pA = 25\%$;
- and $p0 = 10\%$ as low response rate that is expected to be rejected.

In addition, a 1-sided alpha of 4.19% and power of 81.8% are used. The Khan one-stage design for smaller phase 1 trials was selected to calculate the sample size [Khan 2012]. A total of 40 patients will be enrolled. If less than 8 patients out of 40 have ORR, the study will not meet the statistical bar.

11.2. Safety population

The safety population is defined as all patients who receive at least one dose. All safety and tolerability evaluations will be based on this analysis set.

11.3. Efficacy analysis population

Primary efficacy analyses will utilize the mITT population. An exploratory analysis using the ITT population will be done.

Intent to Treat Population: The ITT set is defined as all patients who are enrolled into the study.

Modified Intent to Treat Population: The mITT population is defined as all patients who are enrolled, had at least one dose of trial treatment and undergo at least one post-baseline tumor response assessment.

Per Protocol Population: The PP population is defined as all patients who received trial treatment and fulfil the following criteria:

- The absence of any protocol deviations that could affect the primary efficacy analysis.
- The completion of a minimal exposure to the treatment of 1 cycle (at least three doses of BO-112).

- Availability of baseline and at least one on-treatment / post-randomization imaging assessment.

Important deviations will lead to an exclusion of patients from the PP and will be reviewed at the data review meeting prior to database snapshot for the primary analysis.

11.4. PK analysis population

The PK population includes the subset of at least six patients undergoing these assessments, who received treatment, had at least one measurable serum concentration, and had no protocol deviations affecting interpretability of PK. For non-compartmental assessments, patients must have at least 1 measurable concentration to be evaluable. For model-based evaluations patients will be evaluable if they have at least one documented dose and one measurable concentration.

11.5. PD analysis population

The PD population includes the subset of at least six patients undergoing these assessments without protocol deviations affecting interpretability of the PD. All PD analyses are based on this analysis set.

The PD population will be based on all patients without protocol deviations affecting serum and tissue sampling.

The PD populations for each endpoint will be based on all patients without protocol deviations affecting sampling of the specific biomarker for that analysis.

11.6. Patient disposition and termination status

The number and percentage of enrolled versus screened patients will be summarized. Reasons for screen failure will be documented.

Early withdrawals and the reason for withdrawal will be tabulated. The number and percentage of patients who complete the study and who withdraw from the study will be documented. If dropouts are numerous, safety assessments among dropouts will be listed and summarized by reason for discontinuations (grouped as due to AEs, disease progression, and other).

For a given patient, the end of their participation is the last survival follow-up visit or death, whatever happens first.

11.7. Handling missing data

Missing data will not be imputed in this study. If patients have missing or uninterpretable data, the sponsor may enroll an additional patient to replace the missing information and maintain the planned sample size for the analysis.

11.8. Background and demographic characteristics

Patient baseline characteristics; including demographics, medical history, physical examination, ECG, and vital signs will be summarized descriptively. The descriptive statistics, including n

(number of observations or sample size), mean, standard deviation and/or standard error, median, range (minimum-maximum), geometric means and geometric CV (where applicable) for numerical variables, and frequency and percentages for categorical variables, will be presented.

11.9. Primary outcome measures/analyses

11.9.1. Efficacy analysis

Analyses of efficacy will be conducted on all patients included in the Full Analysis Set as outlined in the table below.

Table 12 Efficacy analyses

Endpoint	Statistical Analysis Methods
Primary	Objective Response Rate based on RECIST 1.1 is defined as the percentage of patients with CR or PR as BOR according to RECIST 1.1. The number and percentage of responders, patients with CR or PR as BOR, and of non-responders, patients with SD, PD or non-evaluable (NE) as BOR, will be provided along with 95% CI for ORR. In addition, number and percentage of patients with CR, PR, SD, PD and NE as BOR according to RECIST 1.1 will be provided.
Secondary	DCR based on RECIST 1.1 is defined as the percentage of patients with CR or PR, and the percentage of patients with SD of at least 12 weeks duration, as BOR according to RECIST 1.1. The number and percentage of patients achieving disease control, patients with CR or PR or SD as BOR, and not achieving disease control, patients with PD or NE as BOR, will be provided along with 95% CI for DCR. • ORR according to RECIST modified for immune-based therapies (iRECIST) is defined as the percentage of patients with iCR or iPR as best overall response (iBOR) according to iRECIST. The number and percentage of responders, patients with iCR or iPR as iBOR, and of non-responders, patients with iSD, iUPD, iCPD or NE as iBOR, will be provided along with 95% CI for ORR. In addition, number and percentage of patients with iCR, iPR, iSD, iUPD, iCPD and NE as iBOR according to iRECIST will be provided. • DCR according to iRECIST is defined as the percentage of patients with iCR or iPR or iSD of at least 12 weeks duration as best overall response according to iRECIST. The number and percentage of patient achieving disease control, patients with iCR or iPR or iSD as iBOR, and not achieving disease control, patients with iUPD or iCPD or NE as iBOR, will be provided along with 95% CI for DCR. • DOR according to RECIST 1.1 is defined as the time in months from the date of first documented response (i.e., overall response = CR or PR) to the earlier between the date of first documented progression (i.e., overall response= PD) and the date of death. DOR will be analyzed using Kaplan-Meier method. Kaplan-Meier estimate of median DOR will be provided with the 95% CIs. Kaplan-Meier DOR curve will be plotted.

Endpoint	Statistical Analysis Methods
	• PFS according to RECIST 1.1 is defined as the time in months from first dose of study treatment (any agent) to first documented radiologic progression or death, whichever occurs first. Subjects without documented radiologic progression or death event will be censored at last evaluable postbaseline tumor assessment. The number and percentage of patients who were progression-free, who progressed, who died and censored for PFS will be provided. Kaplan-Meier estimate of median PFS will be provided with the 95% CIs. The proportion of patients who survived progression-free for at least 12 weeks will be provided along with 95% CI. Kaplan-Meier PFS curve will be plotted. • OS is defined as the time in months from first dose of study treatment (any agent) to death. Subjects without documented death event will be censored at the date he/she was last known to be alive. The number and percentage of patients who died and censored for OS will be provided. Kaplan-Meier estimate of median OS will be provided with the 95% CIs. The proportion of patients who survived for at least 6 and 12 months will be provided along with 95% CI. Kaplan-Meier OS curve will be plotted.
Exploratory	Will be described in the SAP finalized before interim database lock.

Note: the prefix "i" denotes immune-related or immune-based.

11.10. Secondary outcome measures/analyses

11.10.1. Secondary efficacy evaluations.

The secondary efficacy evaluations are described in Table 12.

11.10.2. Safety and tolerability

All safety analyses will be performed on the safety analysis set.

Treatment-emergent adverse events are defined as AEs that first occurred or worsened in severity after the first administration of study treatment (or related to any study specific procedure) and prior to 90 days after the last administration of study treatment.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Numbers of TEAEs and incidence rates will be tabulated by preferred term and system organ class.

Treatment-emergent AEs of any grade, TEAEs with severity ≥ Grade 3 (NCI-CTCAE v 5.0), TEAEs (any grade) by relationship to study treatment, TEAEs related (probable, possible or unknown causality) to study treatment with severity ≥ Grade 3 (NCI-CTCAE v 5.0) SAEs, TEAEs leading to death, and TEAEs leading to discontinuation of study treatment will be tabulated for the number and proportion of patients. Additionally, treatment tolerability will be determined by the number of study discontinuations due to treatment-related TEAEs. Commonly occurring TEAEs, i.e., those that occur in 5% or more of the patients in either treatment group, will be summarized using descriptive statistics.

All laboratory test results, vital signs measurements, ECG results, weight, and body mass index will be summarized using descriptive statistics at each visit for raw numbers and change from

baseline. The incidence of treatment-emergent abnormal laboratory, vital sign, and ECG values will also be summarized using descriptive statistics.

11.11. Other analyses

Biomarker exploratory analyses will be described in the SAP. Pharmacokinetic analyses will be performed in a limited subgroup of patients, and are expected to be absent since no systemic exposure is expected from IT injection of BO-112. There will be no PK analysis of pembrolizumab. Therefore, the PK analysis will be described in the SAP.

11.12. Safety data handling

11.12.1. Adverse events

TEAEs are defined as those AEs that started on or after the first dose of study medication or that worsened after the first dose of study medication, or those which are related to any study specific procedure performed during screening. Only TEAEs will be summarized. The incidence of TEAEs will be presented by the number and percent of patients who experienced the TEAE.

Each patient will be counted only once in the incidence for each term (overall incidence, System Organ Class [SOC] or preferred term). The incidence of treatment-related TEAEs will be summarized by SOC and preferred term. The incidence of TEAEs leading to study withdrawal or dose reduction, the incidence of serious TEAEs, and the incidence of treatment-related SAEs will be tabulated similarly.

In addition, the incidence of TEAEs will be summarized by SOC, preferred term, and maximum intensity based on CTCAE grade, by study cohort and overall, where possible.

11.12.2. Clinical laboratory evaluation

Clinical laboratory results including chemistry, liver function, hematology, clotting and urinalysis will be collected per the Schedules of Assessments (see Table 1).

Laboratory results will be tabulated as necessary or presented as per-patient listings.

11.12.3. Physical examination

Physical examination data will be summarized descriptively by scheduled time point.

11.12.4. Vital signs

Vital signs data will be summarized descriptively by scheduled time point.

Mean change and mean percentage change from baseline will be summarized by descriptive statistics, as appropriate.

11.12.5. 12-lead ECG

12-lead ECG will be categorized as 'normal', 'abnormal, not clinically significant', or 'abnormal, clinically significant' and summarized descriptively.

Intervals of the cardiac cycle will be presented by descriptive statistics.

Mean change and mean percent change from baseline will be summarized by descriptive statistics, as appropriate.

Any abnormal ECG findings will be listed on a per-patient basis.

11.12.6. Prior and concomitant medications

Prior medications are defined as medications that stopped before the date of first dose of study medication. Concomitant medications are defined as any medications taken on or after the first dose of study medication and up to 90 days following the last dose of study medication.

All prior and concomitant medications will be assigned a generic name and a drug class based on the World Health Organization (WHO) Dictionary. Prior and concomitant medications will be listed and summarized by study cohort and drug class, as appropriate.

11.13. Protocol deviations

Protocol deviations are defined as deviations from the procedures outlined in the protocol. Major protocol deviations, such as significant non-compliance or other serious unforeseen deviations deemed to invalidate the data collected in lieu of the purpose of the study may lead to exclusion of data from analysis. In case of minor protocol deviations data will not be excluded from the data analysis.

All decisions regarding the type of deviations (major or minor) will be made prior to commencing the final analysis on the final locked database. A listing of all patients with protocol deviations will be maintained by the Sponsor and a listing of all major protocol violations will be presented in the final study report.

12. STUDY MANAGEMENT

12.1. Approval and consent

12.1.1. Regulatory guidelines

This study will be performed in accordance with the Standard Operating Procedures of the Sponsor (or designee), the EU Clinical Trials Directive and the Code of Federal Regulations, the guidelines of the ICH, and the most recent guidelines of the Declaration of Helsinki.

12.1.2. Independent ethics committee/IRB

Conduct of the study must be approved by an appropriately constituted IEC or IRB. Approval is required for the study protocol, IB, protocol amendments, informed consent forms, patient information sheets, and any advertising materials. No study drug will be shipped to the study site until written IEC or IRB authorization has been received by the sponsor or its representative.

Study progress is to be reported to the IRB/IECs annually (or as required by the committee) by the investigator or sponsor, depending on local regulatory obligations. The investigator or Sponsor will also submit, depending on local regulations, periodic reports and inform the IRB/IEC of any reportable adverse events per ICH guidelines and local IRB/IEC standards of practice.

The IRB/IEC and Competent Authorities will be notified of the end of the trial per local requirements. A summary of the study outcome will be provided, where required.

12.1.3. Informed consent

For each study patient, written informed consent will be obtained prior to any protocol-related activities. As part of this procedure, the principal investigator or one of his/her associates must explain orally and in writing the nature, duration, and purpose of the study, and the action of the study drug in such a manner that the patient is aware of the potential risks, inconveniences, or adverse effects that may occur. They should be informed that the patient may withdraw from the study at any time. They will receive all information that is required by the regulatory authorities and ICH guidelines. The ICF should be signed by the patient and a copy provided to them.

12.2. Protocol amendments and other changes in study conduct

Changes to this protocol that meet the criteria of a substantial amendment in accordance with European Guidance (2010/C 82/01) require a protocol amendment that must be approved by the sponsor, the investigator(s), IEC/IRB and regulatory authorities before implementation. A substantial amendment in the EU will be treated as a protocol amendment in the USA per 21 CFR 312.30(b).

The requirements for approval of the substantial changes should in no way prevent any immediate action from being taken by the investigator or by the sponsor in the interest of preserving the safety of all patients included in the study.

Amendments affecting only administrative aspects of the study that meet the criteria of a non-substantial amendment in accordance with European Guidance (2010/C 82/01) do not require formal protocol amendments or IEC/IRB approval, but the IEC/IRB must be kept informed of such administrative changes, and a log of such changes will be maintained in the case that an audit is required. The sponsor must be consulted and approve of such changes prior to their implementation.

No changes in this protocol can be made without the sponsor’s written approval.

12.3. Protocol deviations

All protocol deviations will be assessed and documented on a case by- case- basis before the database lock.

A protocol deviation is any non-compliance with the clinical trial protocol, GCP, or requirements in other procedures. The non-compliance may be either on the part of the participant, the investigator, or the trial site staff. Because of deviations, corrective actions are to be developed by the site and implemented promptly.

Major protocol deviations are any deviations that might significantly affect the completeness, accuracy, and/or reliability of the trial data or that might significantly affect a subject's rights, safety, or well-being. This includes deviations related to subject eligibility, informed consent, trial drug dosing errors, or failing to perform assessments required to interpret the primary endpoint. Additional categories may be identified as deemed necessary by the Medical Monitor.

All protocol deviations will be reported by the Clinical Research Associate (CRAs) or other trial-involved personnel, such as data manager and statisticians. The protocol deviations will be reviewed by the Medical Monitor. The Medical Monitor determines whether a deviation is major or not. Major deviations are reported to the sponsor as part of the regular reporting. Important protocol deviations will be summarized in the clinical trial report. In accordance with applicable competent authority mandates, the investigator is responsible for reporting protocol deviations to the IEC.

In case of a deviation, the investigator enters a comment in the source documents and the non-compliance will be documented in a Monitoring Visit Report by the CRA. All non-compliance will be followed up and reported to the competent authorities and IEC as per local regulations. In parallel, corrective and/or preventive actions will be undertaken and documented, including any retraining of the investigator and site staff. No waivers for inclusion or exclusion criteria will be given.

The documentation must be kept in the Investigator’s File and the Trial Master File. Deviations should be reported to the IRB/EC per local requirements.

12.4. Discontinuation of the study by the sponsor

The sponsor reserves the right to discontinue the study for safety or administrative reasons at any time. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the sponsor or its representative.

12.5. Data handling

Data will be recorded in an FDA CFR Part 11-compliant eCRF.

All data in the eCRF must reflect the corresponding source data. No data are to be recorded directly on the CRFs (i.e. the CRF is not to be considered as source data).

Data reported on the CRF that are derived from source documents should be consistent with the source documents, or the discrepancies must be explained.

The investigator should agree to have completed source documents and eCRFs available for inspection by the clinical monitor at the time of each scheduled monitoring visit. The investigator must sign the completed eCRF.

12.6. Confidentiality

The contents of this protocol and any amendments and results obtained during the study should be kept confidential and not disclosed, in whole or in part to others, or used for any purpose other than reviewing or performing the study, without written consent of the sponsor.

The investigator agrees to conduct the study according to GCP and comply with all applicable federal, state, and local laws and regulations relating to the privacy of patient health information, including, but not limited to, the EU Clinical Trials Directive or the Code of Federal Regulations. The investigator shall ensure that study patients authorize the use and disclosure of protected health information in accordance with the EU Clinical Trials Directive, and EU Law or the Code of Federal Regulations and in a form satisfactory to the sponsor to allow for review and monitoring of the conduct of the study and to verify the accuracy of data by the sponsor, its representatives, IRBs/ECs, and regulatory authorities.

The Investigator will ensure that all persons assisting in the performance of the study preserve the confidentiality of the patients' data as set forth in the Patient Informed Consent Form and Clinical Trial Agreement. On CRFs or any other documents and biological samples submitted to the Sponsor, the patients will not be identified by their names. Each patient will be assigned an identification number to be used on any data or laboratory samples collected by the Sponsor. Documents not for submission to the Sponsor, e.g. the signed informed consent forms and patient medical records, will be maintained by the Investigator and made available for review and inspection as described above for as long as is required by local regulations.

12.6.1. Data protection

All parties will ensure protection of patient personal data and will not include patient names on any sponsor forms, reports, publications, or in any other disclosures. In case of data transfer, Sponsor will maintain high standards of confidentiality and protection of patient personal data.

The sponsor will ensure the confidentiality of patient's medical information in accordance with all applicable laws and regulations.

The sponsor as data controller according to the European General Data Protection Regulation (regulation EU 2016/697)[1] confirms herewith compliance to regulation EU 2016/697 in all stages of data management.

Data generated within the scope of this study must be available for inspection upon request by representatives of national and local Health Authorities, the sponsor monitors, representatives, and collaborators, and the IEC/IRB for each study site, as appropriate.

12.7. Study monitoring, auditing and inspections

This study will be monitored by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to the protocol and in order to comply with guidelines of GCP. On-site review of eCRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each patient. Note that a variety of original documents, data, and records will be considered as source documents in this study. The eCRF itself is not to be used as a source document under any circumstances.

The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution.

The study may be audited by the sponsor, its designee or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required patient records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in CRF generation, where clinically appropriate.

12.8. Retention of records

The investigator must arrange for retention of study records at the site. The nature of the records and the duration of the retention period must meet the requirements of the relevant regulatory authority. In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority. The site should plan to retain study documents until

[1] *https://www.echodeventer.nl/ecd/_sitefiles/file/1805_Privacy%20and%20personal%20data_Information%20for%20patients.pdf*

directed by the Sponsor that they are no longer required. The investigator should take measures to prevent accidental or premature destruction of these documents.

12.9. Patient insurance

The Sponsor will obtain clinical trial insurance to cover patients participating in the study in accordance with all applicable laws and regulations. The terms of the insurance will be kept in the study files.

12.10. Disclosure of study findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international regulatory filings and registration. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement. A final integrated study report covering clinical and biometric aspects of the study will be prepared by the sponsor or its representative and results of the trial will be disclosed to regulatory authorities and posted on public registries, as required.

12.11. Study disclosure and publication

The study will be posted on www.clinicaltrials.gov and other public databases as required by local regulations.

The sponsor intends to publish the results of this multi-center study upon completion of the analysis and/or clinical study report (CSR). The Sponsor reserves the right to name as authors members of staff at the investigational site in the case that their contribution to the research is significant. Order of authorship will generally be assigned in relation to the relative contribution of each author. Disagreements concerning authorship will be resolved by the Sponsor. All authors will be required to review and agree upon the content of the draft publication prior to its submission to a peer-reviewed congress, journal or posting on the Sponsor's website.

The detailed obligations regarding the publication of any data, material results, or other information generated or created in relation to the study shall be set out in the Clinical Trial Agreement between each investigator and the sponsor/CRO, as appropriate.

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14. APPENDICES

APPENDIX A. CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1.Premenarchal

2.Premenopausal female with 1 of the following:

a) Documented hysterectomy.

b) Documented bilateral salpingectomy.

c) Documented bilateral oophorectomy.

Note: Documentation can come from the study centre personnel's: review of the subject's medical records, medical examination, or medical history interview.

3.Postmenopausal female:

a) A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

b) Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-oestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

Contraception Guidance

Female subjects

Female subjects of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in the table below.

Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of < 1% per year when used consistently and correctly.*
Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation [b] • Oral. • Intravaginal. • Transdermal.
Progestogen only hormonal contraception associated with inhibition of ovulation • Oral. • Injectable.
Highly Effective Methods That Are User Independent [a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD). • Intrauterine hormone-releasing system (IUS). • Bilateral tubal occlusion.
Vasectomised Partner *A vasectomised partner is a highly effective birth control method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual Abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*
NOTES: [a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies.

Pregnancy Testing:

• WOCBP should only be included after a confirmed menstrual period and a negative highly sensitive serum pregnancy test.

• Additional pregnancy testing should be performed at time points specified in the Schedule of Assessments during the treatment period and at the EOS visit and as required locally.

Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

• Pregnancy testing, with a sensitivity of 5, 10, or 25 mIU/mL will be performed and assayed in a certified laboratory.

Collection of Pregnancy Information

Female Subjects who become pregnant

• The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

• While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

• Any post-study pregnancy related SAE considered reasonably related to the study treatment by the investigator will be reported to the Sponsor. While the investigator is not obligated to actively seek this information in former subjects, he or she may learn of an SAE through spontaneous reporting.

• Any female subject who becomes pregnant while participating in the study will discontinue study treatment or be withdrawn from the study.

Continuation of study treatment may only be allowed if either of the following criteria is met:

The study treatment has an approved label that indicates it can be used safely in pregnant females.

OR

All of the following apply:

• The subject has a high mortality disease.

• The investigator determines the subject is benefitting from study participation and there is no other reasonable treatment for her.

• The Sponsor and the relevant IEC give written approval.

• The subject gives signed informed consent.

• The investigator agrees to monitor the outcome of the pregnancy and the status of the subject and her offspring.

• The protocol is amended to allow such participation on a case-by-case basis, if such participation is not already addressed in the protocol.

APPENDIX B. INVESTIGATOR PROTOCOL AGREEMENT PAGE

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by the Sponsor.
- Not to implement any changes to the protocol without written agreement from the Sponsor, and prior review and written approval from the Independent Ethics Committee (IEC) or Institutional Review Board (IRB) except where necessary to eliminate an immediate hazard to study patients.
- That I am thoroughly familiar with the appropriate use of the study drug, as described in this protocol and any other information provided by the Sponsor, including, but not limited to, the current Investigator's Brochure (IB).
- That I am aware of, and will comply with, good clinical practices (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the Sponsor, study drug and of their study-related duties and functions as described in the protocol.

Signature: _____ Date: _____

Name (print): _____

Site Name: _____

APPENDIX C. CKD-EPI CREATININE EQUATION (2009)

Expressed as a single equation:

eGFR = 141 x min(SCr/κ, 1)α x max(SCr /κ, 1)-1.209 x 0.993 Age

x 1.018 [if female] x 1.159 [if Black]

Abbreviations / Units

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m2

SCr (standardized serum creatinine) = mg/dL

κ = 0.7 (females) or 0.9 (males)

α = -0.329 (females) or -0.411 (males)

min = indicates the minimum of SCr/κ or 1

max = indicates the maximum of SCr/κ or 1

age = years

Assays

Creatinine is assayed using methods that are traceable to IDMS assigned NIST certified reference materials. To learn more, go to nkdep.nih.gov.

Clinical Use

Recommended method for estimating GFR in adults.

Designed for use with laboratory creatinine values that are standardized to IDMS.

Estimates GFR from serum creatinine, age, sex, and race.

More accurate than the MDRD Study equation, particularly in people with higher levels of GFR.

Based on the same four variables as the MDRD Study equation, but uses a 2-slope “spline” to model the relationship between estimated GFR and serum creatinine, and a different relationship for age, sex and race.

Some clinical laboratories are still reporting GFR estimates using the MDRD Study equation. The National Kidney Foundation has recommended that clinical laboratories should begin using the CKD-EPI equation to report estimated GFR in adults.

Developed in 2009 by the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI 2009].

APPENDIX D. RESPONSE EVALUATION CRITERIA IN SOLID TUMORS RESPONSE CRITERIA

Response and progression will be evaluated in this study using the international criteria proposed by the RECIST committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST 1.1 criteria.

Measurable disease

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness recommended to be ≤5 mm) and MRI (no less than double the slice thickness and at least 10 mm)
- 10 mm caliper measurement by clinical examination (when superficial)
- 20 mm by chest X-ray (if clearly defined and surrounded by aerated lung)

Lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be ≤5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components that can be evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component meets the definition of measurability. Blastic bone lesions are non-measurable.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Tumor lesions situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion.

Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with □10 to <15 mm short axis) are considered non-measurable disease. Leptomeningeal disease, ascites, pleural or pericardial effusion, lymphangitic involvement of skin or lung, inflammatory breast disease, abdominal masses/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques and blastic bone lesions are all non-measurable.

Target lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total should be identified as target lesions and be recorded and measured at baseline. These 5 lesions should be selected because of their size (lesions with the longest diameter), represent all involved organs and should be suitable for reproducible repeated measurements. 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 of diameters. The baseline sum of diameters will be used as reference to

characterize further any objective tumor regression of the measurable dimension of the disease. If there are >5 measurable lesions, those not selected as target lesions will be considered together with non-measurable disease as non-target lesions.

Non-target lesions

All non-measurable lesions (or sites of disease) plus any measurable lesions over and above the 5 listed as target lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as “present”, “absent”, or in rare cases “unequivocal progression”.

It is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”).

Best response

All patients will have their BEST RESPONSE on study classified as outlined below:

Complete response

Disappearance of all clinical and radiological evidence of tumor (both target and non-target). Any pathological lymph nodes (whether target or non-target) must have a reduction in short axis to <10 mm.

Partial response

At least a 30% decrease in the sum of diameters of target lesions taking as reference the baseline sum, no unequivocal progression of existing non-target lesions and no appearance of new lesions.

Stable disease

Steady state of disease. Neither enough shrinkage to qualify for partial response nor enough increase to qualify for progressive disease, no unequivocal progression of existing non-target lesions, and no appearance of new lesions.

Progressive disease

At least a 20% increase in the sum of 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. Unequivocal progression of existing non-target lesions or the appearance of 1 or more new lesions will also constitute progressive disease.

Table 13 and Table 14 summarize the assessment of best response according to the RECIST 1.1 criteria.

Table 13 Assessment of best response according to the RECIST 1.1 criteria for patients with target and non-target lesions

Target lesions	Non-target lesions	New lesions	Overall response	Best response for this category also requires
Complete response	Complete response	No	Complete response	
Complete response	Non-complete response / Non-progressive disease	No	Partial response	
Complete response	Not evaluated	No	Partial response	
Partial response	Non-progressive disease or not all evaluated	No	Partial response	
Stable disease	Non-progressive disease or not all evaluated	No	Stable disease	Documented at least once at 6 weeks from baseline
Not all evaluated	Non-progressive disease	No	Not evaluable	
Progressive disease	Any	Yes or No	Progressive disease	
Any	Progressive disease	Yes or No	Progressive disease	
Any	Any	Yes	Progressive disease	

Subjects 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.

Table 14 Assessment of best response according to the RECIST 1.1 criteria for patients with non-target lesions only

Non-target lesions	New lesions	Overall response
Complete response	No	Complete response
Non-complete response / non-progressive disease	No	Non-complete response / non-progressive disease [a]
Not evaluated	No	Not evaluable
Unequivocal progressive disease	Yes or No	Progressive disease
Any	Yes	Progressive disease

a "Non-complete response / non-progressive disease" is preferred over "stable disease" for non-target lesions.

Methods of measurement

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline (if available, previous CT / MRI scans may be assessed additionally) and during follow-up.

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

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

CT / MRI - CT is the best currently available and reproducible methods to measure target lesions selected for response assessment. CT scans should be performed with cuts of 5 mm or less in slice thickness. When 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. This applies to the chest, abdomen and pelvis. Head & neck and extremities usually require specific protocols.

Ultrasound - Ultrasound is not useful in assessment of lesion size and should not be used as method of measurement. If new lesions are identified by ultrasound during the study, confirmation by CT or MRI is advised.

Endoscopy / laparoscopy - The use of these techniques for objective tumor evaluation is not advised.

Cytology / histology - These techniques can be used to differentiate between partial response and complete response 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.

APPENDIX E. DESCRIPTION OF THE iRECIST PROCESS FOR ASSESSMENT OF DISEASE PROGRESSION

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic disease progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

For patients who show evidence of radiological PD by RECIST 1.1 as determined by the investigator, the investigator will decide whether to continue a patient on study treatment until repeat imaging is obtained (using iRECIST for patient management; see Table 11). This decision by the investigator should be based on the patient's overall clinical condition.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia or other palliative care. In case of need for palliative radiotherapy, possible treatment continuation should be discussed with Sponsor.

Any patient deemed **clinically unstable** should be discontinued from study treatment at site-assessed first radiologic evidence of PD and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the investigator decides to continue treatment, the patient may continue to receive study treatment and the tumor assessment should be repeated 6 weeks later to confirm PD by iRECIST, per investigator assessment.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥ 20% and ≥ 5 mm from nadir
    - Note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment, the first visit

showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as new lesions – Target. The sum of diameters of these lesions will be calculated and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as new lesions – nontarget.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the patient will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
  - For target lesions, worsening is a further increase in the sum of diameters of ≥5 mm, compared to any prior iUPD time point
  - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
  - For new lesions, worsening is any of these:
    - An increase in the new lesion sum of diameters by ≥5 mm from a prior iUPD time point
    - Visible growth of new non-target lesions
    - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the imaging on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation imaging proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the investigator, and the patient continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, patients will be discontinued from study treatment.

NOTE: If a patient has confirmed radiographic progression (iCPD) as defined above, but the patient is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered following consultation with the Sponsor. In this case, if study treatment is continued, tumor imaging should continue to be performed following the scheduled intervals.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (ie, achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
    - Sum of diameters reaches the PD threshold (≥20% and ≥5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire study, either before or after an instance of pseudo-progression.
- Non-target lesions

  - If non-target lesions have never shown unequivocal progression, their doing so for the first-time results in iUPD.
  - If non-target lesions have shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.

- New lesions
  - New lesions appear for the first time
  - Additional new lesions appear
  - Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
  - Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, with 1 exception: if new lesions occurred at a prior instance of iUPD, and at the confirmatory imaging the burden of new lesions has increased from its smallest value (for new target lesions, the sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication.

APPENDIX F. DATA QUALITY ASSURANCE

All subject data relating to the study will be recorded on printed or eCRFs unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The investigator must permit study-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source data documents.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

APPENDIX G. RECOMMENDATIONS FOR CANCER PATIENT MANAGEMENT IN THE PROTOCOL BOT112-03 DURING SARS-COV-2 PANDEMIC

1-Introduction

Coronaviruses are a group of highly diverse RNA viruses in the Coronaviridae family that are divided in 4 genera: alpha, beta, gamma and delta that cause disease varying from mild to severe in human and animals. However, two zoonotic coronaviruses have emerged causing severe disease in humans: Severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) (1). In January 2020, the etiologic agent responsible for a cluster of severe pneumonia cases in Wuhan, China, was identified as being a novel betacoronavirus, distinct from SARS-CoV and MERS-CoV. On 11 February 2020, the International Committee on Taxonomy of Viruses (ICTV) announced that the virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2) while, on the same day, WHO named the disease as coronavirus disease COVID-19 (3).

The evolution of COVID-19 infection is different to other viruses of the coronavirus family, with a case fatality rate of 1.5-12.3% based on the reporting country, and a worst evolution in older patients. At this moment, a pandemic situation is ongoing and knowledge and recommendations on the management of this illness and its relationship with cancer are in progress. However, there are certain recommendations that it is advisable to follow whenever the resources of the institutions allow, including recommendations regarding the precautions to be taken at enrollment to avoid including in this trial patients infected by COVID-19 and during the treatment period, to reduce risks of infection and describe how to manage the patients in case on infection. These recommendations should be used as guidance for prioritizing the various aspects of cancer care in order to mitigate the negative effects of the COVID-19 pandemic on the management of cancer patients in this trial. The situation is evolving, and pragmatic actions may be required to deal with the challenges of treating patients, while ensuring their rights, safety and wellbeing.

1-1 Clinical manifestations

The percentage of infected people by Covid-19 who are asymptomatic or oligosymptomatic in the general population is currently unknown. These uncertainties are also referring to the prevalence of asymptomatic disease in cancer patients. So far, no systematic reports are available regarding a higher incidence of COVID-19 asymptomatic infections in patients with cancer. Recent limited data from China, and more recently from Italy and the US, do however seem to confirm a higher risk.

When clinical manifestations are present, the most frequent symptoms include fever and persistent cough, with some subjects having dyspnea, sore throat, headache and diarrhea. Another possible symptoms related to COVID-19 infection are muscle pain, tiredness, anosmia and dysgeusia.

In contrast to severe acute respiratory insufficiency related to other coronavirus, more deaths from COVID-19 have been caused by multiple organ dysfunction syndrome rather than respiratory failure, which might be attributable to the widespread distribution of angiotensin converting enzyme 2—the functional receptor for COVID-19—in multiple organs (4).

Males may be affected more severely than females, however most patients (80%) have relatively mild, influenza-like symptoms and achieve full recovery within 10 days. Available data indicate that older people are more vulnerable, with underlying health conditions such as chronic respiratory, cardio-vascular or chronic kidney disease, diabetes, active cancer and more generally severe chronic diseases (5-7).

Cancer patients seem to be at higher risk of infection with COVID-19 because of the immune system impairment related to malignancy and anticancer treatments. More concerning is the increased risk of severe respiratory complications requiring time in the intensive care unit in patients with cancer, as compared with patients without cancer (39% vs 8%, respectively; p=0.0003), as described in a recently published nationwide analysis conducted by the National Clinical Research Center for Respiratory Disease in China (4), which included a prospective cohort to monitor COVID-19 cases throughout the country. As of the data cutoff on Jan 31, 2020, a total of 2007 cases had collected and analyzed from 575 hospitals. From them, 18 cancer patients

were identified in this cohort. Compared with patients without cancer, patients with cancer were older (63.1 years vs 48.7 years), more likely to have a history of smoking, had more polypnea and more severe baseline CT manifestation, but had no significant differences in sex, other baseline symptoms, other comorbidities, or baseline severity of x-ray. Patients with cancer were observed to have a higher risk of severe events (percentage of patients being admitted to the intensive care unit requiring invasive ventilation, or death) compared with patients without cancer (39% cancer patient vs 8%non-cancer patients; p=0·0003). Cancer history represented the highest risk for severe events in this study. Additionally, patients with cancer deteriorated more rapidly than those without cancer (median time to severe events 13 days vs 43 days; p<0·0001; hazard ratio 3.56, 95% CI 1.65–7.69).

In this context, the possible impact that immunomodulatory treatments could have in the risk of infection by COVID-19 and the intensity of the clinical manifestations is not known. Therefore, during this pandemic, the benefit/risk ratio of cancer treatment need to be reconsidered.

1-2 Diagnosis

1-2-1 Molecular methods

Routine confirmation of COVID-19 cases is based on detection of its nucleic acid (RNA) by real time RT-PCR assays.

RT-PCR on respiratory samples is the “gold standard” technique for COVID-19 diagnosis. Recommended samples are those from the lower respiratory tract, including sputum, bronchoalveolar lavage and tracheal aspirate (when possible according to medical criteria). However, when collection of a lower respiratory tract sample is not possible, samples from the upper respiratory tract are also useful. In general, the collection of a combined nasopharyngeal swab and oropharyngeal swab is recommended (8)

This technique has a high specificity; thus, a positive result confirms the detection of the virus. On the contrary, a negative result might not always mean the absence of COVID-19 virus infection. Several reasons might explain a negative result in a person infected with COVID-19 virus, mainly:

- Poor sample quality, handling, transportation and/or storage.
- Poor/failed sample extraction, presence of PCR inhibitors in the extracted RNA.
- The sample was collected at a time where the patient was not shedding sufficient amounts of virus, for instance very early or very late during infection (this point is particularly relevant as the dynamics of the viral presence in different sample types has not been fully established).
- As with any molecular detection assay, virus mutations in the regions that are targeted by the assays might affect the sensitivity of the detection.

Therefore, in case of having a negative RT-PCR result in a patient who has been in contact with a confirmed case or who has suggestive clinical manifestations, the COVID-19 testing should be repeated.

The limitations of this method are that while it confirms the presence of viral RNA, it does not detect prior infection or immunity to future infection. However, it is currently assumed that PCR positive asymptomatic persons have the risk of infecting (it is believed that between 30-50% of infections occur through asymptomatic individuals) and/or develop symptoms later.

1-2-2 Serological methods

Several assays (both ELISA and rapid diagnostic tests) are available for the detection of IgM/IgG antibodies and are marketed for the detection of COVID-19 virus infections.

These tests may be limited due to cross-reactivity with other coronaviruses that are normally present in the community and that make the interpretation of results difficult (9).

Antigen detection

During the first days after symptom onset (approximately 1 to 5), viral proteins are generated and can be detected by different tests (eg, ELISA, immunofluorescence).

Rising IgM levels can be detected from approx. day 3-7 since the infection by COVID-19, with a maximum of 14 days, and frequently coincides with the presence of first clinical manifestations. The maximum levels being detected 8-14 days since the clinical manifestations start date. Secretion of IgG antibodies occurs between days 5-7 after the onset of symptoms. On average, the maximum IgG value is reached between days 10-14.

The detection of an immune response to COVID-19 by serology evaluation allows an understanding of who has been exposed (and been infected) and the degree to which these individuals may have some immunity to future infections. Comparing to RT-PCR, this is a cheaper and more simple technique.

In general, this type of assays has acceptable specificity. In confirmed and probable cases, the efficiency of detection by IgM is greater than that of PCR after 5.5 days of symptom onset. Nevertheless, a negative result (at any stage of infection) should not be used as a criterion to rule out a case, and therefore other criteria must be taken into account. Positive detection rate increases significantly (98.6%) when combining IgM with RT- PCR for each patient compared to a single test (10).

Rapid serologic tests

In general, these types of tests have low sensitivity. Therefore, their positive predictive value is good (they can be used to rule in cases), but their negative predictive value is low (they should not be used to rule out cases).

2- Recommendations to be considered in regards of patients´ eligibility and management during the trial.

2-1 COVID-19 infection diagnosis

Based on ESMO´s guidelines for management of cancer patients during COVID-19 pandemic (11) the following diagnostic procedures should be considered to confirm the diagnosis of COVID-19 infection in this trial:

- RT-PCR testing should be proposed to all potential patients for this trial within the close previous days before submitting the eligibility package. If feasible for each concrete site, and ideally, it should be repeated before each treatment/cycle.

In addition, this test should also be proposed to patients during the treatment period in the trial if they present with symptoms clearly suggestive of COVID-19 infection. In this sense, all patients should be actively asked about the presence of clinical manifestations related to COVID-19 infection by the investigator in ALL visits during the pandemic.

- Serology (ELISA), if available, should be considered to be proposed to identify previous COVID-19 infection in all cancer patients in the trial at screening and, during treatment period, in all those patients with clinical manifestations suggestive of COVID-19 infection or close positive cases. Performing a serology testing could provide us with useful information to differentiate patients with high IgG and little risk of developing the disease, and those patients with high IgM who haves not yet started to develop symptoms and ideally must be studied before the inclusion.

- Ideally, patients should be tested using both techniques, especially those patients with negative IgG and IgM, or with high IgM, although this will be based on the countries and sites´ resources. If only one technique can be done, PCR should be recommended.

2-2 Management of patients with a suspected/confirmed COVID-19 infection.

- Screening period: For patients who are exhibiting symptoms consistent with COVID-19 or have tested positive, enrollment and protocol treatment should not be initiated. After the infection resolution and test negativity, the possibility of enrolment must be discussed case by case with the sponsor Medical Monitor.

- Treatment period: For patients who have a confirmed COVID-19 infection, the decision about having the treatment on hold or not must be discussed case by case with the sponsor Medial Monitor.

2-3 Patients´ visits to the sites

During the COVID-19 pandemic, the duration of the time that the patients spend on the hospital should be reduced as much as possible in order to decrease the probability of infection. Patients should be asked to take all precautionary measures (wearing a face mask, gloves, keeping social distancing…) In addition to that, the investigator should specifically ask the patients about the presence of clinical manifestations suggestive of COVID-19 infection. In this sense, please consider the following recommendations for patients in the trial having fever and/or respiratory symptoms:

- These patients shouldn´t be evaluated in oncology day centers.

- Initial evaluation outside of the area with high concentration of cancer patients or oncology staff.

- Possibility of coronavirus must be considered and evaluated in cancer patients with fever.

- Consider limiting points of entry to the facility.

- Take steps to ensure that all the patients with symptoms of respiratory infection adhere to respiratory hygiene and cough etiquette, hand hygiene, and triage procedures.

o Post visual alerts icon (e.g., signs, posters) at the entrance and in strategic places (e.g., waiting areas, elevators, cafeterias) to provide patients with instructions about hand hygiene and respiratory hygiene.

o Provide supplies for respiratory hygiene and cough etiquette, including alcohol-based hand rub (ABHR) with 60-95% alcohol, tissues, and no-touch receptacles for disposal, at healthcare facility entrances, waiting rooms, and patient check-ins.

o Install physical barriers at the reception areas to limit close contact between triage personnel and potentially infectious patients.

- Ensure rapid safe triage and isolation of patients with symptoms of suspected COVID-19 or other respiratory infection.

o Prioritize assistance of patients with respiratory symptoms.

o Triage personnel should have a supply of facemasks and tissues for patients with symptoms of respiratory infection. These should be provided to patients with symptoms of respiratory infection at check-in.

o Isolate the patient in an examination room with the door closed. If an examination room is not readily available ensure the patient is not allowed to wait with other patients seeking care.

o Identify a separate, well-ventilated space that allows waiting patients to be separated by 2 meters, with easy access to respiratory hygiene supplies.

2-4- Specific treatment for COVID-19

Currently there is no conventional treatment for COVID-19 in addition to supportive treatment. It is therefore not possible to provide any specific recommendations since most of the treatments currently used are based on laboratory data: chloroquine, hydroxychlorocin, anti IL6 antibodies, etc.

In any case, patients should be treated according to the general criteria of the care centers with the resources and means they currently have. Age, comorbidity and life expectancy should be considered.

2-5 Protocol procedures

According to EMA guidance on the management of clinical trials during the COVID-19 pandemic (12), prospective protocol waivers remain unacceptable and all efforts should be done to follow the trial protocol. Nevertheless, patients´ safety and welfare should always prevail. In case of protocol deviations, the classification defined in the Protocol Deviation Plan for this trial will be followed.

In this trial there are some critical laboratory tests, imaging and other diagnostic tests to be performed, (e.g. blood cell count, liver function test, CT, MRI, ECG etc.), e.g. for trial patient´s safety and the integrity of the trial. In case the patient in this trial cannot reach the site to have these performed because of the COVID-19 pandemic, it could be acceptable that laboratory, imaging or ECG tests be done at a local laboratory or relevant clinical facility authorized/certified (as legally required nationally) to perform such tests in these exceptional situations, if this can be done within local restrictions on social distancing. The sites should inform the sponsor about such cases as soon as they be aware of these situations and ask for approval. In these cases, it is important that the sponsor is given access to the normal ranges and certification information of any additional laboratory used in order to support the use and evaluation of results.

2-6 Safety reporting

Sponsors are expected to continue safety reporting in adherence to EU and national legal frameworks. When per protocol physical visits are reduced or postponed, it is important that the investigators continue collecting adverse events from the trial participant through alternative means, e.g phone calls.

At this moment, it is not clear that antineoplastic (mostly immunomodulators) treatments have a special influence on COVID-19 infection over other viruses or vice versa, so for relation assignment it does not seem logical to use criteria other than those previously used.

2-7 Data verification

Whenever possible, data verification will be done by physically visiting the site. Nevertheless, in case of mobility limitations due to the COVID-19 pandemic, remote monitoring visits and data verification will be allowed.

2-8 Audits

During this pandemic, audits will only be conducted if permitted under national, local and/or organizational social distancing restrictions stablished to control COVID-19 pandemic.

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