Protocol Title:

Phase IIa open-label clinical study of intratumoural administration of BO-112 in combination with pembrolizumab in subjects with liver metastasis from colorectal cancer or gastric/gastro-oesophageal junction cancer

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Version:	Version 2.2
Product:	BO-112
Short Title:	Study of BO-112 with pembrolizumab for colorectal or gastric/GEJ cancer with liver metastasis
Study Phase:	IIa
Sponsor Name:	Highlight Therapeutics, S.L.
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I have read this protocol in its entirety and agree to conduct the study accordingly:

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1.0 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: Phase IIa open-label clinical study of intratumoural administration of BO-112 in combination with pembrolizumab in subjects with liver metastasis from colorectal cancer or gastric/gastro-oesophageal junction cancer.

Short Title: Study of BO-112 with pembrolizumab for colorectal or gastric/GEJ cancer with liver metastasis.

Rationale:

Checkpoint inhibition provides significant clinical benefit in various types of cancer, with blockade of the programmed cell death protein 1(PD1) receptor and its ligands being the most widely used approach. Some tumours, however, evade the immune system and are not, or only poorly, responsive to checkpoint inhibition. The innate immune system interacts with the adaptive immune system and responds to different triggers. The purpose of this study is to evaluate if the combination of activation of the innate immune system and blockade of the PD1 immune checkpoint can provide clinical benefit in patients with tumours that are poorly, or only moderately responsive to monotherapy with an anti-PD1 agent. Activation of the innate immune system will be via direct intratumoural (IT) administration of BO-112. Checkpoint inhibition will be via intravenous infusion of pembrolizumab. Metastatic liver lesions have been selected as the site for intratumoural injection for 2 reasons: to reduce the variability of the microenvironment of the injection site, and to evaluate if the TLR3 agonist activity of BO-112 potentially breaks through the generally immune-tolerant hepatic microenvironment.

Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• Investigation of the anti-tumour efficacy and safety of repeated IT administrations of BO-112 in metastatic liver lesions in combination with IV pembrolizumab	• Efficacy: ORR based on the BOR using RECIST 1.1 • Safety: number and proportion of subjects with study treatment-related TEAEs with severity ≥ Grade 3 (NCI-CTCAE v 5.0)
Secondary	
• Further characterisation of safety and of clinical activity of the combination as well as determination of systemic exposure of BO-112	• Safety: number and proportion of subjects with TEAEs (any grade) • Safety: number and proportion of subjects with related TEAEs (any grade) • Tolerability: number of study discontinuations due to related TEAE • Efficacy: disease control rate (DCR = CR, PR and SD of at least 12 weeks duration) using RECIST 1.1 • Efficacy: ORR based on best overall response using RECIST modified for immune-based therapies (iRECIST) • Efficacy: DCR = iCR, iPR + iSD of at least 12 weeks duration) using iRECIST • Efficacy: duration of response • Efficacy: progression-free survival • Efficacy: overall survival rate at 6 months • Pharmacokinetics: systemic exposure after first BO-112 administration
Exploratory	
• Evaluation of antitumoural and immunological effects in the TME of the injected lesion.	• Immune cell profile of TME in injected and noninjected lesions • Genetic analysis of the TME

Abbreviations: BOR=best overall response; CR= complete response; DCR=disease control rate; iRECIST= RECIST modified for immune-based therapies; IT=intratumoural; NCI-CTCAE=National Cancer Institute-Common terminology criteria for adverse events; ORR=objective response rate; PR= partial response; RECIST= Response evaluation criteria in solid tumours; SD=stable disease; TEAE=treatment-emergent adverse event; TME=tumour microenvironment.

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

Overall Design:

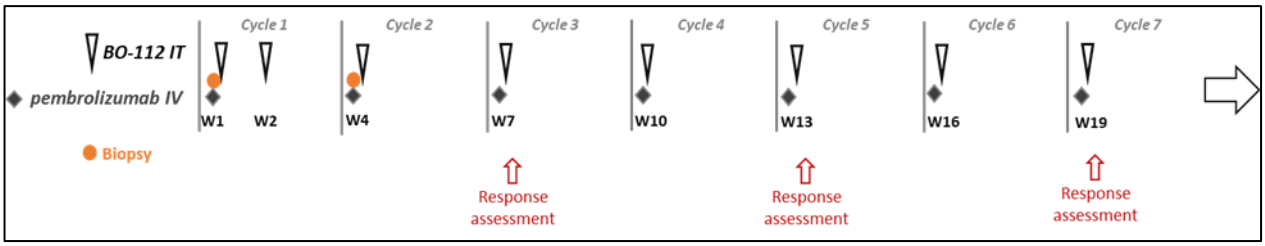
This is an open-label, Phase II, non-comparative, 2-cohort study with a Simon's 2-stage design.

Cohort A: colorectal cancer (CRC)

Cohort B: gastric or gastro-oesophageal junction cancer (GC/GEJ)

Subjects will be enroled in each cohort for Stage 1 of the study up to the predefined sample size and treated according to the treatment schedule. Enrolment will be suspended per cohort and a Data Monitoring Committee (DMC) will review the safety and efficacy data available at the time that the last subject enroled has reached the first on-study response assessment at Week 7 (= Cycle 3). The DMC will make a recommendation to the Sponsor for each cohort regarding expanding to the total foreseen sample size.

Treatment Scheme



Abbreviation: W=week; IT=intratumoural; IV=intravenous

Eligibility

Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria are met:

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. Have nonresectable liver metastasis(es) of colorectal or gastric/gastro-oesophageal junction cancer (GC/GEJ). History of resection for liver metastasis is allowed.
4. Have histological or cytological proof of colorectal (Cohort A) or GC/GEJ cancer (Cohort B).
5. Have progressed during or after, or have not tolerated therapy for advanced/metastatic disease as follows:
    a. Cohort A (CRC): at least 2 lines of fluoropyrimidine, irinotecan and/or oxaliplatin containing therapy with or without bevacizumab according to institutional practice; if epidermal growth factor receptor (EGFR) positive/RAS wild type, prior anti-EGFR treatment is required. In case of prior resection of hepatic metastasis with hepatic recurrence, only 1 prior line of fluoropyrimidine, irinotecan and/or oxaliplatin containing therapy is required.
    b. Cohort B (GC/GEJ): fluoropyrimidine and platinum containing treatment; if Human epidermal growth factor receptor 2 (HER-2) positive, also prior anti-HER-2 treatment is required.
6. Have at least 1 liver metastasis of minimum 20 mm in diameter that is suitable for percutaneous, IT injection (including, but not limited to, absence of tumour infiltration into the main portal vein, hepatic vein or vena cava). Recurrence in the liver of previously resected liver metastasis is allowed.
7. Presence of at least 1 measurable lesion according to RECIST v1.1. Note: this may be the liver metastasis selected for injection if it is the only measurable lesion present.
8. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

9. Adequate haematologic and end-organ function defined by the following laboratory results obtained within 2 weeks prior to the first dose of study treatment:
    a. ANC $\geq 1.5 \times 10^9$/L
    b. Platelet count $\geq 100 \times 10^9$/L
    c. Haemoglobin ≥ 9.0 g/dL
    d. AST and ALT $\leq 5 \times$ upper limit of normal (ULN)
    e. Serum total bilirubin $\leq 2 \times$ ULN (if known Gilbert's syndrome, serum bilirubin level $\leq 3 \times$ ULN)
    f. Prothrombin time (PT) (or international normalised ratio [INR]) within normal limits and activated partial prothrombin time (aPTT) within normal limits
    g. Serum albumin ≥ 2.5 g/dL
    h. Serum creatinine $\leq 1.5 \times$ ULN or creatinine clearance ≥ 30 mL/min (calculated per Cockroft-Gault formula)
10. Female subjects who are not pregnant or breastfeeding and at least 1 of the following conditions applies:
    a) Not a woman of childbearing potential (WOCBP)
    b) WOCBP who agrees to follow contraception guidance during the treatment period and for at least 120 days after the last dose of study treatment.
11. Able and willing to comply with study and follow-up procedures.

Exclusion Criteria

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

1. Prior treatment with an anti-PD1, anti-PDL1 or anti-PDL2 agent, an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX40, CD137) or any Toll-like receptor (TLR) agonist.
2. Liver metastasis(es) with macroscopic tumour infiltration into the main portal vein, hepatic vein or vena cava.
3. Contraindications to tumour biopsy and injections of the hepatic metastasis(es), such as coagulopathy, therapeutic dose anticoagulant treatment and treatment with long-acting agents such as clopidogrel which cannot be safely stopped.
4. 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 adverse events (AEs) due to previous therapies to $\leq$ Grade 1 or baseline. Participants with $\leq$ Grade 2 neuropathy may be eligible.
5. Palliative radiotherapy (≤ 2 weeks of 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.
6. Clinically active central nervous system (CNS) metastases and/or carcinomatosis meningitis. Subjects with previously treated brain metastases may participate provided they are radiologically stable, ie, 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.

7. History of a second malignancy, unless potentially curative treatment has been completed with no evidence of malignancy for 2 years.
8. Life expectancy of < 12 weeks.
9. Severe hypersensitivity ≥ Grade 3 to pembrolizumab and/or any of its excipients.
10. Active infection requiring systemic therapy.
11. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.
12. Active autoimmune disease that required systemic treatment in past 2 years (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
13. 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.
14. Known human immunodeficiency virus (HIV) infection.
15. Known history of hepatitis B (defined as HbsAg reactive) or known active hepatitis C (defined as HCV RNA [qualitative] detected) virus infection.
16. For WOCBP: pregnancy or a positive urine pregnancy test (eg within 72 hours) prior to treatment; or breastfeeding. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
17. Any other medical condition which would impact the safety of the subject or interfere with the subject's ability to comply with the study and follow-up procedures, in the opinion of the investigator.
18. Has received a live vaccine within 28 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines are live attenuated vaccines and are not allowed.
19. 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 (subjects 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).
20. Has had an allogenic tissue/solid organ transplant

Number of Investigators and Study Centers:

Approximately 15 Investigators and 15 study centers in 4 European countries are expected to participate in this study.

Number of Subjects:

Up to a total of 69 evaluable adult subjects who are naive to anti-PD1/PDL1 therapy will be enroled in the study.

For Cohort A: Up to 26 subjects who have CRC with nonresectable liver metastases suitable for IT injection and who have received at least 2 prior standard of care systemic anticancer therapies for advanced/metastatic disease will be enroled. Subjects who have had resection of hepatic metastases and have hepatic recurrence, need to have had only 1 or more prior standard of care systemic anticancer therapies in order to be eligible for this study. Initially, 11 subjects will be enroled and data reviewed by a DMC to recommend on extending the cohort with an additional 15 subjects. Note that for the efficacy analysis, the required number of subjects refers to those with MSS (pMMR) disease. This will be determined during the study and therefore subjects with MSI-H (dMMR) disease will be replaced. Given the low incidence of MSI in stage IV CRC, it is expected that this number will be low, ie, approximately 1 to 2 subjects to be replaced. Subjects with MSI disease will be included in the safety analysis set.

For Cohort B: Up to 43 subjects who have GC/GEJ cancer with nonresectable liver metastases suitable for IT injection and who have received at least 1 prior standard of care systemic anticancer therapy for advanced/metastatic disease will be eligible for this study. Initially, 18 subjects will be enroled and data reviewed by a DMC to recommend on extending the cohort with an additional 25 subjects. Note that for the efficacy analysis, the required number of subjects refers to those with MSS (pMMR) disease. This will be determined during the study and therefore subjects with MSI-H (dMMR) disease will be replaced. Given the low incidence of MSI in GC/GEJ, it is expected that this number will be low, ie, approximately 1 to 2 subjects to be replaced. Subjects with MSI disease will be included in the safety analysis set.

Treatment Groups and Duration:

Study treatment will consist of BO-112 IT injections in combination with intravenous (IV) pembrolizumab infusions and will be administered in 3-week cycles. For each cycle, BO-112 IT injections will be administered after the pembrolizumab infusion, either the same day or within a period of up to 36 hours after the pembrolizumab infusion (for organisational feasibility at the site).

The overall study duration is highly dependent on the duration of achieved clinical benefit, and thus expected to be in the range of 24 to 36 months.

Individual subject study duration will depend on maintenance of clinical benefit and tolerability and is expected to range from 2 to 12 months

Statistical Methods:

Approximately 80 subjects will be screened to achieve a total of 69 evaluable subjects.

The sample sizes of the cohorts were determined using Simon's 2-stage design to enable a first evaluation of the treatment's activity on tumour response. The designs are based on a 1-sided $\alpha = 0.05$; a power 80% and response probabilities based on RECIST 1.1 noted below.

- Cohort A (CRC): response probability based on RECIST 1.1 of $P_0 = 1\%$ to be tested against the alternative (desirable) response probability of $P_1 = 15\%$. For the first stage, 11 subjects* (N_1) will be enroled and if no responses are observed, the study may be stopped for futility. If 1 or more responses are observed, an additional 15 subjects* (N_2) will be enroled for a total of $N = 26$ subjects.

  * The baseline biopsy sample will be evaluated for proficient mismatch repair (pMMR, as proxy of MSS) vs. deficient mismatch repair (dMMR, as proxy of MSI-H) status in Cohort A and subjects who are dMMR will be replaced to ensure the appropriate number of MSS/pMMR subjects for the statistical analysis. Data from subjects with dMMR CRC will be evaluated as part of the safety

population for safety parameters, but separately and descriptively for efficacy endpoints.

- Cohort B (GC): response probability based on RECIST 1.1 of P_0 = 10% to be tested against the alternative (desirable) response probability P_1 = 25%. For the first stage, 18 subjects** (N_1) will be enroled and if 2 or less responses are observed, the study may be stopped for futility. If 3 or more responses are observed, an additional 25 subjects** (N_2) will be enroled for a total of N = 43 subjects.

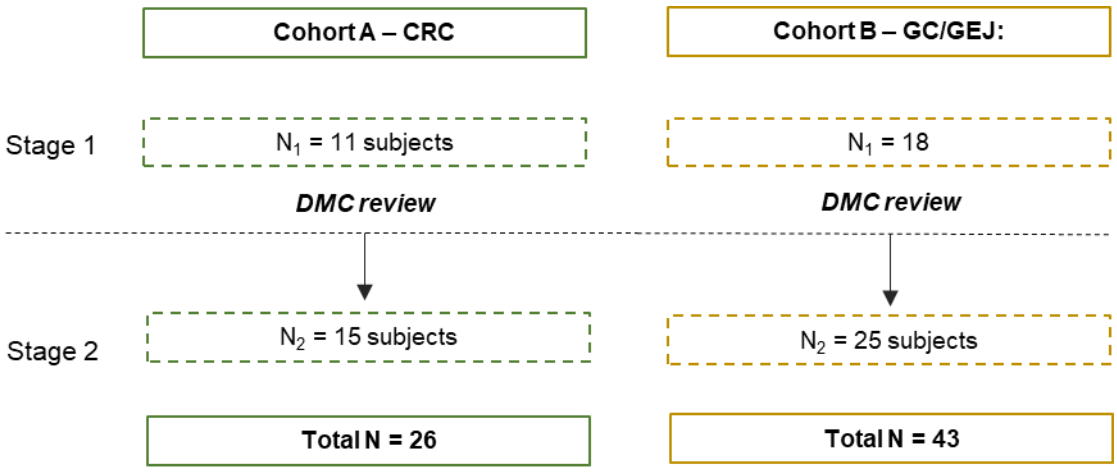
  ** The baseline biopsy sample will be evaluated for pMMR (as proxy of MSS) vs. dMMR (as proxy of MSI-H) status in Cohort B and subjects who are MSI-H/dMMR will be replaced to ensure the appropriate number of pMMR subjects for the statistical analysis. Data from subjects with dMMR GC/GEJ will be evaluated as part of the safety population for safety parameters, but separately and descriptively for efficacy endpoints.

Data Monitoring Committee:

When the prespecified number of subjects for Stage 1 have been enroled and have reached at least the first on-study response assessment, a DMC will review the safety and efficacy data per cohort. Enrolment will be suspended per cohort and the DMC will review the safety and efficacy data available at the time that the last subject enrolled has reached the first on-study response assessment at Week 7 (= Cycle 3). The DMC will make a recommendation to the Sponsor separately for each cohort regarding expanding to the total foreseen sample size.

1.2 Schema

Figure 1 Study Schema



Abbreviations: CRC=colorectal cancer; GC/GEJ= gastric or gastro-oesophageal junction cancer. Note: for Cohort A that number refers to microsatellite stable (MSS) subjects, and actual number may be somewhat higher due to replacement of microsatellite instable (MSI) subjects.

1.3 Schedule of Activities

Table 1 Schedule of Assessments

Treatment Cycle		1		2	3	4	5	6, 8 … 34	7, 9 … 35	EOS
Visit	Screening	V1a Baseline	V1b	V2	V3	V4	V5	V6, V8 … V34 (even numbered visits)	V7, V9 … V35 (odd numbered visits)	EOS
Day of visit within treatment cycle	Day -28 to Day -1	Day 1	Day 8 ± 2 days	Day 1 ± 2 days	Day 1 ± 2 days	Day 1 ± 2 days	Day 1 ± 2 days	Days 1 ± 2 days	Days 1 ± 2 days	28 days after last treatment ± 3 days
Study Week		W1	W2	W4	W7	W10	W13	W16, W22 … W100	W19, W25 … W103	
Administrative procedures										
Informed consent Form	X									
Inclusion/Exclusion criteria	X									
Demography	X									
Medical history	X									
Safety										
Physical examination[a]	X	X	X	X	X	X	X	X	X	X
Vital signs[a]	X	X	X	X	X	X	X	X	X	X
12-Lead ECG	X	X					X		X	
ECOG PS[a]	X	X	X	X	X	X	X	X	X	X
Adverse events[a]		X	X	X	X	X	X	X	X	X
Concomitant medication[a]	X	X	X	X	X	X	X	X	X	X
Coagulation[b]	X	X	X	X	X	X	X	X	X	

Treatment Cycle		**1**		**2**	**3**	**4**	**5**	**6, 8 … 34**	**7, 9 … 35**	**EOS**
Visit	**Screening**	**V1a Baseline**	**V1b**	**V2**	**V3**	**V4**	**V5**	**V6, V8 … V34 (even numbered visits)**	**V7, V9 … V35 (odd numbered visits)**	**EOS**
Day of visit within treatment cycle	**Day -28 to Day -1**	**Day 1**	**Day 8 ± 2 days**	**Day 1 ± 2 days**	**Day 1 ± 2 days**	**Day 1 ± 2 days**	**Day 1 ± 2 days**	**Days 1 ± 2 days**	**Days 1 ± 2 days**	**28 days after last treatment ± 3 days**
Study Week		**W1**	**W2**	**W4**	**W7**	**W10**	**W13**	**W16, W22 … W100**	**W19, W25 … W103**	
Haematology[b, c]	X	X	X	X	X	X	X	X	X	X
Chemistry, CRP[b, d]	X	X	X	X	X	X	X	X	X	X
Thyroid function tests[e]		X			X		X		X	X
Pregnancy (for WOBCP only)[f]	X				X		X		X	X
Efficacy										
Tumour Biopsy[g]		X		X						
Tumour burden and response assessment (per RECIST 1.1)[h,i]	X				X		X	V7 and V9 then V12, V15, V18, V21, V24, V27, V30, V33		
Pharmacokinetics										
PK blood sample[j]		X								
Study drug administration										
Pembrolizumab		X[k]		X	X	X	X	X	X	
BO-112[l]		X	X	X	X	X	X	X	X	

Abbreviations: AE=adverse events; CRP= C-reactive protein; CT= computed tomography; ECG=electrocardiogram; ECOG PS= Eastern Cooperative Oncology Group Performance Status; EOS=End of Study; PK=Pharmacokinetic; RECIST= response evaluation criteria in solid tumours; SAE=serious adverse events; V=visit; WOCBP=woman of childbearing potential

Notes:

a. Physical examination (full examination at baseline, and brief examination subsequently), ECOG PS, AE recording and concomitant medications recordings will be evaluated every 3 weeks (Q3W) prior to pembrolizumab treatment and prior to BO-112 injection at Week 2. Physical examination will include measurement of body weight. Also, vital signs will be collected on the same visits and temperature, pulse rate, and blood pressure, will be assessed.

b. Safety laboratory assessments: standard haematology, chemistry, CRP and coagulation parameters (prothrombin time [PT] and activated partial prothrombin time [aPTT]) will be measured prior to pembrolizumab treatment at baseline (V1a), then prior to pembrolizumab treatment at each cycle. Coagulation tests only need to be done if BO-112 is planned to be administered at that visit. If the safety screening labs are done within 5 days prior to V1a, then these labs do not need to be repeated for V1a, and the screening laboratory results for coagulation, haematology and chemistry results will be used for V1a (baseline). Note: safety labs (coagulation, haematology and chemistry) need to be done within 2 weeks of start of treatment.

c. Haematology assessment will include white blood cell count with differential, platelet counts, red blood cell (RBC) count, haemoglobin, haematocrit, RBC indices (ie, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), % reticulocytes).

d. Clinical chemistry test will include alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, chloride, magnesium, phosphate, bicarbonate, albumin, total protein, and glucose.

e. Thyroid function (triiodothyronine (T3) or free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone [TSH]) will be measured from blood samples at baseline and every 6 weeks.

f. Urine pregnancy test will be performed for all WOCBP. If a urine pregnancy test (within 72 hours prior to treatment) is positive or cannot be confirmed as negative, a serum pregnancy test will be required to confirm negative pregnancy.

g. Biopsy will be done at baseline (Week 1) prior to first administration of BO-112, biopsy of the to be injected (injected) lesion and optionally, biopsy of a nonhepatic, not to be injected (noninjected) lesion. A post-treatment biopsy of the injected lesion and if applicable noninjected lesion will be done prior to BO-112 administration at Visit 2 (Week 4). The biopsy should be done at the same intervention as the BO-112 administration. Biopsy samples will also be used for biomarker analysis.

h. The tumour burden assessment as per Response evaluation criteria in solid tumours version 1.1 (RECIST 1.1) using CT scan (or MRI if CT scan is contraindicated) will be done at screening (within 4 weeks preceding start of study treatment) and on-study every 6 weeks (ie, on V3 W7, V5 W13, V7 W19, and V9 W25 within a window of ± 3 days) and every 9 weeks after V9 W25 within a window of ± 7 days.

i. In case of immune unconfirmed progressive disease (iUPD), a confirmatory CT scan should be done between 4 to 6 weeks after the iUPD scan depending on the clinical status of the subject 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).

j. Timing of PK samples as follows: “Baseline”: Visit 1a (Cycle 1- Day 1), PK sample taken prior to pembrolizumab administration (which is by definition prior to BO-112). This may coincide with the sampling for baseline safety labs. “Post-dose”: Cycle 1, PK sample to be taken 30 to 60 minutes after the first BO-112 administration on Visit 1a (Cycle 1 – Day 1).

k. Treatment should start within 3 business days of enrolment.

l. The BO-112 IT injections is to be administered after pembrolizumab treatment, within a maximum of 36 hours afterwards (ie, same day or following day of pembrolizumab administration).

2.0 INTRODUCTION

Immunotherapy for treating cancer has become an important treatment option for patients with advanced, metastatic disease. Treatment benefit was initially established for immune checkpoint inhibition of CTLA-4 in melanoma and subsequently more widely for inhibition of the programmed cell death protein 1(PD1)/ programmed cell death ligand [PDL]-1 interaction. For the latter, this has been established through improved response rates, durable responses and survival benefit in a number of cancer types, notably melanoma, non-small cell lung cancer (NSCLC), urothelial cancer, squamous cell carcinoma of the head and neck (SCCHN) and renal cell carcinoma to name the most common ones. Other cancer types such as colorectal cancer (CRC) with microsatellite instability (MSI) or deficient mismatch repair (dMMR) and gastric or gastro-oesophageal junction (GC/GEJ) cancer have also shown treatment benefit with anti-PD1 treatment. However, not all patients respond, treatment resistance occurs and some tumour types appear inherently refractory to immunotherapy.

2.1 Study Rationale

Checkpoint inhibition provides significant clinical benefit in various types of cancer, with blockade of the PD1 receptor and its ligands being the most widely used approach. Some tumours, however, evade the immune system and are not, or only poorly responsive to checkpoint inhibition. The innate immune system interacts with the adaptive immune system and responds to different triggers. The purpose of this study is to evaluate if the combination of activation of the innate immune system and blockade of the PD1 immune checkpoint can provide clinical benefit in patients with tumours that are poorly or only moderately responsive to monotherapy with an anti-PD1 agent. Activation of the innate immune system will be via direct intratumoural (IT) administration of BO-112. Checkpoint inhibition will be via intravenous infusion of pembrolizumab. Metastatic liver lesions have been selected as the site for IT injection for 2 reasons: to reduce the variability of the microenvironment of the injection site, and to evaluate if the TLR3 agonist activity of BO-112 potentially breaks through the generally immune-tolerant hepatic microenvironment.

Indication Selection

The response rates observed for anti-PD1 therapy in CRC are strikingly different for tumours characterised by MSI or dMMR compared with microsatellite stability (MSS) or proficient mismatch repair (pMMR). Data from Phase II studies of pembrolizumab and nivolumab in CRC demonstrated an objective response rate (ORR) based on Response evaluation criteria in solid tumours (RECIST) 1.1[1] of approximately 32% in case of MSI CRC [2,3] but essentially non-responsiveness with an ORR of 0% for MSS CRC [4,5]. Unfortunately, in advanced/metastatic CRC, the proportion of patients with MSI tumours is low and estimated at < 5% [6]. Differences in the tumour microenvironment (TME) of MSI CRC compared with MSS CRC tumours were

demonstrated in an analysis of surgically resected primary colorectal cancer from 25 patients naive to chemotherapy. Although higher levels of tumour-infiltrating lymphocytes and higher expression of Th1/Tc1 gene groups (*TBX21* and *IFNG*) and CTL group (*CD8A, GZMB, PRF1* and *IL21*) were found in MSI tumours, they are not absent in MSS/pMMR tumours. These differences may be predictive of response to anti-PD1 treatment [7]. They may also indicate a potential to improve treatment outcomes for the MSS tumours, if the relevant changes in gene expression can be induced. The target population for efficacy endpoints are subjects with pMMR CRC. This is not an eligibility criterium, since MSI/MMR testing is not routine in Europe and requiring this as part of the screening procedure would delay the start of treatment. Subjects will have on-study biopsies and the MMR status will be evaluated to allow replacement of subjects whose disease is dMMR until the required number of MSS (pMMR) subjects for the efficacy analysis is reached. Given 5% frequency of dMMR in stage IV CRC, the number of subjects expected to be replaced is very low (1 to 2 subjects). Furthermore, MSI-high (MSI-H)/dMMR CRC subjects are expected to have better response rates due to pembrolizumab efficacy in this population. As this intended population has limited treatment options, including these dMMR CRC subjects is considered acceptable. Data from these subjects will contribute to the safety data and will not be used for efficacy analysis.

Third line and beyond treatment for metastatic CRC provides limited benefit. Regorafenib, an oral multikinase inhibitor approved in the third line setting, improved overall survival by 1.5 months compared with placebo, but only achieved a median progression-free survival (mPFS) improvement of 1 week versus placebo, an ORR based on RECIST 1.1 of 1% versus 0% for placebo and disease control rate (DCR) of 41% compared with 15% for placebo [8]. More than 50% of subjects experienced a Grade 3 or higher drug-related adverse event (AE).

Gastric and gastro-oesophageal cancer have been shown to have moderate sensitivity to PD1 inhibition. Pembrolizumab has demonstrated an ORR based on RECIST 1.1 of 11.6% in gastric and gastro-oesophageal junction cancer in the third line or beyond setting [9] and has received accelerated approval from the FDA for the PD-L1 positive subgroup. Nivolumab has demonstrated an ORR of 14% in PDL-1 positive or negative gastric cancer or gastro-oesophageal junction cancer (Checkmate 32) and is approved in this indication in Japan.

There is a high unmet medical need in these indications and combined immunotherapy may be able to improve outcomes beyond what monotherapy checkpoint achieves.

Choice of Lesion for Injection

In CRC liver metastases, high immune cell density at the invasive tumour margin has been shown to be associated with better chemotherapy outcome [10]. High immune cell densities, high immunoscore and high T and B cell scores were shown to be associated with prolonged survival in Stage IV CRC [11]. However, the liver is considered an immune privileged organ. In order to deal with the many antigens arising from the gut via the portal circulation, the liver must strike a balance between tolerating foreign antigens and exhibiting some degree of antimicrobial effect without

inducing liver damage. There are complex interactions which are not fully elucidated but suggest that tolerance may be broken through induction of inflammation. TLR3 appears to play a key role in that an antiviral response is generated via TLR3-mediated activation of the TRIF pathway leading to an inflammatory response, whereas bacterial antigens activate the MyD88 pathway which results in a tolerant response [12]. Various nonclinical models suggest that inflammation induced by viral antigens is mediated through increased number of classical dendritic cells, increased levels of IFNγ and tumour necrosis factor (TNF)α as well as decreased number of T-regulatory cell-inducing plasmacytoid dendritic cells [13]. Similarly, a viral infection mimic such as polyinosinic-polycytidylic acid (Poly I:C) results in upregulation of genes involved in T-cell homing and migration [14].

These data provide support for the potential of BO-112 injected directly into a liver metastasis to generate a tumour-targeted immune response, both locally and systemically via migrating immune cells. Potential PD-L1 upregulation by tumour cells or by immune regulatory cells in response to BO-112 treatment suggests the need to combine treatment with an anti-PD1 agent such as prembrolizumab.

From a practical perspective, metastatic lesions in the liver are accessible with ultrasound guidance for IT injections as well as biopsy. The liver as only site for IT injection is also proposed to reduce heterogeneity with regard to the TME.

2.2 Background

BO-112 is noncoding double stranded ribonucleic acid (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 (MDA5) and retinoic acid-inducible gene I (RIG-I). By mimicking the effect of a viral infection, it is aimed at mobilising the immune system to attack tumour cells. This includes activation of dendritic cells, CD8 T-cell infiltration, induction of interferons (IFNs), induction of apoptosis and enhancement of immunogenic cell death [15].

Pembrolizumab is a potent humanised immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the PD1receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications and has a well-defined and acceptable clinical safety profile. For more details on specific indications, refer to the Investigator's Brochure of pembrolizumab.

2.2.1 Nonclinical Studies

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades[16]. Accumulating evidence shows a correlation between tumour-infiltrating lymphocytes in cancer tissue and favourable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells/FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumour-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumour responses in cancers such as melanoma [17,18].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumours 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 Ig superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signalling upon engagement of its ligands (PD L1 and/or PD-L2) [19,20].

The structure of murine PD-1 has been resolved [21]. PD-1 and its family members are type-I transmembrane glycoproteins containing an Ig-variable–type domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [20,22,23,24]. 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 [25,26]. As a consequence, the PD 1/PD-L1 pathway is an attractive target for therapeutic intervention in CRC and GC/GEJ.

BO-112:

In vivo mechanism of action studies have shown a complex of mechanisms potentially involved in the antitumoural effect of intratumourally administered BO 112 [15]. The role of the immune system appears to be predominant with various immune cells contributing. Conventional type-I dendritic cells (cDC1), characterised by the expression of TLR3 in their endosomal compartment and their antigen cross-presenting capacity were shown to be a component of the antitumoural effect of BO-112. In addition, IFN α/β activity appears necessary to potentiate the antitumoural

activity. This was demonstrated in tumour growth models using IFN alpha receptor (IFNAR) knockout mice. Further, in vivo experiments highlighted the role of tumour-specific CD8 T-cells in halting both local (injected) tumour and distant tumour progression. Some additive effects of combining IT BO-112 with systemic anti-PDL1 inhibition have also been observed and tumour specificity of the T-cells was demonstrated using tumour-specific epitope staining.

To investigate whether CD8, CD4 T-cells or IFNγ are involved in the BO-112 antitumour mediated effect, T-cell and IFNγ depletion experiments were performed in colon cancer and melanoma syngeneic tumour models. Tumour 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 antitumoural effect, but not for the initial antitumoural effect. This was further verified by neutralising IFNγ in the model, as this is a cytokine secreted by CD8 Tcells. Administration of anti-IFNγ antibodies resulted in loss of the tumour suppressive effect of BO-112. More details on nonclinicial data for BO-112 can be found in the BO-112 Investigator's Brochure.

Pembrolizumab:

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumour-specific CD8+ T-cells and ultimately leads to tumour rejection, either as a monotherapy or in combination with other treatment modalities [27,28,29,30,31,32,33]. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumour responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute myeloid leukaemia and colorectal carcinoma [30,32,33,34,21]. In such studies, tumour infiltration by CD8+ T-cells and increased IFN-γ, granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumour activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T-cell function in vivo [32]. 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 tumour models (see the pembrolizumab Investigator's Brochure).

2.2.2 Clinical Studies

BO-112: Clinical data are available for BO-112 from single agent Part 1 (N = 16) and from combination treatment Part 2 (N = 28) of a Phase I clinical study (Protocol 112/2016-02, NCT02828098). This study evaluated single agent BO-112 at a dose of 0.6 and 1 mg IT injections, and at a dose of 1 mg IT injections in combination with IV administered pembrolizumab or nivolumab.

Tumour biopsy results from the single agent Part 1 showed increased expression of gene signatures for IFNα, IFNγ, CD8 T-cell activation, CTL effector function and tumour inflammation at both doses evaluated. These subjects had advanced, metastatic solid tumours including melanoma, leiomyosarcoma, breast cancer and others (1 subject had CRC), with metastatic lesions suitable

for IT injection at various sites, mainly lymph nodes and (sub)cutaneous lesions [35,36]. Two subjects in Part 1 had a liver lesion as the site of injection. Neither reported any AEs directly associated with the injection procedure. Mild flu-like symptoms were reported. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin values remained in the normal range for both subjects. Pre- and post- treatment biopsy samples from the injected liver lesions showed some antitumoural and immune-related changes.

Preliminary data from Part 2 in 28 subjects with inherently anti-PD1 refractory tumours (ie, determined to be radiologically progressing with no evidence of prior response) show the potential of 1 mg intratumourally administered BO-112 in combination with PD1 blockade to sensitise the immune system against the tumour with outcomes of disease stabilisation and objective responses ORR of 11% (3 subjects achieved partial response [PR], all had increased CD8 T-cells in the on-treatment tumour biopsy compared to baseline) and DCR of 46%. These subjects had metastatic tumour burden from melanoma, NSCLC, SCCHN, or renal cell cancer which was non-responsive to the ongoing anti-PD1 treatment and radiologically progressing at study entry. Upon study entry, 1 mg BO-112 was administered intratumourally to a single lesion (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 once every 3 weeks (Q3W) schedule at a dose of 200 mg for pembrolizumab and once every 2 weeks (Q2W) at a dose of 3 mg/kg or 240mg for nivolumab.

The safety profile BO-112 (related TEAEs), both as single agent and at the recommended 1 mg dose used in combination with anti-PD1 treatment, is currently characterised by generally Grade 1, fever (65%) and other flu-like symptoms such as asthenia (32%), chills (21%), myalgia (18%), nausea (25%) and vomiting (14%). Injection site pain was reported in 11% of subjects (Grade 1 or 2). Grade 3 and 4 thrombocytopenia was reported as treatment-related for 2 subjects with single-agent BO-112 and Grade 3 pneumonitis was reported as treatment-related in 1 subject in the combination Part 2. No decreases in platelet counts have been reported in the combination Part 2. There were no deaths attributed to study treatment in the Phase 1 study. BO-112 has been repeatedly injected in lung (4 subjects in Part 2) and liver (2 subjects in Part 1 and 1 subject in Part 2) lesions, all with at least 3 injections at 1-week or longer intervals, in the Phase 1 study. These injections were well tolerated. There was only 1 subject with an AE directly related to the injection procedure: Grade 1 injection site pain.

There has been no systemic exposure of BO-112 detected to date after IT administration.

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

Pembrolizumab:

Colorectal cancer: The efficacy of pembrolizumab in patients with colorectal cancer that is mircosatellite stable (MSS) or mismatch repair proficient (pMMR) is poor, with ORR of 0% [4,5]. However, in patients with MSI-H or mismatch repair deficient (dMMR) CRC, pembrolizumab

treatment achieved an ORR of 36% (95% CI: 26, 46). The duration of response ranged from 1.6+ to 22.7+ months. Data were pooled from 3 studies (KEYNOTE-016, KEYNOTE-028 and KEYNOTE-164) which included a total of 90 patients with CRC who were MSI-H or dMMR. Patients received either pembrolizumab 200 mg Q3W week or pembrolizumab 10 mg/kg Q2W week. A maximum of 24-months treatment with pembrolizumab was administered. A total of 84% of patients with metastatic CRC received 2 or more prior lines of therapy.

Gastric Cancer: The efficacy of pembrolizumab was investigated in KEYNOTE-059 (NCT02335411), a multicenter, nonrandomised, open-label multi-cohort study that enroled 259 patients with GC/GEJ adenocarcinoma who progressed on at least 2 prior systemic treatments for advanced disease. Among the 259 patients, 55% (n = 143) had tumours that expressed PD-L1 with a combined positive score of ≥ 1 and MSS tumour status or undetermined MSI or MMR status. Patients received pembrolizumab 200 mg Q3W week. Patients without disease progression were treated for up to 24 months. For the 143 patients, the ORR was 13.3% (95% CI: 8.2, 20.0); 1.4% had a complete response (CR) and 11.9% had a PR. Among the 19 responding patients, the duration of response ranged from 2.8+ to 19.4+ months, with 11 patients (58%) having responses of 6 months or longer and 5 patients (26%) having responses of 12 months or longer. Among the 259 patients enroled in KEYNOTE-059, 7 (3%) had tumours that were determined to be MSI-H. An objective response was observed in 4 patients, including 1 CR. The duration of response ranged from 5.3+ to 14.1+ months.

The safety profile of pembrolizumab as a single agent is characterised by: fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhoea, nausea, rash, pyrexia, cough, dyspnoea, constipation, pain and abdominal pain (ie, reported in ≥ 20% of patients). In addition, immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than 1 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 more details see Section 1.1 and Table 5.

2.3 Benefit/Risk Assessment

The poor prognosis of patients with unresectable liver metastases for CRC or GC/GEJ who have progressed after at least 2 or 1 line of systemic treatment respectively 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 the liver metastases in itself may provide a meaningful clinical benefit. Data from a Phase I study of BO-112 added to either pembrolizumab or nivolumab in subjects with anti-PD1 refractory tumours, suggest the potential of BO-112 to sensitise the immune system against the tumour, including enhanced sensitivity to the anti-PD1 treatment, resulting in halting or reversal of the disease progression. Safety data from that study indicate that IT injections of BO-112 are well tolerated and have an

acceptable safety profile, also when given in the combination with pembrolizumab. The risk/benefit assessment is therefore supportive of further clinical studies.

A specific mention should be done in regards of risk/benefit assessment related to the inclusion and management of patients in this trial in the context of COVID-19 pandemic. Our recommendations as sponsor have been included in Appendix 9.

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.

3.0 OBJECTIVES AND ENDPOINTS

Table 2 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• Investigation to the anti-tumour efficacy and safety of repeated IT administrations of BO-112 in metastatic liver lesions in combination with IV pembrolizumab	• Efficacy: ORR based on BOR using RECIST 1.1 • Safety: number and proportion of subjects with study treatment-related TEAEs with severity ≥ Grade 3 (NCI-CTCAE v 5.0)
Secondary	
• Further characterisation of safety and of clinical activity of the combination as well as determination of systemic exposure of BO-112	• Safety: number and proportion of subjects with TEAEs (any grade) • Safety: number and proportion of subjects with related TEAEs (any grade) • Tolerability: number of study discontinuations due to related TEAE • Efficacy: disease control rate (DCR = CR, PR and SD of at least 12 weeks duration) using BOR based on RECIST 1.1 • Efficacy: ORR based on best overall response using RECIST modified for immune-based therapies (iRECIST) • Efficacy: DCR = iCR, iPR and iSD of at least 12 weeks duration) using iRECIST • Efficacy: duration of response • Efficacy: progression-free survival • Efficacy: overall survival rate at 6 months • Pharmacokinetics: systemic exposure after first BO-112 administration
Exploratory	
• Evaluation of antitumoural and immunological effects in the TME of the injected lesion	• Immune cell profile of TME in injected and noninjected lesions • Genetic analysis of the TME

4.0 STUDY DESIGN

4.1 Overall Design

The purpose of this Phase II study is to evaluate the safety, tolerability, antitumoural activity and systemic exposure of repeated IT administrations of BO-112, percutaneously injected into a hepatic metastatic lesion in combination with IV administered pembrolizumab.

This is an open-label, non-comparative, 2-cohort study with a Simon's 2-stage design and will include up to 69 evaluable adult subjects with CRC (MSS) or GC/GEJ who are naive to anti-PD1/PDL1 therapy. Cohort A will consist of up to 26 subjects with CRC (MSS) with nonresectable liver metastases suitable for IT injection and who have received at least 2 prior standard of care systemic anticancer therapies for advanced/metastatic disease. Subjects who have had resection of hepatic metastases and have hepatic recurrence, need to have had only 1 or more prior standard of care systemic anticancer therapies in order to be eligible for this study. Note that for the efficacy analysis, the required number of subjects refers to those with pMMR disease. This will be determined during the study and therefore subjects with dMMR disease will be replaced. Given the low incidence of dMMR in stage IV CRC, it is expected that this number will be low, ie, approximately 1 to 2 subjects to be replaced. Subjects with dMMR disease will be included in the safety analysis set.

Cohort B will consist of up to 43 subjects with gastric or GC/GEJ with nonresectable liver metastases suitable for IT injection and who have received at least 1 prior standard of care systemic anticancer therapy for advanced/metastatic disease. Note that for the efficacy analysis, the required number of subjects refers to those with pMMR disease. This will be determined during the study and therefore subjects with dMMR disease will be replaced. Given the low incidence of dMMR in GC/GEJ, it is expected that this number will be low, ie, approximately 1 to 2 subjects to be replaced. Subjects with dMMR disease will be included in the safety analysis set. See Section 1.2 for study Schema.

The overall duration of the study until completion of data analysis is highly dependent on the duration of achieved clinical benefit, and therefore expected to be between 24 and 36 months.

Screening

The screening period will be from Days -28 to -1, ie, within 4 weeks before the start of the treatment. The screening assessments will be performed according to the Schedule of Assessments (SoA; see Section 1.3). Baseline will be at Week 1.

Study Treatment Period

The study treatment period will comprise 3-week cycles. Treatment and assessment schedules vary somewhat over time and are described in detail in Section 6.0 and section 1.3, respectively. The overall study duration could be extended up to 2 years (ie, Visit 35 plus the end of study [EOS]

visit 28 days after last dose of treatment) depending on the achieved clinical benefit. Individual subject study duration will depend on maintenance of clinical benefit and tolerability and is expected to range from 2 to 12 months, although the study foresees 2 years of treatment.

The study treatment will consist of BO-112 IT injections in combination with IV infusion of pembrolizumab infusions and will be administered in 3-week cycles for both Cohort A and Cohort B subjects.

BO-112 (1 mg) and pembrolizumab (200 mg) will be administered as described in Section 6.1.

Blood samples will be collected for haematology, renal function and hepatic functions tests, C-reactive protein (CRP), and coagulation parameters measurements, prior to any study drug administration at any visit for the first 13 weeks and subsequently every 6 weeks.

Urine pregnancy test will be performed for all women of childbearing potential (WOCBP). If a urine pregnancy test (within 72 hours prior to treatment) is positive or cannot be confirmed as negative, a serum pregnancy test will be required to confirm negative pregnancy.

Tumour biopsies will be performed at baseline (Week 1) prior to first administration of BO-112, on the "to be injected (injected)" lesions and optionally, of a non-hepatic "not to be injected (noninjected)" lesion. On the Week 4, 1 post-treatment biopsy of the injected lesion and if applicable, of the noninjected lesion will be performed.

Physical examinations (full at baseline and brief at subsequent visits), Eastern Oncology Cooperative Group (ECOG) performance status[37], AEs, concomitant medications will be evaluated at screening and each visit (ie, at Week 1, Week 2, and Q3W week thereafter) prior to the pembrolizumab treatment.

End of Study

An EOS visit will be performed for all subjects at 4 weeks after last administered study treatment.

4.2 Scientific Rationale for Study Design

Treatment with pembrolizumab has led to long term durable responses in patients with dMMR CRC (estimate of progression-free survival [PFS] at 1 year is 76%[4]) and with MSI and PD-L1 positive gastric or gastro-oesophageal junction cancer (median duration of response 8.4 months[9]). However, patients with pMMR CRC have not achieved objective responses with pembrolizumab and only around 12% of GC/GEJ patients have achieved an objective response. Clinical activity of BO-112 in anti-PD1 refractory patients suggests that combining BO-112 with pembrolizumab may provide clinical benefit in non- or poorly responsive tumour types such as dMMR CRC and GC/GEJ. To limit the number of subjects exposed to a potentially non-effective treatment, a Simon's 2-stage design has been applied with a DMC review of available safety and efficacy data of the Stage 1 subjects for each cohort separately.

4.3 Justification for Dose

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 is 1 mg administered in 1.7 mL volume as an IT injection. 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 I study of subjects with advanced/metastatic solid tumours (see Section 2.2).

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

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

Among the 8 randomised dose-comparison studies, a total of 2262 subjects were enroled with melanoma and NSCLC, covering different disease settings (treatment naive, 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 these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5 folds 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 tumour 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 tumour 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 tumour PD-1 saturation over a wide range of tumour penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumour.

Finally, population PK analysis of pembrolizumab, which characterised the influence of body weight and other subject 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 many pembrolizumab protocols.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has

- Completed the full 35 cycles of treatment

or

- Discontinued treatment and study due to confirmed disease progression and has completed the EOS visit.

Therefore, subjects are not considered to have completed if any of the following apply:

- Withdrawal due to AE
- Withdrawal of consent (patient decision to stop)
- Unconfirmed PD
- Lost to follow-up
- Any other reason

The end of the study is defined as the date of the last visit of the last subject in the study or last scheduled procedure shown in the SoA for the last subject in the study globally.

5.0 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is in general not permitted. Minor deviations from timing and some laboratory parameters may be discussed with the Sponsor in exceptional cases.

5.1 Inclusion Criteria

Subjects will be included in the study only if all the following inclusion criteria are met:

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. Have nonresectable liver metastasis(es) of colorectal or GC/GEJ cancer. History of resection for liver metastasis is allowed.
4. Have histological or cytological proof of colorectal (Cohort A) or GC/GEJ cancer (Cohort B).
5. Have progressed during or after, or have not tolerated therapy for advanced/metastatic disease as follows:
   a. Cohort A (CRC): at least 2 lines of fluoropyrimidine, irinotecan and/or oxaliplatin containing therapy with or without bevacizumab according to institutional practice; if epidermal growth factor receptor (EGFR) positive/RAS wild type, prior anti-EGFR treatment is required. In case of prior resection of hepatic metastasis with hepatic recurrence, only 1 prior line of fluoropyrimidine, irinotecan and/or oxaliplatin containing therapy is required.
   b. Cohort B (GC/GEJ): fluoropyrimidine and platinum containing treatment; if HER-2 positive, also prior anti-HER-2 treatment is required.
6. Have at least 1 liver metastasis of minimum 20 mm in diameter that is suitable for percutaneous, IT injection (including, but not limited to absence of tumour infiltration into the main portal vein, hepatic vein or vena cava). Recurrence in liver of previously resected liver metastasis is allowed.
7. Presence of at least 1 measurable lesion according to RECIST v1.1. Note: this may be the liver metastasis selected for injection if it is the only measurable lesion present.
8. ECOG performance status of 0 or 1 (see Appendix 8).
9. Adequate haematologic and end-organ function defined by the following laboratory results obtained within 2 weeks prior to the first dose of study treatment:
   a. ANC ≥ 1.5×10^9/L
   b. Platelet count ≥ 100×10^9/L
   c. Haemoglobin ≥ 9.0 g/dL
   d. AST and ALT ≤ 5 × upper limit of normal (ULN)
   e. Serum total bilirubin ≤ 2 × ULN (if known Gilbert's syndrome, serum bilirubin level ≤ 3 × ULN)
   f. Prothrombin time (PT) (or international normalised ratio [INR]) within normal limits and activated partial prothrombin time (aPTT) within normal limits

g. Serum albumin ≥ 2.5 g/dL
h. Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 30 mL/min (calculated per Cockroft-Gault formula)

10. Female subjects who are not pregnant or breastfeeding and at least 1 of the following conditions applies:
    a) Not a WOCBP
    b) WOCBP who agrees to follow contraception guidance during the treatment period and for at least 120 days after the last dose of study treatment.
11. Able and willing to comply with study and follow-up procedures.

5.2 Exclusion Criteria

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

1. Prior treatment with an anti-PD1, anti-PDL1 or anti-PDL2 agent, an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX40, CD137) or any Toll-like receptor (TLR) agonist.
2. Liver metastasis(es) with macroscopic tumour infiltration into the main portal vein, hepatic vein or vena cava.
3. Contraindications to tumour biopsy and injections of the hepatic metastasis(es), such as coagulopathy, therapeutic dose anticoagulant treatment and treatment with long-acting agents such as clopidogrel which cannot be safely stopped.
4. 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.
5. Palliative radiotherapy (≤ 2 weeks of 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.
6. Clinically active central nervous system (CNS) metastases and/or carcinomatosis meningitis. Subjects with previously treated brain metastases may participate provided they are radiologically stable, ie, 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.
7. History of a second malignancy, unless potentially curative treatment has been completed with no evidence of malignancy for 2 years.
8. Life expectancy of < 12 weeks.
9. Severe hypersensitivity (≥ Grade 3) to pembrolizumab and/or any of its excipients.
10. Active infection requiring systemic therapy.
11. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.

12. Active autoimmune disease that required systemic treatment in past 2 years (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
13. 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 to exceed 10 mg/day of prednisone or equivalent.
14. Known human immunodeficiency virus (HIV) infection.
15. Known history of hepatitis B (defined as HbsAg reactive) or known active hepatitis C (defined as HCV RNA [qualitative] detected) virus infection.
16. For WOCBP: pregnancy or a positive urine pregnancy test (eg within 72 hours) prior to treatment; or breastfeeding. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
17. Any other medical condition which would impact the safety of the subject or interfere with the subject's ability to comply with the study and follow-up procedures, in the opinion of the investigator.
18. Has received a live vaccine within 28 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines are live attenuated vaccines and are not allowed.
19. 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, (subjects 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).
20. Has had an allogenic tissue/solid organ transplant.

5.3 Lifestyle Considerations

Meals and Dietary Restrictions

Study subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhoea, nausea or vomiting.

Contraception

BO-112 and pembrolizumab may have adverse effects on a foetus in utero. Study subjects should be informed that taking the study drug may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement from the day of study drug initiation (or 14 days prior to the initiation of study drug for oral contraception) throughout the

study period up to 120 days after the last dose of study drug. If there is any question that a study subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Pregnancy

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.

Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breastfeeding are not eligible for enrolment.

5.4 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may or may not be rescreened depending on the reasons for screen failure. Rescreened subjects should not be assigned the same subject number as for the initial screening.

6.0 STUDY TREATMENT

Study treatment is defined as any investigational treatment(s) or marketed product(s) intended to be administered to a subject according to the study protocol.

6.1 Study Treatment(s) Administered

The recommended dose for further clinical development of BO-112 is 1 mg administered in 1.7 mL volume. The planned dose of pembrolizumab for this study is 200 mg (See Table 4). Study treatment will consist of BO-112 IT injections in combination with IV pembrolizumab infusions and will be administered in 3-week cycles. For each cycle, BO-112 IT injections will be administered after the pembrolizumab infusion, either the same day or within a period of up to 36 hours after the pembrolizumab infusion (for organisational feasibility at the site).

BO-112 IT Injections

The BO-112 IT injections will be administered by an interventional radiologist under ultrasound guidance, or occasional CT scan guidance at the discretion of the interventional radiologist. Preparatory and post-procedural measures such as those routinely used in institutional practice regarding sterility measures and local anaesthetic use for percutaneous biopsy of the liver will be applied for each IT injection. There are no specifications regarding needle size or length as this will be dependent on the depth of the lesion to be injected and will be determined at the discretion of the interventional radiologist. 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.

- Cycle 1: BO-112 1 mg in 1.7 mL volume will be percutaneously injected intratumourally in the liver lesion on Days 1 or 2 (V1a, W1) up to 36 hours after pembrolizumab infusion and on Day 8 (V1b, W2).
- Cycle 2 and all further cycles: BO-112 1 mg in 1.7 mL volume will be percutaneously injected intratumourally in the liver lesion(s) on the first or second day of each 3-week cycle, up to 36 hours after pembrolizumab infusion.
    - If there is more than 1 injectable liver lesion, the injected lesion may be alternated across cycles.
    - If the previously injected liver lesion becomes too small to inject the full volume, the volume may be divided across other liver lesions if present, to ensure the total IT administered dose of 1 mg.
    - If the full dose (volume) cannot be safely administered to 1 or more liver lesions, then that dose should be omitted.

Pembrolizumab Intravenous Infusions

For all cycles: pembrolizumab will be administered using IV infusion on Day 1 of each 3-week treatment cycle prior to the BO-112 IT injection, starting in Visit 1a Week 1. Pembrolizumab will be administered at a dose of 200 mg using a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (ie, infusion time is 30 minutes (-5 min/+10 min).

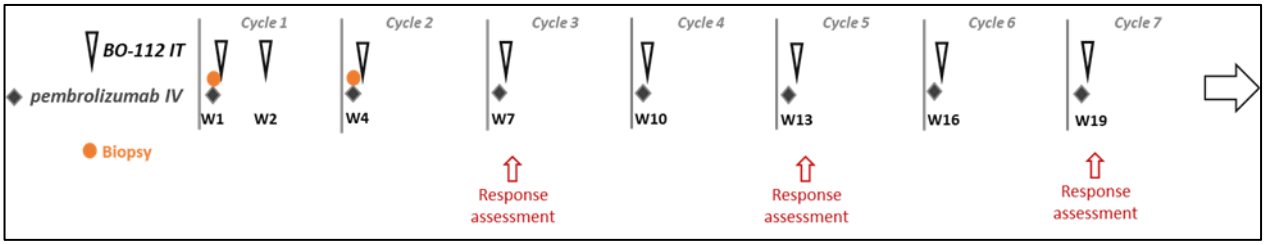
Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on anti-PD1 associated infusion reaction are provided in Table 3.

Table 3 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 subject is deemed medically stable in the opinion of the Investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hours.	• **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 subject 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 (eg from 100 to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5 hours (± 30 minutes) prior to infusion of anti-PD1with: 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 (ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalisation indicated for other clinical sequelae (eg, 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 subject is deemed medically stable in the opinion of the investigator. • Hospitalisation may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing
Abbreviations: IV=intravenous; NCI-CTCAE=National Cancer Institute-Common Terminology Criteria for Adverse Events; NSAID=nonsteroidal anti-inflammatory drug Note: 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 v4.0 (CTCAE) at http://ctep.cancer.gov		

Treatment Scheme



Abbreviation: W=week; IT=intratumoural; IV=intravenous

Study treatment should continue as long as there is clinical benefit and it is tolerated, up to a maximum of approx. 2 years (corresponding to 35 treatment cycles). In addition, for IT administration of BO-112, IT administration continues as long as it is feasible to inject the liver lesion(s), including temporary interruptions.

Table 4 Study Treatment Details

Study Treatment Name:	BO-112	Anti-PD1
	Active ingredient: BO-112 is a noncoding dsRNA based on polyinosinic-polycytidylic acid (Poly I:C), formulated with polyethylenime (PEI)	Active ingredient: pembrolizumab (MK-3475) is a humanised IgG4mAb
Dosage Formulation:	Sterile isosmotic suspension. A clear to opalescent liquid, with a colourless to pale milky aspect and may contain functional visible particles	Solution for infusion. A clear to slightly opalescent, colorless to slightly yellow solution.
Unit Dose Strength(s)/Dosage Level(s):	At a dose of 1 mg in 1.7 mL volume	At a dose of 200 mg using a 30-minute IV infusion
Route of Administration	Intratumoural	IV infusion
Dosing Instructions:	For each cycle, BO-112 IT injections will be administered after the pembrolizumab infusion, either the same day or within a period of up to 36 hours after the pembrolizumab infusion	For all cycles: pembrolizumab will be administered using IV infusion on Day 1 of each 3-week treatment cycle prior to the BO-112 IT injection, starting in Visit 1a Week 1
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.	Pembrolizumab will be provided in a 10 mL vial containing 4 mL of liquid with 100 mg of pembrolizumab. Each vial will be labelled as required per country requirement.
Manufacturer	Highlight Therapeutics S.L.	Merck Sharpe & Dohme, Corp.

Abbreviations: IgG4=immunoglobulin G4; IT=intratumoural; IV=intravenous; mAb=monoclonal antibody; PEI= polyethyleneimine; Poly I:C= polyinosinic-polycytidylic acid

6.2 Treatment Continuation Decisions Based on Efficacy

The decision to continue study treatment will be based on iRECIST guidelines [38]. Treatment will be discontinued if progressive disease is confirmed (iCPD) within 4 to 6 weeks of immune unconfirmed progressive disease (iUPD) or if iUPD is associated with clinical instability, (eg, worsening of performance status, need for increased palliative intervention or more intensive management of disease-related symptoms).

6.3 Dose Modification

6.3.1 Dose Modification and Toxicity Management for Immune-related TEAEs

Treatment-emergent AEs (TEAEs) associated with pembrolizumab exposure may represent an immunologic aetiology. These irAEs may occur shortly after the first dose or several months after

the last dose of pembrolizumab treatment and may affect more than 1 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. The role of BO-112 in inducing or potentiating the occurrence of anti-PD1 associated irAEs is currently unknown and therefore BO-112 treatment must also be interrupted in case of any irAE. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, and skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and BO-112, and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab and/or BO-112 are provided in Table 5.

Individual subject decisions may be discussed with the Sponsor in case of a clinically stable patient or one who has clinical benefit which needs to be balanced against the potential risk of continued or re-instated study treatment (also see below for additional special cases).

Table 5 Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab

<table>
<tr><td colspan="5">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. Anti-PD1 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 anti-PD1 treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If anti-PD1 has been withheld, anti-PD1 may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.</td></tr>
<tr><td>irAEs</td><td>Toxicity grade (CTCAE V5.0)</td><td>Action with anti-PD1</td><td>Corticosteroid and/or other therapies</td><td>Monitoring and follow-up</td></tr>
<tr><td rowspan="2">Pneumonitis</td><td>Grade 2</td><td>Withhold</td><td rowspan="2">Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper
Add prophylactic antibiotics for opportunistic infections</td><td rowspan="2">Monitor subjects for signs and symptoms of pneumonitis
Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment</td></tr>
<tr><td>Recurrent Grade 2, Grade 3 or 4</td><td>Permanently discontinue</td></tr>
<tr><td>Diarrhoea / Colitis</td><td>Grade 2 or 3</td><td>Withhold</td><td></td><td></td></tr>
</table>

	Recurrent Grade 3 or Grade 4	Permanently discontinue	Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	Monitor subjects for signs and symptoms of enterocolitis (ie, diarrhoea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) Participants with ≥ Grade 2 diarrhoea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis Participants with diarrhoea/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
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 - 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)
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 - 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 subjects with T1DM Administer anti-hyperglycemic in participants with hyperglycemia	Monitor subjects for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold		

	Grade 3 or 4	Withhold or permanently discontinue [d]	Administer corticosteroids and initiate hormonal replacements as clinically indicated	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
Hyperthyroidism	Grade 2	Continue	Treat with non-selective beta-blockers (eg, 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 (eg, 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 – 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm aetiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm aetiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

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 anti-PD1 is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, anti-PD1 may be resumed.

e Events that require discontinuation include but are not limited to: Guillain-Barre Syndrome, encephalitis, Stevens-Johnson Syndrome and toxic epidermal necrolysis.

6.3.2 Temporary Discontinuation/Interruption

BO-112: IT administration 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 administer either the full dose, or to divide the full dose across the previously injected lesion and another amenable liver lesion,
- Or the lesion(s) has become non-injectable for other reasons such as density.

Intratumoural 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 Sponsor if 2 or more consecutive BO-112 administrations are omitted. Pembrolizumab treatment should continue as scheduled.

Both BO-112 and pembrolizumab may be interrupted in a subject 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 Sponsor. Subjects should be placed back on study treatment within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the subject's medical and study record.

6.4 Preparation/Handling/Storage/Accountability

BO-112:

BO-112 drug product is a sterile isosmotic suspension for IT administration, presented 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. It is a clear to opalescent liquid, with a colourless to pale milky aspect and may contain functional visible particles. The clear glass vials (20R size) are sealed using 20 mm grey bromobutyl stoppers and aluminium caps.

BO-112 must be stored in the original carton at 2ºC to 8ºC and protected from light. Before being administered, vials should be gently mixed a couple of times using a sideways motion for homogenisation (avoid stirring, upside down mixing or vigorous mixing). BO-112 must be filtered prior to administration (within a time window of 2 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 cm² of filtration area. The filter should have a bidirectional use and should be provided in a single blister pack.

Current shelf-life is 21 months. This will be updated as stability data accrue.

Pembrolizumab:

Pembrolizumab is a solution for IV infusion, presented in a 10 mL clear glass vial containing 4 mL of liquid (100 mg drug product). It is a clear to slightly opalescent, colourless to slightly yellow solution.

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.

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 enroled in the study may receive study treatment and only authorised 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 authorised 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 inspection at any time.

6.5 Measures to Minimise Bias: Randomisation and Blinding

Not applicable as this is an open-label study.

6.6 Study Treatment Compliance

The prescribed dosage, timing and mode of administration may not be changed, except as defined in Section 1.1. Any departures from the intended regimen must be recorded in the electronic case report forms (eCRFs).

6.7 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrolment (within 28 days before the time of enrolment) or receives during the study must be recorded on the eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

A list of excluded medications/therapy is provided in Appendix 5.

6.8 Treatment after the End of the Study

The Sponsor will not provide any additional care to subjects after they leave the study because such care should not differ from what is normally expected for subjects with CRC or GC/GEJ.

7.0 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Treatment

7.1.1 Permanent Discontinuation

BO-112 should be permanently discontinued when a subject meets 1 of the conditions below or if the investigator believes that it is in best interest of the subject:

- Grade 3 AST/ALT elevation which does not resolve to Grade 2 or less within 1 week of onset
- Grade 4 AST/ALT increase
- Grade 3 or 4 increased total bilirubin
- Clinically significant hepatic bleeding/haematoma in or around injected lesion in the liver.

Note: Pembrolizumab must also be permanently discontinued in the above situations (see also Table 5**Error! Reference source not found.**), except for hepatic bleeding/haematoma in or a round injected lesion, where pembrolizumab may be continued as long as the subject is clinically stable and there is no confirmed disease progression.

Individual subject decisions regarding the above may be discussed with the Sponsor in case of a clinically stable patient or one who has clinical benefit which needs to be balanced against the potential risk of continued or re-instated study treatment.

BO-112 must be permanently discontinued when a subject meets 1 of the conditions below or if the investigator believes that it is in best interest of the subject:

- Pregnancy: See Appendix 6 and Section 8.3.5
- Lost to follow-up
- Inadvertent enrolment.

If a subject who does not meet enrolment criteria is inadvertently enroled, that subject must be discontinued from study treatment and the Sponsor or Sponsor designee must be contacted. An exception may be granted in rare circumstances for which there is a compelling safety reason to allow the subject to continue. In these rare cases, the investigator must obtain documented approval from the Sponsor or Sponsor designee to allow the subject to continue in the study. Subjects who discontinue study treatment will not be replaced.

See the SoA (Table 1) for data to be collected at the time of treatment discontinuation and follow-up and for any further evaluations that need to be completed.

7.1.2 Rechallenge

In case of temporary discontinuation, subjects should be placed back on study treatment within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor (see Section 6.3). The subjects should be closely monitored for any signs of AEs.

7.2 Subject Discontinuation/Withdrawal from the Study

- A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons.
- If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the study center study records.
- See SoA table (Table 1 in Section 1.3) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

7.3 Lost to Follow-up

A subject 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 subject fails to return to the clinic for a required study visit:

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

8.0 STUDY ASSESSMENTS AND PROCEDURES

A written informed consent will be obtained from the subjects prior to any assessments. Screening assessments will be performed according to the SoA. At the enrolment, demographic information and medical history will be collected. Subsequent study procedures and their timing are also summarised in the SoA (Table 1 in Section 1.3).

- Protocol waivers or exemptions are not allowed for this study.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of the informed consent form (ICF) may be utilised for Screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

8.1 Efficacy Assessments

Tumour imaging is strongly preferred to be acquired by CT scan. For the abdomen and pelvis, contrast-enhanced magnetic resonance imaging (MRI) may be used when CT with iodinated contrast is contraindicated, or when mandated by local practice. Magnetic resonance imaging 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 in a subject throughout the study to optimise the reproducibility of the assessment of existing and new tumour burden and improve the accuracy of the assessment of response or progression based on imaging. Imaging should include the chest, abdomen, and pelvis.

Tumour assessments will be conducted at time points stipulated in the SoA (Table 1) based on RECIST 1.1. Initial tumour imaging at screening must be performed within 28 days prior to the date of treatment allocation. The site study team must review screening images to confirm the subject has measurable disease per RECIST 1.1. The first on-study imaging assessment should be performed at 6 weeks (42 days ±3 days) from the date of start of treatment. Subsequent tumour imaging should be performed every 6 weeks (42 days ±3 days) or more frequently if clinically indicated. After 24 weeks (168 days ±7 days), subjects who remain on treatment will have imaging performed every 9 weeks (63 days ±7 days). 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.

In case of iUPD, a confirmatory CT scan should be done between 4 to 6 weeks after the iUPD scan depending on the clinical status of the subject 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, tumour imaging should resume at the next scheduled timepoint ensuring an interval between imaging of at least 6 weeks.

For subjects who show evidence of radiological PD by RECIST 1.1 as determined by the investigator, the investigator will decide whether to continue a subject on study treatment until repeat imaging is obtained (using iRECIST for subject management; see Table 6).

Table 6 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 6 weeks to confirm PD.	May continue study treatment at the investigator's discretion while awaiting confirmatory tumour imaging by site by iRECIST.	Repeat imaging at 4 to 6 weeks to confirm PD per the investigator's discretion only.	Discontinue treatment
Repeat tumour 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 tumour imaging shows iUPD by iRECIST per the investigator's assessment	Repeat imaging at 4 to 6 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 6 weeks to confirm PD per investigator's discretion only.	Discontinue treatment

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
Repeat tumour 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 tumour imaging should occur according to the regular imaging schedule.

Abbreviations: iCPD = iRECIST confirmed progressive disease; iCR = iRECIST complete response; iRECIST = modified Response Evaluation Criteria in Solid Tumours 1.1 for immune-based therapeutics; iSD = iRECIST stable disease; iUPD = iRECIST unconfirmed progressive disease; PD = progressive disease; RECIST 1.1 = Response Evaluation Criteria in Solid Tumours 1.1.; VOP=verification of progression

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 tumour response, date of disease progression, and as a basis for all protocol guidelines related to disease status except for discontinuation of study treatment. Given the interval of 6 weeks between tumour imaging in the first 24 weeks, there is no requirement to confirm objective response within 4 weeks.

The hepatic lesion that is the site of BO-112 IT administration, if measurable must be included as a target lesion to ensure that any direct effect of the injection is recorded. This lesion(s) will be identified as the site of injection and will also be reported separately.

iRECIST is based on the RECIST 1.1 but adapted to account for the unique tumour response seen with immunotherapeutic drugs. iRECIST will be used by the investigator to make treatment decisions. When clinically stable, subjects should not be discontinued until progression is confirmed by the investigator, according to the rules outlined in Appendix 1. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some subjects can have a transient tumour 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.

For the assessments of secondary efficacies or clinical activity of the combination therapy in terms of tumour burden and response, following parameters will be determined from CT scan results:

- Disease control rate comprising best response for CR, PR as well as stable disease (SD) lasting at least 12 weeks using RECIST 1.1

- Objective response rate based on best overall response (all time points) using RECIST modified for immune-based therapies (iRECIST)
- Disease control rate comprising best response for CR, PR as well as SD lasting at least 12 weeks using iRECIST
- Duration of response
- PFS
- Overall survival rate 6 months

Exploratory efficacy: Immune cell profile of the TME in injected and noninjected lesions and genomic analysis of the TME will be done on tumour biopsy samples. See Section 8.8 for further details.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1).

8.2.1 ECOG Performance Status

Eastern Cooperative Oncology Group performance status will be measured in a 0 to 5 scale (See Appendix 8) at time points stipulated in SoA (Table 1).

8.2.2 Physical Examinations

Physical examination will be performed at time points stipulated in SoA (Table 1).

- A full physical examination will include, assessments of the skin, cardiovascular, respiratory, gastrointestinal, musculoskeletal and neurological systems. Body weight will also be measured and recorded.
- A brief physical examination will include examination of any system that had abnormality at previous visits or reported by subjects, as well as assessments of the lungs and abdomen (liver and spleen).
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.3 Vital Signs

Vital signs will be measured at time points stipulated in SoA (Table 1).

- temperature, pulse rate and blood pressure will be assessed.
- Blood pressure and pulse rate measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones).
- Vital signs (to be taken before blood collection for laboratory tests) will consist of 1 pulse rate and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at

intervals of at least 1 minute). The average of the 3 blood pressure readings will be recorded on the eCRF.

8.2.4 Electrocardiograms

Electrocardiogram (ECG) will be performed at time points stipulated in SoA (Table 1).

- Single 12-lead ECG will be obtained as outlined in the SoA (see Table 1 in Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 7.0 for QTc withdrawal criteria and any additional QTc readings that may be necessary.

8.2.5 Clinical Safety Laboratory Assessments

A complete list of clinical laboratory tests is provided in Appendix 3. Blood and urine samples will be collected at time points stipulated in the SoA (Table 1) and sent to local laboratories as appropriate.

- Safety laboratory assessments will include standard haematology, chemistry, hepatic function, CRP, renal function and coagulation parameters, pregnancy tests and thyroid tests.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 28 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or Medical Monitor.
  - If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the aetiology should be identified, and the Sponsor notified.
  - All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the laboratory manual and the SoA.
  - If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.3 Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorised representative).

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment or the study (see Section 7.0).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

- All AEs or ECIs (Events of Clinical Interest) from the time of treatment start through 30 days following cessation of study treatment must be reported by the investigator. All AEs will be collected at the time points specified in the SoA (Section 1.3).
- All AEs meeting serious criteria, from the time of treatment start 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 SAEs will be collected at the time points specified in the SoA (Section 1.3).
- All pregnancies and exposure 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.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the medical history/Current Medical Conditions section of the ECRF, not the AE section.

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the Sponsor.

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

8.3.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilisation, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Independent Ethics Committees (IECs), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

- Details of all pregnancies in female subjects and, if indicated, female partners of male subjects will be collected after the start of study treatment and until 120 days after the last dose.
- If a pregnancy is reported, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 7.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.3.6 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

Events of clinical interest for this trial include:

- An overdose of BO-112 and/or pembrolizumab, as defined in Section 8.4, that is not associated with clinical symptoms or abnormal laboratory results.
- 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 Sponsor. However, abnormalities of liver blood tests that do not meet the criteria noted above are not ECIs for this trial.

8.3.7 Disease-related Events and/or Disease-related Outcomes Not Qualifying as SAEs

The following disease-related events (DREs) are common in subjects with colorectal and GC/GEJ cancer and can be serious/life-threatening:

- Signs and symptoms unambiguously associated with disease progression.
- Death due to disease progression.

Because these events are associated with the disease under study, they will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. These events will be recorded on the corresponding AE page of the subject's eCRF within 14 days.

NOTE: However, if either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- *The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual subject.*
  OR
- *The investigator considers that there is a reasonable possibility that the event was related to study treatment.*

8.4 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 tumour into circulation and this may be volume related. Note there is only 1 dose/volume allowed (1 mg in 1.7 mL) in this protocol. An overdose is therefore defined for this protocol as an IT injection volume in mL which exceeds the approximate volume of the tumour 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 tumoural lesion into normal liver 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 subject 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 subject.

8.5 Pharmacokinetics

- Plasma samples of approximately 3 mL will be collected to evaluate if BO-112 is detectable in plasma concentrations of BO-112 as specified in the SoA (Table 1). A baseline sample will be collected at Visit 1a (Cycle 1 – Day 1) at the same time as the baseline safety laboratory tests, namely before the first treatment with pembrolizumab. A unique sample will be collected during the study at Visit 1a (Cycle 1 – Day 1). This PK sample must be taken 30 to 60 minutes after the first BO-112 administration. Instructions for the collection and handling of biological samples will be provided by the Sponsor. The actual date and time (24-hour clock time) of each sample will be recorded. The time and date of study treatment administration will also be recorded.
- 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.

Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the Sponsor and study center study files but will not constitute a protocol amendment. The IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

8.6 Pharmacodynamics

Evaluation procedure of antitumoural and immunological effects of BO-112 in the TME of the injected lesion has been described in Section 8.8.

8.7 Genetics

Not applicable to this study.

8.8 Biomarkers

- The following samples for biomarker research are required and will be collected from all subjects in this study as specified in the SoA (Table 1):
  - Tumour biopsy samples to be collected at the same intervention as the BO-112 administration at the applicable visit.
- Samples will be tested for evaluation of antitumoural effects of BO-112 in the TME of the injected lesion.

Samples may be stored for a maximum of 5 years (or according to local regulations) following the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of biomarker responses to BO-112.

8.8.1 Immune Cell Profile of the Tumour Microenvironment

Immune cell profile of the TME in BO-112 injected and noninjected lesions will be evaluated in biopsy samples collected from all subjects according to the SoA. These samples will be tested by the Sponsor or Sponsor's designee.

The detection and characterisation of the immune cell profile of the TME in BO-112 injected and noninjected lesions will be performed using an immunohistochemistry method by or under the supervision of the Sponsor. Samples may be stored for a maximum of 5 years (or according to local regulations) following the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of immune responses to BO-112.

8.8.2 DNA Research

DNA studies will be conducted to analyze the tumor microenvironment with the aim of gain insights into the activation of the immune response and identify possible biomarkers related to the

efficacy of BO-112. The samples may also be used to confirm findings by application of alternative technologies.

8.9 Health Economics OR Medical Resource Utilisation and Health Economics

Health Economics/Medical Resource Utilisation and Health Economics parameters are not evaluated in this study.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses and Sample Size Determination

Approximately 80 subjects will be screened to achieve a total of 69 evaluable subjects. For both Cohort A (CRC) and Cohort B (GC/GEJ), subjects must have MSS (pMMR) disease to be considered evaluable for efficacy. This will be determined during the study and therefore subjects with MSI (dMMR) disease will be replaced. Given the low incidence of dMMR in stage IV CRC and in GC/GEJ, it is expected that this number will be low, ie, approximately 1 to 2 subjects to be replaced per cohort. Subjects with dMMR disease will be included in the safety analysis set.

The sample sizes of the cohorts were determined using Simon's 2-stage design [39] to enable a first evaluation of the treatment's activity on tumour response. The designs are based on a one-sided $\alpha = 0.05$; a power 80% and response probabilities based on RECIST 1.1 noted below.

When the prespecified number of subjects for Stage 1 have been enroled and have reached the first on-study response assessment, an interim review of available data will be done by a DMC per cohort. The DMC will take the number of responses defined by the Simon's 2-stage design into consideration together with the ORR, DCR, number of subjects with Grade 3 to 5 AEs, SAEs, and individual subject tumour burden profiles to make a reasoned recommendation to the Sponsor regarding extension of each cohort to the foreseen total number of subjects. The stopping rules for futility based on the observed number of responses are considered nonbinding, leaving the final recommendation for stopping the study after Stage 1 at the discretion of the DMC members.

- Cohort A (CRC): response probability based on RECIST 1.1 of P_0 = 1% (for anti-PD1 monotherapy in MSS CRC: ORR of 0% based on RECIST 1.1 for pembrolizumab [4,5]) to be tested against the alternative (desirable) response probability of P_1 = 15%. For the first stage, 11 subjects* (N_1) will be enroled and if no responses are observed, the study will be stopped for futility. In case there is no treatment effect (P_1=P_0 = 1%), the study entails a 90% chance of early stopping (after Stage 1). If 1 or more responses are observed, an additional 15 subjects* (N_2) will be enroled for a total of N = 26 subjects. If 2 or more subjects have a response, then the study will have shown that with 95% confidence, the response probability is above 1%. For 2 responses, the observed response rate is 7.7% and the 90% exact confidence interval (CI) is [5.8%, 10.9%]. However, accounting for the adaptive 2-stage feature of the design, the corrected 90% CI is [1.7%, 24.2%] [40].

  * The baseline biopsy sample will be evaluated for pMMR (as proxy of MSS) versus dMMR (as proxy of MSI-H) status in Cohort A and subjects who are MSI-H/dMMR will be replaced to ensure the appropriate number of pMMR subjects for the statistical analysis. Data from subjects with dMMR CRC will be evaluated as part of the safety population for safety parameters, but separately and descriptively for efficacy endpoints.

- Cohort B (GC/GEJ): response probability based on RECIST 1.1 of P_0 = 10% (expected under anti-PD1 monotherapy where pembrolizumab treatment has achieved ORR based on RECIST 1.1 of 12%, [9]) to be tested against the alternative (desirable) response probability P1 = 25%. For the first stage, 18 subjects** (N1) will be enroled and if 2 or less responses are observed, the study will be stopped for futility. In case there is no treatment effect (P_1=P_0= 10%), the study entails a 73% chance of early stopping (after Stage 1). If 3 or more responses are observed, an additional 25 subjects** (N_2) will be enroled for a total of N = 43 subjects. If 8 or more subjects have a response, then the study will have shown that with 95% confidence, the response probability is above 10%. Note that for 8 responses, the observed response rate is 18.6% with 90% exact CI [17.0%, 20.8%]. However, accounting for the adaptive 2-stage feature of the design, the corrected 90% CI is [10.1%, 32.1%] [40].

  ** The baseline biopsy sample will be evaluated for pMMR (as proxy of MSS) versus dMMR (as proxy of MSI-H) status in Cohort B and subjects who are MSI-H/dMMR will be replaced to ensure the appropriate number of pMMR subjects for the statistical analysis. Data from subjects with dMMR GC/GEJ will be evaluated as part of the safety population for safety parameters, but separately and descriptively for efficacy endpoints. The baseline biopsy sample will be evaluated for PD-L1 status in Cohort B; however, there will be no replacement of subjects based on this since the ORR reported the PD-L1 positive subgroup is 15.5% and for the PD-L1 negative subgroup is 6.4% [9].

9.2 Populations for Analyses

For purposes of analysis, the analysis sets in Table 7 are defined.

Table 7 Analysis Sets

Analysis Set	Description
Entered Analysis Set	All subjects who sign the informed consent form.
Full Analysis Set	All enroled subjects who receive at least 1 dose of study treatment (BO-112 or pembrolizumab). pMMR and dMMR subjects will be evaluated separately for efficacy endpoints. dMMR subjects will only be analysed descriptively for efficacy endpoints.
Safety Analysis Set	All enroled subjects who receive at least 1 dose of study treatment (BO-112 or pembrolizumab), i.e. same population as FAS. dMMR subjects will be evaluated together with pMMR subjects for safety endpoints.

9.3 Statistical Analyses

The Statistical Analysis Plan (SAP) will be developed and finalised before interim database lock and will describe the subject analysis sets to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

All analyses, summaries, and listings will be performed using SAS® software (version 9.4 or higher).

The following descriptive statistics will be used as applicable to summarise the study data unless otherwise specified:

- Continuous variables: sample size (n), mean, standard deviation (SD), median, minimum (min), and maximum (max).
- Categorical variables: frequencies and percentages.

Individual subject data will be presented in listings.

9.3.1 Efficacy Analyses

Analyses of efficacy will be conducted separately for each cohort based on all subjects included in the Full Analysis Set. In Cohort A, subjects who are dMMR will be evaluated separately and descriptively only. See Table 8 below.

Table 8 Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	Objective Response Rate based on RECIST 1.1 is defined as the percentage of subjects with CR or PR as BOR according to RECIST 1.1. The number and percentage of responders, subjects with CR or PR as BOR, and of non-responders subjects with SD, PD or NE as BOR, will be provided along with 95% CI for ORR. In addition, number and percentage of subjects with CR, PR, SD, PD and non-evaluable (NE) as BOR according to RECIST 1.1 will be provided.
Secondary	Disease Control Rate (DCR) based on RECIST 1.1 is defined as the percentage of subjects with CR or PR, and the percentage of subjects with SD of at least 12 weeks duration, as BOR according to RECIST 1.1. The number and percentage of subjects achieving disease control, subjects with CR or PR or SD as BOR, and not achieving disease control, subjects 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 subjects with iCR or iPR as best overall response (iBOR) according to iRECIST. The number and percentage of responders, subjects with iCR or iPR as iBOR, and of non-responders, subjects with iSD, iUPD, iCPD or NE as iBOR, will be provided along with 95% CI for ORR. In addition, number and percentage of subjects 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 subjects with iCR or iPR or iSD of at least 12 weeks duration as best overall response according to iRECIST. The number and percentage of subject achieving disease control, subjects with iCR or iPR or iSD as iBOR, and not achieving disease control, subjects 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 (ie, overall response = CR or PR) to the earlier between the date of first documented progression (ie, overall response= PD) and the date of death. DOR will be analysed using Kaplan-Meier method. Kaplan-Meier estimate of median DOR will be provided with the 95% Cis. Kaplan-Meier DOR curve will be plotted. • 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 tumour assessment. The number and percentage of subjects 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 subjects 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 subjects 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 subjects who survived for at least 6 months will be provided along with 95% CI. Kaplan-Meier OS curve will be plotted.
Exploratory	Will be described in the SAP finalised before interim database lock.

Abbreviations: BOR=best overall response; CI=confidence interval; DCR=disease control rate; DOR=duration of overall response; iRECIST= RECIST modified for immune-based therapies; PR= partial response; RECIST= Response evaluation criteria in solid tumours; SD=stable disease; ORR=overall response rate; OS=overall survival.

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

9.3.2 Safety Analyses

All safety analyses will be performed on the safety analysis set separately for each cohort. In Cohort A, subjects who are dMMR will be evaluated together with pMMR subjects.

Treatment-emergent adverse events are defined as AEs that first occurred or worsened in severity after the first administration of study treatment and prior to 30 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 subjects. Additionally, treatment tolerability will be determined by the number of study discontinuations due to treatment-related TEAEs. Commonly occurring TEAEs,

ie, those that occur in 5% or more of the subjects in either treatment group, will be summarised using descriptive statistics.

All laboratory test results, vital signs measurements, ECG results, weight, and body mass index will be summarised 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 summarised using descriptive statistics.

9.3.3 Other Analyses

Biomarker exploratory analyses will be described in the SAP. Pharmacokinetic analyses are expected to be limited 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.

9.3.4 Missing, Unused and Spurious Data

Statistical analysis will be performed using available data only; missing values will not be imputed. Data from subjects who withdraw from the study, including AEs and any follow-up, will be included in the analyses of primary and secondary outcomes.

9.4 Interim Analyses

An interim analysis for futility will be carried out in each cohort separately when the prespecified number of subjects for Stage 1 have been enroled and have reached at least the first on-study response assessment. Enrolment will be suspended per cohort at the Stage 1 sample size pending the outcome of the DMC review. The DMC will review the safety and efficacy data per cohort. The number of responses together with the ORR, DCR, number of subjects with Grade 3 to 5 AEs, SAEs and individual subject tumour burden profiles will be taken into account to make a reasoned recommendation to the Sponsor regarding extension of each cohort to the foreseen total number of subjects. The stopping rules for futility based on the observed number of responses are defined in Section 9.2 and considered non-binding, leaving the final recommendation for stopping the study after Stage 1 at the discretion of the DMC members

The SAP will describe the planned interim analyses in greater detail.

9.5 Data Monitoring Committee

A DMC consisting of members who are independent from the Sponsor will be established. The DMC membership will have 3 members, including specialists with expertise in CRC, gastric/GEJ cancer and immuno-oncology. The study statistician will provide statistical support.

When the prespecified number of subjects for Stage 1 have been enroled and have reached at least the first on-study response assessment, the DMC will review the safety and efficacy data per cohort. Enrolment will be suspended per cohort and the DMC will review the safety and efficacy data available at the time that the last subject enrolled has reached the first on-study response

assessment at Week 7 (= Cycle 3). The DMC will make a recommendation to the Sponsor separately for each cohort regarding expanding to the total foreseen sample size.

The DMC will be responsible for making recommendations as to whether it is scientifically and ethically appropriate to continue enrolment, discontinue treatment groups, or stop the study.

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11.0 APPENDICES

Appendix 1 Abbreviations

Abbreviation	Definition
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BOR	Best overall response
CI	Confidence interval
CR	Complete response
CRC	Colorectal cancer
CRO	Contract research organisation
CRP	C-reactive protein
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease control rate
DMC	Data monitoring committee
dMMR	Deficient mismatch repair
DRE	Disease-related event
ECG	Electrocardiogram
ECOG PS	Eastern Cooperative Oncology Group Performance Status
eCRF	Electronic case report form
EGFR	Epidermal growth factor receptor
EOS	End of Study
GC/GEJ	Gastric or gastro-oesophageal junction cancer
GCP	Good Clinical Practice
ICF	Informed consent form
ICH	International Council for Harmonisation
iCPD	Confirmed progressive disease
IEC	Independent Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
irAE	Immune-related adverse event
iRECIST	RECIST modified for immune-based therapies
IT	Intratumoural

Abbreviation	Definition
DNA	Deoxyribonucleic acid
iUPD	Immune unconfirmed progressive disease
IV	Intravenous (ly)
mAb	Monoclonal antibody
mPFS	Median progression-free survival
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
MSS	Microsatellite stability
NCI	National Cancer Institute
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
PD1	Programmed cell death Protein 1
PDL	Programmed cell death ligand
PEI	Polyethyleneimine
PFS	Progression-free survival
PK	Pharmacokinetics
pMMR	Proficient mismatch repair
Poly I:C	polyinosinic-polycytidylic acid
PR	Partial response
Q2W	Once every 2 weeks
Q3W	Once every 3 weeks
RBC	Red blood cell
RECIST	Response evaluation criteria in solid tumours
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCCHN	Squamous cell carcinoma of the head and neck
SD	Stable disease
SoA	Schedule of Assessments
TEAE	Treatment-emergent adverse event
TME	Tumour microenvironment
ULN	Upper limit of normal
V	Visit

Abbreviation	Definition
W	Week
WOCBP	Woman of childbearing potential

Appendix 2 Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organisations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable International Council for Harmonisation (ICH) Good Clinical Practice Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (eg, advertisements) must be submitted to an IEC by the investigator and reviewed and approved by the IEC before the study is initiated.
- Any amendments to the protocol will require IEC and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IEC.
  - Notifying the IEC of SAEs or other significant safety findings as required by IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or representative (Appendix 10). The study will not start at any study center at which the investigator has not signed the protocol.

Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Insurance

Sponsor will provide insurance in accordance with local guidelines and requirements as a minimum for the subjects in this study. The terms of the insurance will be kept in the study files.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorised representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, and the IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was entered in the study and the date the written consent was obtained. The authorised person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's legally authorised representative.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs within 28 days from the previous ICF signature date.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorised designee will explain to each subject the objectives of the exploratory research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining specimens to be used for exploratory research. Subjects who decline to participate in this optional research will not provide this separate signature.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

- The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.
- The Sponsor or its representative will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician, or any other third party, unless required to do so by law.
- Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, the Sponsor or representative physician or an investigator might know a subject's identity and also have access to his or her genetic data. Also regulatory authorities may require access to the relevant files.

Administrative Structure

Table 9 Study Administrative Structure – 3rd parties external to Sponsor

Activities	External Service Provider
Study Operations Management Medical Monitoring	Pivotal Pivotal
Study Master File	Pivotal
Data Management	IDDI
Clinical Supply Management	IDDI/Alcura
Biostatistics	IDDI
Medical Writing	IDDI
Laboratory Assessments	Local labs for safety parameters Covance and Pangaea labs for tumour biopsy samples
Pharmacokinetic Sample Testing	Accelero

Dissemination of Clinical Study Data

The results of the study should be reported within 1 year from the end of the clinical study. Irrespective of the outcome, the Sponsor will submit to the EU database a summary of the results of the clinical study within 1 year from the end of the clinical study. It shall be accompanied by a summary written in a manner that is understandable to laypersons.

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 authorised 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.

Source Documents

The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (eg, via an audit trail).

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's study center.
- Data reported on the eCRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in clinical monitoring plan.

Study and Study Center Closure

The Sponsor designee reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed.

The investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study center by the Sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of subjects by the investigator.
- Discontinuation of further study treatment development.

Publication Policy

The data generated by this study are confidential information of the Sponsor. The Sponsor will make the results of the study publicly available. The publication policy with respect to the investigator and study center will be set forth in the Clinical Trial Agreement.

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual study center data. In this case, a Coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Appendix 3 Clinical Laboratory Tests

- The tests detailed in Table 10 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5.0 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10 Protocol-required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Haematology	Platelet count Red blood cell (RBC) count Haemoglobin Haematocrit	RBC Indices: Mean corpuscular volume (MCV) Mean corpuscular haemoglobin (MCH)	White blood cell count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical chemistry[a]	Blood urea nitrogen	Potassium	Aspartate aminotransferase (AST)/ Serum glutamic-oxaloacetic transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine aminotransferase (ALT)/ Serum glutamic-pyruvic transaminase (SGPT)	Total protein
	Glucose (nonfasting)	Calcium	Alkaline phosphatase GGT (gamma glutamyl transferase)	
Urine and serum pregnancy test (HCG)	• At screening, within 72 hours prior to treatment, then every 6 weeks and at EOS			
Thyroid function test (blood)	• At Visits 1a, 3, 5, 7 (Weeks 1, 7, 13, and 19) and each 6 weeks intervals			
C-reactive protein (CRP)	• At screening and at each visit during the treatment period and at EOS			
NOTES: Hematology, chemistry and CRP will be done at each visit during the treatment period and at EOS visit. In addition, if it is scheduled to administer BO-112, a coagulation test will also be done. [a] Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1. All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalised ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).				

Investigators must document their review of each laboratory safety report.

Appendix 4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, and vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgement of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalisation for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a) Results in death
b) Is life-threatening The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c) Requires inpatient hospitalisation or prolongation of existing hospitalisation In general, hospitalisation signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalisation" occurred or was necessary, the AE should be considered serious. Hospitalisation for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d) Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e) Is a congenital anomaly/birth defect
f) Other situations: • Medical or scientific judgement should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

Recording and Follow-up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the eCRF. Each event must be recorded separately.
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to Highlight Therapeutics in lieu of completion of the BOT112-02/AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by Highlight Therapeutics. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to Highlight Therapeutics.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilised for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.
- National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.0 will be used for severity grading.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE. The AE must be characterised as unrelated, unlikely to be related, possibly related, probably related, or unknown (unable to judge).
  - "Probably related" conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
  - "Possibly related" suggests that the association of the AE with the study treatment is unknown; however, the AE is not reasonably supported by other conditions.
  - "Unlikely to be related" suggests that 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" is used if there is not a reasonable possibility that the study treatment caused the AE.
  - All efforts should be made to classify the AE according to the above categories. The category "unknown" (unable to judge) may be used only if the causality is not assessable, eg, because of insufficient evidence, conflicting evidence, conflicting data, or poor documentation.
- The investigator will use clinical judgement to determine the relationship.

- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to appropriate regulatory authorities. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to appropriate regulatory authorities.
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Highlight Therapeutics to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognised follow-up period, the investigator will provide Highlight Therapeutics with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to Highlight Therapeutics, S.L within 24 hours of receipt of the information.

Reporting of SAEs

SAE Reporting to appropriate regulatory authorities

- Please follow the adverse event and serious adverse event guidelines.

Appendix 5 Excluded Medications/Therapy

Excluded medications/therapy is listed below. The use of an excluded medication/therapy is a protocol violation and must be recorded in the eCRF.

- Chemotherapy, definitive (curative) radiation, or biological cancer therapy within 4 weeks prior to the first dose of study treatment and during the study.
- Palliative radiotherapy (≤ 2 weeks of radiotherapy) within 1 week of start of study treatment. Palliative radiotherapy during the study may be allowed and should be discussed with the Sponsor. Lesions which receive palliative radiotherapy will be considered not evaluable as of the first day of the radiotherapy.
- Corticosteroids at physiologic doses exceeding 10 mg/day of prednisone or equivalent prior to start of treatment. Corticosteroid therapy is allowed during the study for the treatment of immune-related adverse events (See Section 1.1).
- Contraindications to tumour biopsy and injections of the hepatic metastasis(es), such as coagulopathy, therapeutic dose anticoagulant treatment and treatment with long-acting agents such as clopidogrel which cannot be safely stopped.
- Another investigational agent or device.

Appendix 6: 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 subjects who show evidence of radiological PD by RECIST 1.1 as determined by the investigator, the investigator will decide whether to continue a subject on study treatment until repeat imaging is obtained (using iRECIST for subject management; see Table 6). This decision by the investigator should be based on the subject'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 subject deemed **clinically unstable** should be discontinued from study treatment at site-assessed first radiologic evidence of PD and is not required to have repeat tumour imaging for confirmation of PD by iRECIST.

If the investigator decides to continue treatment, the subject may continue to receive study treatment and the tumour assessment should be repeated 4-6 weeks later to confirm PD by iRECIST, per investigator assessment.

Tumour 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 subject 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 subject continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, subjects will be discontinued from study treatment.

NOTE: If a subject has confirmed radiographic progression (iCPD) as defined above, but the subject 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, tumour 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 7 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 center 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 (28 days after the last dose of study treatment) 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 as described in Section 8.3.4. 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 8 Eastern Cooperative Oncology Group (ECOG)Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, ie, light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

For detail, see Oken et al., 1982 (Reference 37)

Appendix 9 Recommendations for cancer patient management in the protocol BOT112-02 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.
    - 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.
    - 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.
    - 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.
    - Prioritize assistance of patients with respiratory symptoms.
    - 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.
    - 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.
    - 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 authorised/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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Appendix 10 Signature of Investigator

PROTOCOL TITLE:

Phase IIa open-label clinical study of intratumoural administration of BO-112 in combination with pembrolizumab in subjects with liver metastasis from colorectal cancer or gastric/gastro-esophageal junction cancer.

PROTOCOL NO: BOT112-02

VERSION: Amendment 1 Version 2.1

This protocol is a confidential communication of Highlight Therapeutics, S.L. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to Pivotal.

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Center: _____

_____

_____