Clinical Trial Protocol

AN EXPLORATORY FIRST IN HUMAN PHASE I CLINICAL AND PHARMACOKINETIC STUDY OF INTRATUMORAL ADMINISTRATION OF BO-112 IN ADULT PATIENTS WITH AGGRESSIVE SOLID TUMORS, WITH AN EXTENSION COHORT IN COMBINATION WITH ANTI-PD1 TREATMENT

Sponsor: BiOncoTech Therapeutics S.L.
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EudraCT: 2016-000527-24

Study drug: BO-112

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SPONSOR CODE: 112/2016-IT

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This clinical study protocol has been reviewed and approved by the Sponsor to ensure compliance with International Conference on Harmonization (ICH) guidelines for Good Clinical Practices (GCP) and applicable regulatory requirements

SPONSOR: BiOncoTech Therapeutics S.L.

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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>ANC</td>
<td>Absolute neutrophil count</td>
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<td>Maximum tolerated dose</td>
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SYNOPSIS

TITLE OF CLINICAL TRIAL
An exploratory first in human Phase I clinical and pharmacokinetic study of intratumoral administration of BO-112 in adult patients with aggressive solid tumors, with an extension cohort in combination with anti-PD1 treatment.

PROTOCOL CODE
112/2016-IT.

VERSION DATE
23 May 2018

VERSION NUMBER
Version 7.0

SPONSOR
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CLINICAL TRIAL PHASE
Phase I

CLINICAL TRIAL SITES
A list of participating sites will be provided in a document apart of this protocol.

CEIm
Cómite de Ética de la Investigación con medicamentos de Navarra.
INDICATION

Part 1: Single agent dose escalation and multiple dose: Patients with aggressive solid tumors from whom biopsies can be obtained. Injected lesions must be palpable and biopsiable at the time of injection, and biopsied after 7-14 days. Patients will not receive an alternative therapy during the period comprising from first and second biopsy.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab: Patients with aggressive solid tumors who are clinically stable but who have disease progression on treatment with either pembrolizumab or nivolumab for an approved indication. Patients must have at least 1 lesion which is accessible for intra-tumoral injection and biopsy.

EXPERIMENTAL PRODUCT

Parts 1 and 2: BO-112.

BACKGROUND TREATMENT

Part 2 only: pembrolizumab (Keytruda) or nivolumab (Opdivo)

OVERALL STUDY DESIGN

Part 1: Single agent dose escalation and multiple dose:

This is a first-in-human Phase I exploratory study of biopsy accessible tumors to determine the biological effect of a intratumoral (IT) administration of BO-112. Additionally, this study will also study BO-112 biological activity, the innate and adaptive immune system response and signaling pathways, as well as signs of clinical relevance, will be studied.

BO-112 will be administered at a starting dose of 0.6 mg. Upon confirmation of the safety profile of the starting dose and evaluation of the pharmacokinetic (PK) profile, three additional dose levels (1 mg, 1.6 mg and 2.4 mg) are expected to be tested.

Between each dose level, an interim analysis of safety and tolerability will be performed for all dosed patients. A Dose Escalation Meeting will be held between the investigator and the sponsor to evaluate the available data. Once a dose level is judged to be safe, the next dose level can be administered. Pharmacokinetic data will be taken into account within each cohort of subjects. An external Safety Review Committee will review the conclusions made by the Dose Escalation Meeting, providing guidance when required.

Upon completion of the first cohort (0.6mg) with a single intratumoral (IT) administration and after relevant information obtained regarding the biological effect of BO-112, a multi-dose
scheme (two-three administrations, one administration per week) will be settled for all cohorts. The single-dose cohort ongoing upon approval of this protocol amendment will be completed. Subsequent cohorts will be opened following a multi-dose scheme. Previous completed single-dose cohorts could also be opened in a multi-dose scheme. Dose escalation and DLT criteria for a multi-dose scheme will be followed as described in section 6.2.

Patients that have received BO-112 in a single-dose scheme could be included in the next cohorts testing multi-dose scheme if there is any benefit and no additional treatment can be offered to them.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab:

Preliminary data from the single and multiple dose cohorts from Part 1 will guide the selection of the BO-112 dose to be used in an extension cohort of BO-112 in combination with continued treatment of pembrolizumab or nivolumab in patients who have disease progression at the time of study entry. Pembrolizumab or nivolumab will be administered as per the SmPC, which allows for continuation of treatment in case of signs of progression in patients who are clinically stable. For the purposes of this Phase I study, additional eligibility criteria are added regarding the presence at study entry of at least 1 lesion that is accessible for IT injection and biopsy.

The safety and tolerability of multiple IT administrations of 1 mg BO-112 in combination with pembrolizumab or nivolumab will be assessed for future use as the recommended dose in Phase 2 combination treatment clinical trials. BO-112 will be administered IT at a dose of 1 mg for 5 administrations over a period of 8 – 9 weeks. Pembrolizumab or nivolumab will be administered IV every 3 weeks or every 2 weeks respectively as per the SmPC. During this period, safety and tolerability will be monitored. In addition, immunological biomarkers will be evaluated in blood and tumor tissue samples. At the end of the 8 – 9 week period of combined treatment and before Visit 7 (pembrolizumab) or Visit 8 (nivolumab) in Week 12, tumor response will be evaluated by physical examination and by CT scan during an ‘unscheduled’ visit, to allow continuation of treatment in case of an objective response (partial or complete) or stable disease based on RECIST v 1.1. In case of treatment continuation, IT administrations of 1 mg BO-112 will be given at regular intervals for as long as there is a clinical benefit, the treatment is tolerated and there is a lesion (or lesions) amenable for IT injection; pembrolizumab or nivolumab will continue as per the SmPC; all for up to a total of 1 year. In case of disease progression, before or at any response assessment, treatment with BO-112 and pembrolizumab or nivolumab will be discontinued and the subject will be taken off the study.
In case the lesion used for IT injection is no longer amenable for IT injection, then another lesion which is amenable should be used. If no lesion is accessible for IT injection at the scheduled visit, then that BO-112 dose administration will not be done at that visit and only pembrolizumab or nivolumab will be administered. Subsequent, scheduled administrations of BO-112 should continue per protocol if accessible lesions are present.

**STUDY OBJECTIVES**

**Primary objective:**

Part 1: **Single agent dose escalation and multiple dose**: To determine the biological effect of an IT administration of BO-112 as single agent.

Part 2: **Extension cohort of BO-112 in combination with pembrolizumab or nivolumab**: To determine the safety and tolerability of combination of BO-112 with pembrolizumab or nivolumab.

**Secondary objectives:**

Part 1 **single agent dose escalation and multiple dose**:

- To determinate the safety profile and tolerability of BO-112 in patients enrolled in the study.
- To establish the PK profile of BO-112 administered intratumorally
- To explore pharmacodynamics (PD) markers of the BO-112
- To monitor changes in the profiles of innate, specific, complement, and NK mediated immune response
- To evaluate the preliminary antitumor activity of the drug

Part 2: **extension cohort of BO-112 in combination with pembrolizumab or nivolumab**:

- To evaluate the biological activity of BO-112 in combination with pembrolizumab or nivolumab
- To establish the PK profile of BO-112 administered intratumorally
- To explore pharmacodynamics (PD) markers of BO-112 and pembrolizumab or nivolumab
- To monitor changes in the profiles of innate, specific, complement, and NK mediated immune response
- To evaluate the preliminary antitumor activity of BO-112 in combination with pembrolizumab or nivolumab
PRIMARY ENDPOINT

Part 1 single agent dose escalation and multiple dose:

• BO-112 biological effect.

Part 2 extension cohort of BO-112 in combination with pembrolizumab or nivolumab:

• Safety and tolerability

SECONDARY ENDPOINTS

Part 1 single agent dose escalation and multiple dose

• Safety profile and tolerability
• PK profile
• PD markers
• Immune profiles changes
• Preliminary antitumor activity

Part 2 extension cohort of BO-112 in combination with pembrolizumab or nivolumab:

• Biological effect
• PK profile
• PD markers
• Immune profiles changes
• Preliminary antitumor activity

STUDY POPULATION

Inclusion criteria

1. Able and willing to give voluntary written informed consent before being enrolled in the study. The subject must sign the informed consent form (including their consent for the analysis of biological samples of tumor and blood) prior to any study-related procedures and agree to the schedule of assessments.

2. Patients age 18 years or more on the day of signing informed consent form.

3. Histologically or cytologically confirmed aggressive solid malignancy

4. Patients must have:
   • Biopsy-accessible tumors
   • No prior anticancer treatment during the last 14 days; except for Part 2 extension cohort where ongoing treatment with pembrolizumab or nivolumab is required (in case of nivolumab treatment in combination with ipilimumab, then
5. Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≥ 2.

6. An expected survival of ≥ 3 months.

7. Patients with the following hematologic values:
   - Hemoglobin (Hb) ≥ 10 g/dL
   - Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L
   - Platelets ≥ 100 x 10^9/L

8. Patients with the following biochemical values:
   - Bilirubin ≤ 1.5 x upper limit of normality (ULN)
   - Aspartate aminotransferase (AST) and Alanine transaminase (ALT) ≤ 3 x ULN.
     Nevertheless, in patients with documented liver metastases, AST and/or ALT could be ≤ 5 x ULN.
   - Serum creatinine ≤ 1.5 x ULN or CrCl ≥ 40 mL/min on the basis of measured CrCl from a 24-hour urine collection or Cockroft-Gault glomerular filtration rate estimation: (140 - age) x (weight in kg) x (0.85 if female) / 72 x (serum creatinine in mg/dL).

9. Female patients of childbearing potential and male patients with partners of childbearing potential must agree to the use of 2 highly effective forms of contraception in combination (male condom plus one of the methods listed in Appendix 4) or must totally/truly abstain from any form of sexual intercourse, throughout their participation in the study and for at least 3 months after last dose of study drug. For Part 2, if combination with pembrolizumab this is 4 months after last dose of pembrolizumab; if combination with nivolumab, this is 5 months after last dose of nivolumab as per SmPC.
   - Females of childbearing potential are defined as sexually mature women without prior oophorectomy or hysterectomy who have had menses within the last 12 months.
   - Females are not considered to be of childbearing potential if they have been with continuous spontaneous amenorrhea for > 12 months and follicle-stimulating hormone (FSH) level ≥ 40 IU/L.
   - For females who have been with continuous spontaneous amenorrhea for ≥ 2 years, the requirement for FSH measurement at screening will be waived.

10. Patients should be able to report on their condition while on the treatment.

11. Additional inclusion criteria for Part 2 extension cohort:
   1. At study entry, patients must have radiologic disease progression during ongoing monotherapy with pembrolizumab or nivolumab (monotherapy or in combination with ipilimumab) for any approved indication as described in the
SmPC at the time of study entry, and have never had a partial or complete response to the current pembrolizumab or nivolumab treatment;

- In case of stable disease on current treatment: only initial stable disease followed by disease progression within 6 months of the start of pembrolizumab or nivolumab is allowed.

2. Clinically stable at the time of study entry.
3. Able to continue treatment with pembrolizumab or nivolumab.
4. Progression amenable to further immunotherapy in the opinion of the Investigator.
5. At least 1 lesion which is accessible for intra-tumoral injection and biopsy (visible, palpable or with the aid of imaging techniques).

Exclusion criteria

1. Other relevant and clinically significant concomitant diseases or adverse clinical conditions which may jeopardize patient safety:

- Increased cardiac risk: congestive heart failure; or unstable angina pectoris; or arrhythmia requiring treatment or uncontrolled arterial hypertension; or myocardial infarction within 12 months before inclusion in the study.

- Patients with active central nervous system (CNS) lesions (including carcinomatous meningitis) will be excluded. However, patients will be eligible if:
  - Clinical interventions with surgery and/or radiotherapy and/or radiosurgery had finalized at least 4 weeks prior to receive BO-112 and they do not require treatment with high doses of steroids (>10mg/24h prednisone or equivalent).
  - There is not requirement, per investigator criteria, of immediate clinical interventions with surgery and/or radiotherapy and/or radiosurgery and they do not require treatment with high doses of steroids (>10mg/24h prednisone or equivalent).

- Active infection.

- Significant non-neoplasic liver disease (e.g., cirrhosis, active chronic hepatitis B or C).

- Any clinically significant abnormality on history or examination including diagnosis of immunodeficiency or receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study medication (physiologic doses of corticosteroids may be approved after consultation with the Sponsor).
2. Patients who are pregnant or breastfeeding. Women of childbearing potential must have a negative blood pregnancy test performed within 7 days before the onset of treatment for Part 1. For Part 2 pregnancy test is to be done during screening and repeated on Day 1 if screening test was done more than 3 days before.

3. Substance abuse or clinical, psychological or social conditions that can undermine the validity of the informed consent or protocol compliance.

4. Patients who present any contraindication or suspected allergy to the product compounds under investigation in the study.

5. Simultaneous participation in any other study involving an investigational medicinal product, or having participated in a study less than 14 days prior to the start of study treatment.

6. Impossibility to comply with treatment due to cultural or geographic circumstances.

7. Any condition that is unstable or could endanger the patient’s safety and/or the patient’s compliance with the study.

8. Additional exclusion criteria for Part 2 extension cohort:
   1. Grade 3-4 toxicity during treatment with pembrolizumab or nivolumab (monotherapy or in combination with ipilimumab) which has only recovered to grade 2 or more.
   2. Permanent discontinuation of pembrolizumab or nivolumab due to immune-related or other adverse reaction.
   3. Use of chronic steroids >10mg/24h prednisone or equivalent.

**DISCONTINUATION CRITERIA**

Subjects may withdraw their consent to participate in this study at any time without prejudice. The investigator must withdraw from the study any subject who requests to be withdrawn (preferably after consultation with the study medical monitor). A subject’s participation in the study may be discontinued at any time at the discretion of the investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the End of Study (EOS) visit should be carried out.

BiOncoTech must be notified of all subject withdrawals as soon as possible. BiOncoTech also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual investigator or site for poor enrollment or noncompliance.

Reasons for which the investigator or BiOncoTech should withdraw a subject from the study include, but are not limited to, the following:

- Subject experiences disease progression
• Subject experiences toxicity that is determined by the investigator to be no longer safe for the subject to continue therapy
• Subject requests to withdraw from the study treatment
• Subject requires or has taken medication prohibited by the protocol
• Subject is unwilling or unable to comply with the study requirements
• Subject was erroneously admitted into the study or does not meet entry criteria
• Subject is lost to follow-up
• Subject becomes pregnant

PLANNED NUMBER OF SUBJECTS
Part 1: The total number of patients finally included will range between 9 to 24. Patients will be in the study between 2 to 12 weeks.

Part 2: Up to a total of 30 patients will be included, or less if the number of patients with NSCLC reaches 20 first.

STUDY AND TREATMENT DURATION
Screening period will take maximum 28 days. Exposure to the experimental product BO-112 is expected to be 8 or 9 weeks for patients whose disease continues to progress, and up to a total of one year for patients whose disease becomes stable or who demonstrate a partial or complete response. Study background treatment duration will be either 8 or 9 to 12 weeks for patients whose disease continues to progress or up to a total of 1 year for patients whose disease becomes stable or who demonstrate a partial or complete response.
1 GENERAL INFORMATION

1.1 Title, code and date.
An exploratory first in human Phase I clinical and pharmacokinetic study of intratumoral administration of BO-112 in adult patients with aggressive solid tumors, with an extension cohort in combination with anti-PD1 treatment.

Code: 112/2016-IT
EudraCT: 2016-000527-24
Version: 7.0 (23 May 2018)

1.2 Name and addresses of the Sponsor and monitor.

Sponsor
BiOncoTech Therapeutics S.L.
Parque Científico de la Universidad de Valencia
C/ Catedrático Agustín Escardino, 9 46980
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Monitor
Monitor designed by Pivotal
C/ Gobelas 19 Madrid 28023
Tel: 91 708 12 50
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C/ Entença 332-334 7º 4º
08029 Barcelona
Tel: 93 444 17 47
Fax: 93 444 17 46

1.3 Identification data of the authorized people for sign the protocol and amendments in name of the Sponsor.

PIVOTAL S.L.
C/ Gobelas 19
28023 Madrid, Spain
Tel: 91 708 12 50
Fax: 91 708 13 08
C/ Entença 332-334 7º 4º
08029 Barcelona
Tel: 93 444 17 47
Fax: 93 444 17 46
1.4 Identification data of the clinical trial investigators and sites.
A list of participating sites will be provided in a document apart of this protocol.

2 BACKGROUND AND JUSTIFICATION

2.1 Name and description of the investigational product.

**BO-112**

BO-112 is a pharmaceutical product formed by a specific non-coding dsRNA based on polyinosinic-polycytidylic acid (Poly I:C), as active pharmacological ingredient (API), formulated with polyethylenimine (PEI), a polycation that stabilizes the API, and glucose. It is manufactured as a sterile isosmotic light suspension in a glass vial containing 7.2 mg of active component (Poly I:C) at a concentration of 0.6 mg/ml.

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
<th>Amount per vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly I:C on dry basis</td>
<td>Active substance</td>
<td>7.2 mg</td>
</tr>
<tr>
<td>PEI</td>
<td>Stabilizer of Poly I:C</td>
<td>5.849 mM</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>Isosmotic agent</td>
<td>0.66 g</td>
</tr>
<tr>
<td>Water for injection</td>
<td>Solvent</td>
<td>c.s.p. 12 mL</td>
</tr>
</tbody>
</table>

More detailed information of the product is contained in the Investigator Brochure (IB) attached as an Appendix to this protocol (Appendix 1).

2.2 Summary of the studies of interest to the present trial.

Poly I:C is a non-coding double stranded RNA not complementary to any known human DNA or RNA sequence and it does not selectively bind to any DNA. Its therapeutic target has been discovered by BiOncoTech’s team to promote a selective killing of tumor cells (1). Polyethylenimine is an excipient/adjuvant which protects Poly I:C from nucleases and helps deliver the API into the cytosol of cancer cells. Both compounds, poly I:C and PEI, have been clinically tested ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). A summary of nonclinical data is provided below and more details are available in the Investigator Brochure.

BO-112 is a first-in-class agonist of the cytosolic receptor melanoma differentiation-associated gene 5 (MDA-5). MDA-5 has been shown to be effective to treat a variety of tumors. BO-112 mechanism of action includes:

- Selective induction of apoptosis and autophagy in tumor cells independent from the mutational status of classical oncogenes, such as BRAF.
• Systemic effects such as immune stimulation and inhibition of lymphangiogenesis and angiogenesis.

Therefore, BO-112 is a nanomedicine that has been designed to target the cytosolic receptor MDA-5 (1) capable of stimulating innate and adaptive immune responses and triggering apoptosis in cancer cells (2). BO-112 overcomes nuclease degradation and cytosolic delivery by engulfing a specific chain of Poly I:C with a polycationic polymer. The nanoparticle protects Poly I:C from nucleases by modifying Poly I:C physicochemical properties without affecting its inherent anti-tumoral properties. In addition, the new formulation engages Poly I:C to cytosolic sensors in malignant cells, which are otherwise maintained in a silent state. In this context, BO-112 not only induced activation of the immune system by interferons (IFNs) but also activated programs of sustained autophagy and apoptotic cell death in cancer cells. This is a new strategy to promote tumor cell death. BO-112 in the cytosol activates the helicase MDA-5 (specific sensor of Poly I:C, active component of BO-112, in tumor cells). Accordingly, a subsequent activation of apoptotic and autophagy-dependent cancer cell death is induced. Within 2-3 hours after incubation with BO-112, tumor cells undergo massive ultrastructural changes in the endosomal compartment. Although MDA-5 could facilitate autophagosome formation, MDA-5 actively induces the pro-apoptotic factor NOXA (which has an important role in BO-112 dependent tumor cells death). Thus, the activation of apoptotic caspases accompanied by a sustained lysosomal-dependent degradative process can ultimately converge in an efficient tumor cell death in presence of BO-112. Interestingly enough, MDA-5 mediates Poly I:C dependent tumor cell death in different types of cancer, such as melanoma (1;3), ovarian cancer (4), and breast cancer (5).

BO-112 activity in a large panel of different melanoma tumor cells was selective and very efficient. Cell death was induced at different concentration levels which were not toxic for non-tumor cells like melanocytes or fibroblasts. It is also noteworthy that BO-112 is also very effective in BRAF mutant model of melanoma cells. BO-112 was also shown to be very effective in pancreatic cancer cells lines. Additional data also reveal BO-112 the capability to induce cell death in cancer stem cells (CSCs). BO-112 can also compromise the viability of some triple negative breast tumor cells for which no effective treatments are available. The anti-tumor activity of the BO-112 in full battery of tumor cells in vitro was also observed in vivo. In this context, BO-112 was very selective and efficient in xenografts of melanoma and bladder generated in immunocompetent mice. Lung metastases of aggressive human melanomas grown in immunsuppressed mice also responded to BO-112.

BO-112 was found to reduce tumor growth in a more effective and sustained manner than vemurafenib, a currently approved BRAF\(^{V600E}\) inhibitor. These results are highly significant in
the context of BO-112 as a valuable new therapeutic strategy focused on cancer patients with short life expectancy, due to its demonstrated increased efficiency in comparison with current treatments.

Many clinical trials are currently ongoing using intratumoral (IT) immunostimulatory products, including pharmaceutical products based on Poly-ICLC (trial nº NCT01976585). Preclinical models have recently demonstrated that the efficacy of immunostimulatory drugs is potentiated upon IT injections. The hypothesis behind such a practice is that by delivering locally high concentrations of immunomodulatory drug, we could trigger a more efficient antitumor immune response. As opposed to conventional anticancer drugs, these immunostimulatory drugs can be delivered directly into the tumor, even at a single site, and generate a systemic antitumor immune response, inducing tumor responses in distant, un.injected, tumor sites. Local delivery of immunostimulating drugs should prevent their circulation at high concentrations in the blood, causing less autoimmune toxicity. Therefore, local injections allow much higher concentrations of the immunostimulatory products in the tumor microenvironment than do systemic infusions, providing lower toxicity and better efficacy (6). Intra-tumoral administrations bring additional benefits when planning to conduct studies in combination with other agents. In this regard BO-112 has proven to have an additive anti-tumoral effect in vivo when combined with an immune checkpoint inhibitor (aPDL-1 Ab). The effectiveness of BO-112 was evaluated in a melanoma model using two different administrations routes. Inhibition of tumor growth following the intra-tumoral administration of BO-112 in combination with the anti-PDL1 antibody was more impressive than that observed when BO-112 was administered systemically.

The IT route is a parenteral route where the tissue exposed to the highest concentration of the pharmacological agent is the tumor itself, that is, the tissue that wants to be reduced or eliminated. In contrast, the rest of organs and tissues in the body are expected to be less exposed. In comparison to intravenous route, it can be assumed that healthy organs of patients will be less exposed following IT route. Based on this data BiOncoTech will like to conduct this study to assess the pharmacokinetics (PK) profile of a IT administration of BO-112.

Regarding target population, available data suggest a broad efficacy of BO-112 in a wide range of aggressive tumors, including pancreatic cancer, metastatic melanoma, glioblastoma and bladder cancer. By not depending on classical signaling cascades [i.e. driven by classical oncogenes such as BRAF, NRAS or AKT (MDA5 not found mutated or deleted in human cancers)], BO-112 may avoid potential resistance mechanisms that limit the action of targeted therapies.
In summary, BO-112 constitutes a novel paradigm of a potent anti-tumoral mechanism with an innovative mode of action that promotes (i) tumor self degradation by autophagy and apoptosis, while (ii) inducing innate immunity programs that ultimately blunt neovascularization, and (iii) help eliminate the treated cells. These activities can also be exploited in adjuvant settings, particularly for individuals at risk of metastatic development.

2.3 Justification of Part 1: Single agent dose escalation and multiple dose

Considering the short-term action and/or toxicity of current treatments, we believe that BO-112 is a valuable new therapeutic strategy for those cancers that pose a significant unmet medical need.

The purpose of this trial is to evaluate the safety and tolerability, pharmacokinetics and pharmacodynamics of intra-tumoral administration of BO-112, administered to patients with aggressive solid tumors from whom biopsies can be obtained. In all cases the investigators will justify the risk of participating in a Phase I first in human trial. Moreover, this study will address BO-112 biological activity, the innate and adaptive immune system response and signaling pathways, as well as signs of clinical relevance.

The doses and schedule of BO-112 administration for subsequent development phases will be determined according to its safety and tolerability profile as well as signs of biological activity.

2.4 Justification of Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab

Based on the mechanism of action of BO-112 and further to the results of the nonclinical studies noted above, it is expected that BO-112 will be used in combination with other immunotherapy agents, such as PD1 or PDL1 checkpoint inhibitors to improve patient outcomes. The rationale for the combination relies on the cytotoxic effect via MDA-5 mediated apoptosis and autophagy generating new tumor antigens and the immune stimulatory effect of BO-112 in an IFN-dependent manner. Although immune checkpoint inhibitors have demonstrated improved survival in patients with advanced melanoma, non-small cell lung cancer and other difficult to treat cancer indications, not all patients respond to treatment. As observed by Teng et al, (7) it is key for immune checkpoint inhibitor activity that the immune cells have infiltrated into the tumours. Certain tumor types are also less responsive than others. Even in the most responsive types of melanoma, renal cell carcinoma, bladder cancer, non-small cell lung carcinoma (NSCLC) and Hodgkin lymphoma, not all patients respond to therapy. For example, from the registrational trial for pembrolizumab, KEYNOTE-006 in patients with advanced melanoma, the objective response rate was 33%. In KEYNOTE-010 in
patients with advanced NSCLC treated with 2nd line pembrolizumab, the objective response rate overall was 18%, but reached 30% in the subgroup that was strongly PDL1 positive.

Resistance to immunotherapy has been described as primary, adaptive or acquired. Clinically, primary resistance presents as a cancer which does not respond to immunotherapy (8). Adaptive resistance is found in a cancer which has adapted to protect itself against the immune system which has recognised it. Acquired resistance describes a cancer which initially responded to immunotherapy but which has subsequently lost that response.

A recently proposed classification of tumors according to the presence/absence of tumor-infiltrating lymphocytes (TILs) and PD-L1 may help guide treatment with immunotherapy (7):

- type I where the adaptive immune system is driven by PD-L1 and TILs
- type II where the immune system is “ignorant” with absent PD-L1 and absent TILs
- type III where the tumors have intrinsic induction of PD-L1 with no TIL’s
- type IV where other suppressors play a role with presence of TILs in the absence of PD-L1.

This classification is not yet used in clinical practice, but it illustrates the evolving understanding of the tumor microenvironment and potential for improving the outcomes of immunotherapy. Immune stimulators could be used to attract T cells to the tumor in types II and III, thereby potentially making these tumors more responsive to PD1/PDL1 inhibition. This classification has been investigated mostly in melanoma, where it has been reported that ~ 41% of tumors are type II and are predicted to have a poor prognosis (1). NSCLC is expected to have a higher frequency of type III tumors.

We have shown in a mouse melanoma model that stimulation of the innate immune system by BO-112 improves the outcome of PD1 inhibition. In a genetically engineered mouse model, Bald et al demonstrated that poly(I:C) can engage the cellular immune system to melanomas via type I IFN-dependent activation of different immune cell subsets, with dendritic cells playing a key role (9). This resulted not only in recruitment of cytotoxic, but also up-regulation of PD-L1 expression in tumor tissue and prolongation of survival in the mice.

The safety profile of PD1/PDL1 inhibitors is characterised by immune-related adverse reactions and include dermatologic, gastrointestinal, hepatic, endocrine, and other less common inflammatory events. When combining treatments, it is important to assess if there is a potential for overlapping toxicity. Review of the safety and pharmacodynamic data to date of the Part 1 of this trial, reveals thrombocytopenia as a clinically significant finding (see section 2.4).
Under the careful monitoring of this first-in-human clinical trial and utilising the experience gained by the investigators and site staff participating in the trial, an extension cohort will investigate the safety and tolerability of multiple dose administrations of BO-112 in combination with continued pembrolizumab or nivolumab. This will be evaluated in patients who are currently receiving pembrolizumab or nivolumab for an approved indication (see the respective SmPC), and are clinically stable, but who have not obtained a partial or complete response since the start of pembrolizumab or nivolumab treatment and who either have disease that has continued to progress, or who initially had stable disease with subsequent disease progression observed within 6 months of the start of treatment. Patients who show no response or no durable stable disease after initiating treatment with a PD1 inhibitor potentially may have their treatment 'rescued' by addition of an immune stimulator such as BO-112 and it is important to evaluate the safety and tolerability of such a combination. Continuation of pembrolizumab or nivolumab after first signs of disease progression is described in the SmPC and is considered acceptable clinical practice for an immunotherapy since progression may be 'pseudo-progression’ and some patients have delayed response (10).

The continuation of anti-PD1 treatment with addition of BO-112 is expected to be for 8 or 9 to 12 weeks with a tumor response assessment performed (physical examination and CT scan) according to RECIST v1.1 in the period between Weeks 8/9 and 12. Subjects who show disease progression at this assessment will be discontinued from treatment and the study. For subjects who achieve stable disease or an objective response, combination treatment will continue for up to a total of 1 year (see Sections 2.6 and 6 for details). Preliminary data from 8 subjects enrolled in Part 2 have so far demonstrated an acceptable safety and tolerability profile of BO-112 in combination with nivolumab or pembrolizumab. There have also been some cases of stable disease at the first response assessment. Therefore, treatment with BO-112 will continue in combination with pembrolizumab or nivolumab in cases of stable disease or response, to allow maintenance of a potentially achieved clinical benefit.

2.5 Risks and benefits of the investigational product.

2.5.1 Nonclinical

The toxicology of BO-112 has been adequately evaluated during the nonclinical development following current regulatory requirements for the support of a phase I clinical trial. Relevant toxicity studies included: preliminary dose range finding studies in rats and a maximum tolerated dose (MTD) study in dog and GLP 28-day repeated-dose toxicity in rats and dogs. The pharmacokinetic (PK) profile of the parent compound has been evaluated in 28-day toxicity studies.
In the dose range finding (DRF) study in rats, intravenous (bolus) administration of BO-112 at the dose of 0.7, 1.0 and 1.4 mg/kg twice/week for 14 days (days 1, 4, 8 and 11) resulted in minor and transient treatment related clinical signs such as chromorhinorrhea and piloerection. A remarkable decrease in body weight, body weight gain and food consumption, a statistically significant decrease in RBC, HGB, HCT and reticulocytes, and a significant increase in liver enzymes were found in animals from all treatment groups. At terminal sacrifice, enlargement of the spleen and a decrease in seminal vesicle size was evident. Inflammatory changes in blood vessels, mainly small calipered arteries and capillaries considered to be test item-related were found in the lungs, liver, kidneys and spleen from animals belonging to all treated groups. Based on these results, dose levels of 0.2, 0.45 and 1 mg/kg (2 administrations/week) were selected for further repeated 28-day toxicity study in rats.

In the first of two 28–day repeated dose toxicity study in rats, intravenous (bolus) administration of BO-112 at the dose of 0.2, 0.45 and 1 mg/kg twice/week for 28 days, showed treatment related mortality within 24 hours after second administration in 4 females from the low dose group (0.20 mg/kg) and in 4 males and 6 females from the mid dose group (0.45 mg/kg). All of the decedents showed heavy inflammatory changes at least in the liver. Therefore, liver failure was considered to be the main cause of deaths, likely supported by further inflammatory lesions in other organs (lungs, liver, kidneys and spleen). Furthermore, two additional deaths were recorded upon completion of dosing period, one female from mid dose group and one female from high dose group, belonging to PK and immunostimulation allocation respectively, probably related to the blood sampling procedures although a test item relationship cannot be fully discarded.

Findings reported were quite similar to those reported in the previous DRF study and affected in a different degree all treatment groups (males and females). These findings included piloerection, reduced mobility and apathy mainly appearing after second administration, reduction in body weight, body weight gain and food consumption, decreases in RBC, HGB and HTC, accompanied by an increase in reticulocyte counts and higher ALT, AST, ALP, total bilirubine and GGT levels. Macroscopic findings at terminal sacrifice suggest a dose-dependent enlargement of the spleen, and a decrease of the seminal vesicles and prostate gland. Inflammatory changes in blood vessels, small calipered arteries and capillaries were reported mainly in the lungs, liver, kidneys, and spleen. An explanation for the lack of dose-dependent relationship in mortality could not be determined. However, the primary adverse findings in liver responsible of the main toxicity of BO-112 followed a clear dose-dependent
relationship. Based on these it can be concluded that the STD10 may be the low dose, 0.2 mg/kg. The no observed adverse effect level (NOAEL) could not be established.

A second 28-day repeated dose toxicity study in rats was performed given BO-112 intravenously (bolus) at the dose of 0.09, 0.2 and 0.45 mg/kg once/week and so mimicking the intended dose regime for the phase I clinical trial. No treatment related mortality was observed throughout the study period and only minor and transient treatment related clinical signs such as chromorhinorrhea and piloerection were observed in animals from high dose group (0.45 mg/kg). A slight reduction in body weight, body weight gain and estimated daily food consumption throughout the dosing period was observed in all treatment groups. A dose-dependent statistically significant increase in reticulocytes along with a decrease in red series parameters was observed in males from all test item treated groups (0.09, 0.20 and 0.045 mg/kg) and in females from the high dose group. Additionally, ALP levels were statistically significant higher in males receiving the test item at the three dose levels and AST only at the high dose group. Enlarged spleen size was observed in males from all treated groups and in females from high dose group, and lesions in the liver, kidneys, and spleen were reported and related to inflammatory changes in blood vessels, mainly small arteries and capillaries at all doses. It's noteworthy that the type, incidence and severity of findings following a once weekly dose interval did not differ essentially from those observed at the same dose (i.e., 0.20 and 0.45 mg/kg) following twice weekly interval. An additional non-GLP study suggests a significantly decrease of the severity of findings after 8 weeks of recovery under this schedule of administration, although a complete remission is not observed. However, the mortality was prevented by reducing the frequency of administration. Based on the results reported an under these experimental conditions the STD10 following a once weekly dose regimen can be considered 0.45 mg/kg. The NOAEL could not be established under these conditions.

In a MTD dog study, doses of 0.15 and 0.30 mg/kg were given intravenously (by bolus) twice/week. Higher transaminase levels, and periportal vacuolation, single cell necrosis and hepatocyte pigment were observed in the liver in animals given 0.30 mg/kg/day. Moreover, in the thymus, marked or moderate atrophy and arteritis, characterised by intimal thickening, was reported at this high dose (0.30 mg/kg).

In a 28-day repeated dose study in dogs, the administration of BO-112 intravenously (bolus) at dose levels of 0.1, 0.2 or 0.4 mg/kg twice weekly for four weeks was generally tolerated, all dose levels tested were associated with post dosing observations, body weight loss, and sporadic reductions in food consumption. The severity of these findings led to the early termination of one male and one female given 0.4 mg/kg in Week 3 of the dosing period. On
macroscopic examination, dark areas and pale and/or mottled livers were present in animals given 0.2 or 0.4 mg/kg. On microscopic examination, findings related to administration of BO-112 were noted in liver, thymus, heart and injection sites. Degeneration/regeneration and periportal vacuolation in liver was recorded in the two animal terminal kill given 0.4 mg/kg, and multifocal inflammation, present in all treated animals. The haematological changes, together with the higher liver enzyme activities and total bilirubin levels observed as well in BO-112 treated animals (0.2 and 0.4 mg/kg), were possibly associated with the macroscopic and microscopic findings recorded in the liver, and related to liver damage (correlating to degeneration and necrosis of hepatocytes). Based on the results reported and under these experimental conditions, the NOAEL could not be established, and the highest non-severely toxic dose (HNSTD) was 0.2 mg/kg.

In all toxicity studies both in rats and dogs, lymphoid organ sections stained with antibodies against CD3, CD20 and CD68 were evaluated indicating a normal distribution and quantity of positive staining T-cells, B-cells and macrophages. Therefore, an immune stimulating effect by the test article was excluded. In addition, evaluation of INF-a suggests no stimulation observed after treatment of BO-112 in both species.

Plasma levels of BO-112 were evaluated as part of the 28-day toxicity studies in rats (after once and twice administrations/week) and dogs (twice/week). In both species, exposure increased with increasing doses indicating and approximate dose-related exposure. Overall, T_{max} in rat were within 0.25-3 hours (twice administration/week) and 0.25 to 0.5 hours (once administration/week), while in dog the T_{max} was observed between 0.08 and 0.25 hours. Overall, no accumulation with time was observed after the last administration compared to test day 1 (C_{max}, AUC) neither in rats not in dogs. Both C_{max} and AUC values of BO-112 appeared to be partly lower after the last administration compared to test day 1 in all the studies performed. No noteworthy differences between the genders were observed.

All the toxicity findings observed in both rats and dogs were reversible, totally or partially after 4 weeks of recovery period. However, liver inflammation in rats and dogs, and presence of brown pigment in the liver in rat require a longer period to recover completely.

The vehicle (PEI) was devoid of any toxicity effects when given alone, in rats and dogs at the same dose regime used for BO-112.

Local tolerance was evaluated after intravenous administration in the 28-day repeated dose toxicity studies. Subcutaneous inflammation was present at injection sites of animals given the test article or vehicle, and was not strictly dose-related, suggesting that the local effects were rather related with the method of administration itself. Subcutaneous inflammation was
characterised by oedema and inflammatory cell infiltration in the subcutis in the vicinity of the cephalic vein.

A more detailed description could be consulted in the IB of BO-112 attached as Appendix to this protocol (Appendix 1).

2.5.2 Clinical

Part 1 has been completed and an interim analysis of the data performed. A total of 16 subjects were treated in Part 1: 6 subjects with a single 0.6 mg IT dose, 3 subjects with 3 x 0.6 mg IT dose weekly and 7 subjects with 3 x 1 mg IT dose weekly. It was concluded that single agent treatment with BO-112 was generally well tolerated. The most frequently reported TEAEs (in >15% of subjects overall) were nausea, asthenia, pyrexia (each 4 subjects, 25%), fatigue, and headache (each 3 subjects, 19%). Most TEAEs were grade 1 or grade 2 in severity. A total number of 13 grade 3/4/5 TEAEs occurred in 6 (38%) subjects. Five (31%) subjects experienced a total of 6 serious TEAEs. In 2 (13%) subjects, the serious TEAE had a fatal outcome (intestinal ischaemia and respiratory failure), these were assessed by the Investigator as not related to the study drug. Study drug-related TEAEs occurred in 10 (63%) subjects. The most frequently reported study drug-related TEAEs (in >10% of subjects overall) were pyrexia (3 subjects, 19%), chills, platelet count decreased, and headache (each 2 subjects, 13%). Grade 3/4 study drug-related TEAEs occurred in 2 (13%) subjects (both had platelet count decreased/thrombocytopenia). Systemic exposure appears negligible as all samples analyzed for PK were below the lower limit of detection (62.5 ng/mL). Biological activity was observed in evaluations including immunohistochemistry evaluation of tumor tissue, phenotyping and genetic testing of circulating and tumor microenvironment immune cells. Among these observations were apoptosis and necrosis in the injected lesion, increases in tumor infiltrating CD4+ and CD8+ T- cells and in circulating immune cells, as well as upregulation of genes associated with an anti-tumor immune response.

2.6 Description and justification of method of administration and posology.

Part 1: Single agent dose escalation and multiple dose

BiOncoTech has estimated the starting dose according to the ICH S9 Guideline.

“The goal of selecting the start dose is to administer a pharmacologically active dose that is reasonably safe to use.

…. A common approach for many small molecules is to set a start dose at 1/10 the Severely Toxic Dose in 10% of the animals (STD 10) in rodents. If the non-rodent is the most sensitive species then 1/6 the Highest Non- Severely Toxic Dose (HNSTD) is considered an appropriate start.
The HNSTD is defined as the highest dose level that does not produce evidence of lethality, life-threatening toxicities or irreversible findings.

The preclinical toxicology studies established the STD10 in rat at 0.2 mg/Kg and the HNSTD in dog 0.2 mg/Kg. Considering the S9 recommendation the starting dose could be set at 0.02 mg/kg. Taking into consideration the recommendation made by the AEMPS as part of a Scientific Consultation, Bioncotech has decided to define the starting dose from the lowest dose tested in the most sensitive species (rats), 0.09 mg/kg with a correction of 1/10. The suggested starting dose for this study has been fixed at 0.6mg (0.009 mg/kg).

BiOncoTech proposes to administer the agent intratumorally. This approach has two main benefits i) to concentrate the effect in the tumor itself and ii) to reduce toxicities at relatively high concentrations. The design we are proposing aims to study the PK profile of IT administration of BO-112. Our strategy is to validate the safety profile of the IT administration by gaining a complete understanding of the PK profile.

In this context, we claim a STARTING DOSE of 0.009 mg/Kg.

Update on initial single agent study design:
Review of available data during the conduct of Part 1 led to moving from single (0.6 mg) to multiple administrations (3 x weekly 0.6 or 1 mg) of BO-112 within Part 1 and ultimately to the decision to extend the study with Part 2 to evaluate the combination of intra-tumoral administration of 1 mg BO-112 with systemic anti-PD1 treatment in a new cohort. An overview of the safety data from Part 1 is provided in section 2.5.2.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab
Based on the data presented in section 2.5 and the mechanistic rationale described in section 2.2, the safety and tolerability of combination treatment of BO-112 with pembrolizumab or nivolumab, approved PD1 inhibitors, will be investigated in an extension cohort of this Phase I study.

The extension cohort will evaluate the safety and tolerability of 5 IT administrations of BO-112 at a dose of 1 mg during a period of 8 or 9 weeks, in combination with continued treatment of pembrolizumab or nivolumab in patients who are progressing at the time of study entry. Pembrolizumab or nivolumab will be administered as per the EU SmPC, which allows for continuation of treatment in case of signs of progression in patients who are clinically stable. For the purposes of this Phase I study, additional eligibility criteria are included regarding presence of at least 1 lesion that is accessible for IT injection and biopsy.
At the end of the 8 or 9 week period of combined treatment and before Visit 7 (pembrolizumab) or Visit 8 (nivolumab) in week 12, tumor response will be evaluated by physical examination and by CT scan according to RECIST v1.1 during an ‘unscheduled’ visit to allow continuation of treatment in case of an objective response (partial or complete) or stable disease. In such cases, IT administration of BO-112 will be continued at regular intervals (see section 6 for details) for up to one year as long as there is clinical benefit, it is tolerated and there is a lesion (or lesions) amenable for IT injection, (note that complete response means that there is no lesion available for injection and BO 112 can then not be administered). Background treatment with pembrolizumab or nivolumab will also continue as per the SmPC for up to a total of 1 year. Treatment will continue as per protocol or until dose limiting toxicity, disease progression or subject/investigator withdrawal for other reasons.

In case the lesion used for IT injection is no longer amenable for IT injection, then another lesion which is amenable should be used. If no lesion is accessible for IT injection at the scheduled visit, then that BO-112 dose administration will not be done at that visit and only pembrolizumab or nivolumab will be administered. Subsequent, scheduled administrations of BO-112 should continue if accessible lesions are present.

For details of the treatment schedule, see section 6.

2.7 Ethics and loyal aspects.

This trial will be carried out following the content of the present protocol, according to ICH Good Clinical Practice Guidelines and all required laws (see details in Section 12).

2.8 Description of the study population.

Part 1: Single agent dose escalation and multiple dose: Patients with aggressive solid tumors from whom biopsies can be obtained. Injected lesions must be palpable and biopsiable at the time of injection, and biopsied after 7-14 days. Patients will not receive an alternative therapy during the period comprising from first and second biopsy.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab: Patients with radiologic disease progression during ongoing treatment with pembrolizumab or nivolumab for any approved indication as described in the SmPC, and who have never had a partial or complete response to the current pembrolizumab or nivolumab treatment. In case of stable disease on current anti-PD1 treatment, only initial stable disease followed by disease progression within 6 months of the start of pembrolizumab or nivolumab is allowed. Patients
must be clinically stable at time of study entry and must have at least 1 lesion which is accessible for intra-tumoral injection and biopsy. This lesion may be the only lesion present. Lesions are not required to be measurable as per RECIST v1.1 criteria, since the primary endpoint is safety. The response assessment may therefore be based on non-measurable lesions if these are the only ones present.

3 STUDY OBJECTIVES

Primary objective

Part 1 single agent dose escalation and multiple dose: To determine the biological effect of a IT administration of BO-112 as single agent.

Part 2: extension cohort of BO-112 in combination with pembrolizumab or nivolumab: To determine the safety and tolerability of combination of BO-112 with pembrolizumab or nivolumab

Secondary objectives

Part 1 single agent dose escalation and multiple dose:

- To determinate the safety profile and tolerability of BO-112 in patients enrolled in the study.
- To establish the PK profile of BO-112 administered intratumorally
- To explore pharmacodynamics (PD) markers of the BO-112
- To monitor changes in the profiles of innate, specific, complement, and NK mediated immune response
- To evaluate the preliminary antitumor activity of the drug

Part 2: extension cohort of BO-112 in combination with pembrolizumab or nivolumab:

- To evaluate the biological activity of BO-112 in combination with pembrolizumab or nivolumab
- To establish the PK profile of BO-112 administered intratumorally
- To explore pharmacodynamics (PD) markers of BO-112 and pembrolizumab or nivolumab
- To monitor changes in the profiles of innate, specific, complement, and NK mediated immune response
- To evaluate the preliminary antitumor activity of the BO-112 in combination with pembrolizumab or nivolumab
4 OVERALL STUDY DESIGN

4.1 Study endpoints.

Part 1 single agent dose escalation and multiple dose:

**Primary endpoint**
- BO-112 biological effect.

**Secondary endpoints**
- Safety profile and tolerability
- PK profile
- PD markers
- Immune profiles changes
- Preliminary antitumor activity

Part 2: extension cohort of BO-112 in combination with pembrolizumab or nivolumab:

**Primary endpoint**
- Safety & tolerability

**Secondary endpoints**
- Biological effect
- PK profile
- PD markers
- Immune profiles changes
- Preliminary antitumor activity

4.2 Study design.

This is a first-in-human Phase I exploratory study of biopsy-accessible tumors to determine the safety of IT administration of BO-112 as a single agent in a standard 3 + 3 subjects design, both as single dose and multiple dose administrations, and subsequently in an extension cohort in combination with pembrolizumab or nivolumab, both PD1-inhibitors. Additionally, this study will also study BO-112 biological activity, the innate and adaptive immune system response and signalling pathways, as well as signs of clinical relevance.
Part 1: Single agent dose escalation and multiple dose

BO-112 will be administered at a starting dose of 0.6 mg. Upon confirmation of the safety profile of the starting dose and evaluation of the PK profile, three additional dose levels (1 mg, 1.6 mg and 2.4 mg) may be tested, following a modified Fibonacci design with dose increases of 60% of previous doses. The protocol was amended after the first cohort of 0.6 mg single dose administration, based on initial safety data and signs of biological activity to allow multiple dose IT administrations (2 – 3) at a dose level of 0.6 and 1 mg.

Between each dose level, an interim analysis of safety and tolerability will be performed for all dosed subjects. A Dose Escalation Meeting will be held between the investigator and the sponsor to evaluate the available data. Once a dose level is judged to be safe, the next dose level can be administered. Pharmacokinetic data will be taken into account within each cohort of subjects. An external Safety Review Committee will review the conclusions made by the Dose Escalation Meeting, providing guidance when required.

Upon completion of the first cohort (0.6mg) with a single intra-tumoral (IT) administration and after relevant information obtained regarding the biological effect of BO-112, a multi-dose scheme (two-three administrations, one administration per week) will be applied for the 0.6 mg and 1 mg dose cohorts. Dose escalation and DLT criteria for a multi-dose scheme will be followed as described in section 6.2.

Patients that have received BO-112 in a single-dose scheme could be included in the next cohorts testing multi-dose scheme if there is any benefit and no additional treatment can be offered to them.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab

Study Flow:
Preliminary data from the single and multiple dose cohorts from Part 1 will guide the selection of the BO-112 dose to be used in an extension cohort of BO-112 in combination with continued treatment of pembrolizumab or nivolumab in patients who have disease progression at the time of study entry. Pembrolizumab or nivolumab will be administered as per the SmPC, which allows for continuation of treatment in case of signs of progression in patients who are clinically stable. For the purposes of this Phase I study, additional eligibility criteria are added regarding the presence at study entry of at least 1 lesion that is accessible for IT injection and biopsy.

The safety and tolerability of multiple administrations of 1 mg BO-112 in combination with pembrolizumab or nivolumab will be assessed for future use as the recommended dose in Phase 2 combination treatment clinical trials. BO-112 will be administered IT at a dose of 1 mg for 5 administrations over a period of 8 weeks (in combination with nivolumab) or 9 weeks (in combination with pembrolizumab). Pembrolizumab or nivolumab will be administered IV every 3 weeks or every 2 weeks respectively as per the SmPC. During this period, safety and tolerability will be monitored. In addition, immunological biomarkers will be evaluated in blood and tumor tissue samples. At the end of the 8 or 9 week period of combined treatment and before Visit 7 (pembrolizumab) or Visit 8 (nivolumab) in week 12, tumor response will be evaluated by physical examination and by CT scan according to RECIST v1.1 criteria to allow continuation of treatment in case of an objective response (partial or complete) or stable disease. In case of treatment continuation, IT administrations of 1 mg BO-112 will be given at regular intervals for up to one year for as long as there is a clinical benefit, it is tolerated and there is a lesion or lesions amenable for IT injection (note that complete response means that there is no lesion available for injection and BO-112 can then not be administered). Background treatment with pembrolizumab or nivolumab will continue as per the SmPC for up to a total of 1 year. In case of disease progression at any response assessment, treatment with BO-112 and pembrolizumab or nivolumab will be discontinued and the subject taken off the study.

In case the lesion used for IT injection is no longer amenable for IT injection, then another lesion which is amenable should be used. The 1 mg dose to be administered in a volume of 2 mL may also be divided across the original and other lesion(s) in case the original lesion has decreased in size and cannot be safely injected (risk of extravasation) with the full dose. If no lesion is accessible for IT injection at the scheduled visit, then that BO-112 dose administration will not be done at that visit and only pembrolizumab or nivolumab will be administered. Subsequent, scheduled administrations of BO-112 should continue per protocol if accessible lesions are present.
4.3 Study treatments: doses and schedules.
Treatment is described in detail in Section 6.1.

4.4 Criteria for discontinuation from study.
Subjects may withdraw their consent to participate in this study at any time without prejudice. The investigator must withdraw from the study any subject who requests to be withdrawn (preferably after consultation with the study medical monitor). A subject’s participation in the study may be discontinued at any time at the discretion of the investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the End of Study (EOS) visit should be carried out.

BiOncoTech must be notified of all subject withdrawals as soon as possible. BiOncoTech also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual investigator or site for poor enrollment or noncompliance.

Reasons for which the investigator or BiOncoTech should withdraw a subject from the study include, but are not limited to, the following:

- Subject experiences disease progression
- Subject experiences toxicity that is determined by the investigator to be no longer safe for the subject to continue therapy
- Subject requests to withdraw from the study treatment
- Subject requires or has taken medication prohibited by the protocol
- Subject is unwilling or unable to comply with the study requirements
- Subject was erroneously admitted into the study or does not meet entry criteria
- Subject is lost to follow-up
- Subject becomes pregnant

4.5 Drug accountability and control of the study products.
Investigational Product Supplies (IPS) will be provided to the participant hospitals. The Sponsor will provide labelled drug. Each container of BO-112 will have an investigational-use label permanently affixed to the outside and will be labelled in accordance with local regulations, stating that the drug is for clinical trial use only and should be kept out of reach of children. Labels will include blank lines for the patient enrolment code, visit and date dispensed. The medication provided for this study is for use only as directed in the protocol. It is the investigator/institution’s responsibility to establish a system for handling investigational medicinal products, so as to ensure that:
• Deliveries of such products from BiOncoTech are correctly received by a responsible person
• Such deliveries are recorded
• Study treatments are handled and stored safely and properly
• Study treatments are only dispensed to study patients in accordance with the protocol.
• Any unused supply of BO-112 will be returned to the Sponsor or its representative upon completion of the trial or destroyed at site following written approval from the Sponsor.

Background treatment: pembrolizumab (Keytruda) or nivolumab (Opdivo) will be sourced via the hospital pharmacy and will be reimbursed by the Sponsor. In line with the Guidance on Investigational Medicinal Products (IMPs) and Non-Investigational Medicinal Products (NIMPs) (rev 1 March 2011), pembrolizumab and nivolumab are considered NIMPs with a marketing authorisation in the EU. Data on which background treatment, pembrolizumab or nivolumab, and on date, dose and route of administration during the study will be collected. Both products are subject to restricted medical prescription and will be dispensed by the hospital pharmacy and administered at the investigational site.

4.6 Maintenance and opening of the randomization codes.
Not applicable: this is an open labelled study design.

4.7 Source data.
It is a prerequisite of this study that the study monitor has direct access to source data for data verification. This will be done by comparing data from the CRFs with those in the subject’s medical notes.

4.8 Trial end.
The end of the study is defined as last visit last patient.

5 SELECTION CRITERIA
All patients must fulfill ALL inclusion criteria and NONE exclusion criteria. To avoid the inclusion of ineligible patients, any doubts should be discussed with the Sponsor. If a patient does not fulfill eligibility criteria and is included inadvertently in the study, the Sponsor should be informed. The Sponsor will decide whether the patient should continue in the study by evaluating the risks and benefits for the patient and guaranteeing the patient’s maximum safety.
5.1 Inclusion criteria.

1. Able and willing to give voluntary written informed consent before being enrolled in the study. The subject must sign the informed consent form (including their consent for the analysis of biological samples of tumor and blood) prior to any study-related procedures and agree to the schedule of assessments.

2. Patients age 18 years or more on the day of signing informed consent form.

3. Histologically or cytologically confirmed aggressive solid tumors

4. Patients must have:
   1. Biopsy-accessible tumors
   2. No prior anticancer treatment during the last 14 days; except for Part 2 extension cohort, where ongoing treatment with pembrolizumab or nivolumab is required (in case of nivolumab treatment in combination with ipilimumab, then ipilimumab must be stopped at time of study entry).

5. Eastern Cooperative Oncology Group (ECOG) performance status (PS) 2.

6. An expected survival of ≥ 3 months.

7. Patients with the following hematologic values:
   • Hemoglobin (Hb) ≥ 10 g/dL
   • Absolute Neutrophil Count (ANC) ≥1.5 x 10^9/L
   • Platelets ≥ 100 x 10^9/L

8. Patients with the following biochemical values:
   • Bilirubin ≤ 1.5 x upper limit of normality (ULN)
   • Aspartate aminotransferase (AST) and Alanine transaminase (ALT) 3 x ULN. Nevertheless, in patients with documented liver metastases, AST and/or ALT could be ≤ 5 x ULN.
   • Serum creatinine ≤ 1.5 x ULN or CrCl ≥ 40 mL/min on the basis of measured CrCl from a 24-hour urine collection or Cockcroft-Gault glomerular filtration rate estimation: (140-age) x (weight in kg) x (0.85 if female) / 72 x (serum creatinine in mg/dL).

9. Female patients of childbearing potential and male patients with partners of childbearing potential must agree to the use of 2 highly effective forms of contraception in combination (male condom plus one of the methods listed in Appendix 4) or must totally/truly abstain from any form of sexual intercourse, throughout their participation in the study and for at least 3 months after last dose of study drug. For Part 2, if combination with pembrolizumab this is 4 months after last dose of pembrolizumab; if combination with nivolumab, this is 5 months after last dose of nivolumab as per SmPC.
1. Females of childbearing potential are defined as sexually mature women without prior oophorectomy or hysterectomy who have had menses within the last 12 months.

2. Females are not considered to be of childbearing potential if they have been with continuous spontaneous amenorrhea for >12 months and follicle-stimulating hormone (FSH) level ≥ 40 IU/L.

3. For females who have been with continuous spontaneous amenorrhea for ≥ 2 years, the requirement for FSH measurement at screening will be waived.

10. Patients should be able to report on their condition while on the treatment.

11. Additional inclusion criteria for Part 2 extension cohort:

   1. At study entry, patients must have radiologic disease progression during ongoing monotherapy with pembrolizumab or nivolumab (monotherapy or in combination with ipilimumab) for any approved indication as described in the SmPC at the time of study entry and have never had a partial or complete response to the current pembrolizumab or nivolumab treatment;
      * In case of stable disease on current treatment: only initial stable disease followed by disease progression within 6 months of the start of pembrolizumab or nivolumab is allowed.

   2. Clinically stable at the time of study entry.

   3. Able to continue treatment with pembrolizumab or nivolumab.

   4. Progression amenable to further immunotherapy in the opinion of the Investigator.

   5. At least 1 lesion which is accessible for intra-tumoral injection and biopsy (visible, palpable or with the aid of imaging techniques).

5.2 Exclusion criteria.

   1. Other relevant and clinically significant concomitant diseases or adverse clinical conditions which may jeopardize patient safety:
      * Increased cardiac risk: congestive heart failure; or unstable angina pectoris; or arrhythmia requiring treatment or uncontrolled arterial hypertension; or myocardial infarction within 12 months before inclusion in the study.
      * Patients with active central nervous system (CNS) lesions (including carcinomatous meningitis) will be excluded. However, patients will be eligible if:
         * Clinical interventions with surgery and/or radiotherapy and/or radiosurgery had finalized at least 4 weeks prior to receive BO-112 and they do not require treatment with high doses of steroids (>10mg/24h prednisone or equivalent).
There is not requirement, per investigator criteria, of immediate clinical interventions with surgery and/or radiotherapy and/or radiosurgery and they do not require treatment with high doses of steroids (>10mg/24h prednisone or equivalent).

- Active infection.
- Significant non-neoplastic liver disease (e.g., cirrhosis, active chronic hepatitis B or C).
- Any clinically significant abnormality on history or examination including diagnosis of immunodeficiency or receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study medication (physiologic doses of corticosteroids may be approved after consultation with the Sponsor).

2. Patients who are pregnant or breastfeeding. Women of childbearing potential must have a negative blood pregnancy test performed within 7 days before the onset of treatment for Part 1. For Part 2 pregnancy test is to be done during screening and repeated on Day 1 if screening test was done more than 3 days before.

3. Substance abuse or clinical, psychological or social conditions that can undermine the validity of the informed consent or protocol compliance.

4. Patients who present any contraindication or suspected allergy to the product compounds under investigation in the study.

5. Simultaneous participation in any other study involving an investigational medicinal product, or having participated in a study less than 14 days prior to the start of study treatment.

6. Impossibility to comply with treatment due to cultural or geographic circumstances.

7. Any condition that is unstable or could endanger the patient’s safety and/or the patient’s compliance with the study.

8. Additional exclusion criteria for Part 2 extension cohort:
   1. Grade 3-4 toxicity during treatment with pembrolizumab or nivolumab which has only recovered to grade 2 or more.
   2. Permanent discontinuation of pembrolizumab or nivolumab due to immune-related or other adverse reaction.
   3. Use of chronic steroids >10mg/24h prednisone or equivalent.

5.3 Withdrawal procedures.

Subjects should be withdrawn from the study treatment under any of the circumstances specified in Section 4.4.
Subjects who themselves decide to discontinue should always be asked about the reasons for their discontinuation and the presence of any AE. The date and reasons for any premature interruption will be recorded in the CRF and will be taken into account in the final evaluation.

If possible, subjects who withdraw should be seen and assessed by an investigator. After discontinuation from treatment, patients must be followed up for all existing and new AEs for 30 calendar days and followed until resolution unless, in the opinion of the investigator, the condition is unlikely to resolve due to the patient's underlying disease.

If treatment is terminated, follow-up of the patient will continue according to section 6.2.

6 TREATMENT DESCRIPTION

6.1 Study treatment

Part 1: Single agent dose escalation and multiple dose

BO-112 will be administered at a starting dose of 0.6mg (0.009 mg/kg)*: the volume to be administered will be fixed at every dose level as follows: 0.6mg – 1ml and 1mg – 2ml; (1.6mg-3ml and 2.4mg – 4ml dose levels have not been evaluated due to biological activity already observed at both 0.6 mg and 1 mg dose levels).

(*) Dose increase will be confirmed after a dose escalation meeting between sponsor and sites.

Accessible palpable site of disease will be selected by the principal investigator. Selected sites of injection will be based on the longest lesion dimension being 1cm or more. An ultrasound guided puncture could be performed at the investigator election. BO-112 injection volume could be split in different lesions.

Suggested injection volume based on lesion size (this table is only a guidance and is not mandatory)

<table>
<thead>
<tr>
<th>Lesion size (longest dimension)</th>
<th>BO-112 injection volume</th>
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</thead>
<tbody>
<tr>
<td>&gt;1 cm-2cm</td>
<td>1mL</td>
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<tr>
<td>&gt;2 cm-3cm</td>
<td>2mL</td>
</tr>
<tr>
<td>&gt;3 cm-4cm</td>
<td>3mL</td>
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<tr>
<td>&gt;4 cm</td>
<td>4mL</td>
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</tbody>
</table>

Doses of BO-112 will be administered in the presence of a physician or nurse who will monitor the subject for at least 30 minutes after injection, including a determination of blood pressure, heart rate, and respiratory rate before and after injection.

Upon confirmation of the safety profile of the starting dose of 0.6 mg and evaluation of the PK profile, three additional dose levels (1 mg, 1.6 mg and 2.4 mg) may be tested. Subsequently only two dose levels, 0.6 mg and 1 mg, have been tested as multiple doses. Cohorts of three
subjects per dose level will be treated consecutively, in the absence of Dose Limiting Toxicity (DLT). If none of the three subjects in a cohort experiences DLT, three more subjects will be treated at the next higher dose level. However, if DLT is observed in only 1 subject in a cohort of 3 subjects, 3 additional subjects will be enrolled up to a total of 6 subjects at this dose level. The dose escalation will continue until at least two subjects among a cohort of three to six subjects experience a DLT within the 7 days post BO-112 first administration (i.e. ≥ 33% of patients with DLT at that dose level).

The sponsor will assess the data obtained from the different cohorts to decide when the clinical trial must be finalized.

The first subject in every cohort will be observed during at least 7 days post-injection before including the remaining two patients. All subjects within a dose level will be followed for at least 7 days before any dose escalation is defined for the next dose level.

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab

BO-112:
The IT injection will be administered by a physician. The subject should remain under observation by a physician or nurse for 30 minutes after each IT injection. Specific monitoring of heart rate, blood pressure, or respiratory rate should be done as clinically indicated. Each subject will be treated with 1 mg BO-112 administered as a 2 ml IT injection in a lesion or lesions amenable to injection. In case the initial lesion is no longer amenable to injection, an additional or alternative, amenable lesion(s) should be used for injection. In case the original lesion is no longer present and there is no alternative lesion for injection, then BO-112 will not be administered at that visit. Subsequent scheduled doses should be administered per protocol if an accessible lesion is present. There will be no replacement visits or replacement dose administrations for missed doses due to absence of accessible lesion.

Pembrolizumab or nivolumab:
These will be administered in accordance with the SmPC and institutional practice as valid at the time of study entry:
- pembrolizumab: as an intravenous (IV) infusion over 30 minutes every 3 weeks at the dose administered prior to study entry: 200 mg for NSCLC that has not been previously treated with chemotherapy, classical Hodgkin lymphoma or urothelial carcinoma; 2mg/kg for NSCLC that has been previously treated with chemotherapy or for melanoma (see Appendix 6 for SmPC).
- nivolumab: 3 mg/kg nivolumab administered intravenously (IV) over 60 minutes every 2 weeks; the dosing regimen has been updated on 23 April 2018 with 240 mg as a 30-minute IV
infusion every 2 weeks for melanoma, renal cell carcinoma, NSCLC, classical Hodgkin lymphoma, squamous cell carcinoma of the head and neck and urothelial carcinoma; or 480 mg as a 60-minute IV infusion every 4 weeks for melanoma and renal cell carcinoma. Depending on the institutional standard of care, the previous or new 2-weekly dose regimens are both acceptable for this study: the subject should continue with the dose regimen as started prior to the study. In case the 480 mg 4-week regimen was started, then this should be switched to the 240 mg 2-week regimen to be able to follow the combined treatment schedule (see Appendix 7 for SmPC).

The administration schedule is as follows (also see schematic representation below and tables 4 and 5 in section 7):

First 12-week ‘cycle’:

- BO-112 (background treatment pembrolizumab): IT administration on Days 1, 8, 15, 36 and 57 (5 administrations in total) over a period of 9 weeks; in case of treatment continuation after response assessment see below.

- BO-112 (background treatment nivolumab): IT administration on Days 1, 8, 22, 36 and 50 (5 administrations in total) over a period of 8 weeks; in case of treatment continuation after response assessment see below.

- pembrolizumab: IV infusion every 3 weeks (re-)starting on Day 15, at least 2 hours after the BO-112 administration, up to the response assessment; in case of treatment continuation after response assessment see below.

- nivolumab: IV infusion every 2 weeks (re-)starting on Day 8, at least 2 hours after the BO-112 administration up to the response assessment; in case of treatment continuation after response assessment, see below.

At the end of the 8 or 9 week period of combined treatment and before Visit 7 (pembrolizumab) or Visit 8 (nivolumab) in week 12, tumor response will be evaluated by physical examination and by CT scan, to allow continuation of treatment for subjects who have an objective response (partial or complete) or stable disease according to RECIST v1.1. Subsequent response assessments will be done between 10 – 14 weeks after the previous assessment (or earlier if clinically indicated) to justify continued treatment.

Subsequent 12-week ‘cycles’ in case of treatment continuation:

- BO-112 will be administered IT at regular intervals for 4 administrations over a 12-week period (see schematic for separate pembrolizumab and nivolumab groups since intervals differ) for up to a total of 1 year, as long as there is a clinical benefit, it is tolerated and there is a lesion or lesions amenable for IT injection (note that complete
response means that there is no lesion available for injection and BO-112 cannot then be administered)

- pembrolizumab or nivolumab will continue as long as there is a clinical benefit, it is tolerated as per the SmPC, for up to a total of 1 year.

In case of disease progression before or at the scheduled response assessments, treatment with BO-112 and pembrolizumab or nivolumab will be discontinued and the subject taken off the study.

Schematic: treatment and assessment schedule for Part 2 extension cohort

**Pembrolizumab group**

<table>
<thead>
<tr>
<th>STUDY PERIOD</th>
<th>1st response assessment W5-12</th>
<th>Maintenance treatment period</th>
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<tbody>
<tr>
<td>Routine care</td>
<td>Screening</td>
<td>Dose intense treatment period</td>
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- Pembrolizumab start q3w

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<th>Week</th>
<th>Pembrolizumab IV infusion</th>
<th>Biopsy pre-BO-112</th>
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**Nivolumab group**

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</table>
**Treatment interruption (study drug and/or background treatment)**

In case a scheduled administration of BO-112, in combination or not with pembrolizumab or nivolumab, cannot be given due to an adverse event, then the combined treatment should be given as soon as the adverse event resolves sufficiently in the opinion of the Investigator and as follows:

- if the combined treatment can be given within 3 days of the originally scheduled date, then the subsequent treatment and assessment schedule should be followed without change and on the originally scheduled dates;

- if the combined treatment can only be given 4 days or more after the originally scheduled date, then the subsequent treatment and assessment schedule should be shifted out by an interval of 1 calendar week, up to a maximum of 4 calendar weeks. If the interruption lasts longer than 4 weeks, the case should be discussed between the Investigator and the Sponsor.

If the adverse event is an immune-related adverse event as described in section 4.2 of the SmPC for pembrolizumab or nivolumab for which treatment with pembrolizumab or nivolumab should be withheld, then BO-112 administration should also be interrupted (if applicable) and treatment should only be re-started with pembrolizumab or nivolumab as described in the SmPC and with BO-112 if applicable and following the instructions in the preceding paragraph. **Important exception:** if it is an immune-related adverse reaction for pembrolizumab or nivolumab as described in the SmPC section 4.2 which requires permanent discontinuation, then refer to section 6.2 below.

**BO-112 preparation and storage**

BO-112 will be supplied as a single-in-use vial ready to be diluted in glucose 5% (control article) to reach the volume needed for the administration. BO-112 should be stored at 4°C in dark. The initial test item light suspension (0.6 mg/mL) will be diluted with 5% glucose and mixed thoroughly until homogenization (avoid vortex).

The formulation will be performed just before each administration, which will be performed within 2 hours after preparation.

BO-112 light suspension for injection can be filtered just before administration to reassure absence of adventitious contamination or particles.

Formulated light suspension could be storage at room temperature for a period of 2 hours prior to administration.

Standard aseptic technique to make injectable dilutions will be used.

Suitable, adequate precision, sterile syringes and connections with sterile needles will be used.

Suitable sterile pipettes will be used for the dilution procedures.
Empty sterile vials, which have a suitable volume in accordance with the envisaged total volume of injection will be prepared.

6.2 Dose modifications and management of dose limiting toxicity.

Dose limiting toxicity

Parts 1 and 2: Dose limiting toxicity (DLT) is defined as any one of the following BO-112 related toxicities that occur after BO-112 administration and requires discontinuation of BO-112. These should be reported as SAEs:

- Hematological treatment-related AEs:
  - Any grade 4 neutropenia (ANC < 0.5 x10⁹/L) lasting more than 5 days.
  - Febrile neutropenia (ANC <1.0 x10⁹/L with a single temperature of >38.3 °C or a sustained temperature of ≥38.0 °C for more than one hour.
  - Any grade 4 thrombocytopenia.
  - Grade 3 thrombocytopenia associated with bleeding requiring platelet transfusion, or lasting more than 7 days.

- Any grade 3-4 non-hematological AE, except for the following:
  - Grade 3 nausea and vomiting, diarrhea, with appropriated prophylactic or therapeutic measures been administered
  - Grade 3 elevation of hepatic transaminases lasting less than 7 days, and
  - Non-clinically relevant biochemical abnormalities [i.e., isolated increase of gamma-glutamyltransferase (GGT)]

- Inability to complete the treatment due to toxicity (only to be reported as a SAE if other criteria for being assessed as ‘serious’ are applicable).

In addition for Part 2 only:
Pembrolizumab or nivolumab treatment may be continued in case of a DLT related to BO-112 which requires discontinuation of BO-112, if the DLT is assessed by the Investigator as not related to pembrolizumab or nivolumab and if it is not an immune-related adverse reaction as described in section 4.2 of the SmPC.
Pembrolizumab or nivolumab treatment should be permanently discontinued for any immune-related or other adverse reaction as described in section 4.2 of the SmPC. The event leading to the discontinuation will be reported as a SAE. If this occurs during the period of combined treatment with BO-112, also BO-112 must be discontinued and the subject taken off study with completion of an end-of-treatment visit as described in section 7.4. The reason for discontinuation will be documented at the end-of-treatment visit.
Management of dose limiting toxicity – Part 1 only

In order to define DLT, patients should not be prophylactically prescribed G-CSF support, antiemetics, anti-diarrhoeals or antipyretics during therapy. If a patient experiences grade 3 or greater nausea and/or vomiting and/or diarrhoea, medical intervention should occur, including prophylactic administration of these agents for subsequent doses as indicated.

As soon as one dosed patient shows a DLT in a cohort of 3 subjects, 3 additional subjects will be enrolled up to a total of 6 subjects at this dose level. If 2 of 3 patients show DLT, the next 3 patients (and remaining patient in the cohort) will be treated with a dose 25% lower than the dose used in the cohort ongoing.

In the event a patient included in a multi-dose cohort will present a DLT after first administration, treatment will be discontinued and patient will be replaced.

The establishment of the next escalation steps will be discussed between sponsor and sites during a review dose escalation meeting. An external Safety Review Committee will review the conclusions made by the Dose Escalation Meeting, providing guidance when required.

In case of DLT, at least four patients will be observed during 7 days post-injection before including the remaining patients.

All toxic effects will be graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0, and will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

6.3 Concomitant medication permitted and prohibited.

Any medications, with the exceptions noted below (“prohibited treatments”), which are considered necessary for the patient’s welfare, and which it is believed will not interfere with the study medication, may be given at the discretion of the investigator. All relevant concomitant medications should be recorded in the CRF.

In addition, any unplanned diagnostic, therapeutic or surgical procedure performed during the study period should be recorded in the CRF, including the date, indication, description of the procedure(s) and any clinical finding.

During the study visits special attention will be paid to questioning patients in relation to any self-medication.

Prohibited treatments

- No other chemotherapy, immunotherapy – except for pembrolizumab or nivolumab in Part 2 extension cohort –, hormonal therapy or other novel agent is to be permitted during the course of the study for any patient. Moreover, patients must not receive any other concurrent anti-cancer therapy while on study treatment. Patients may continue
the use of biphosphonates for bone disease, as well as ongoing luteinizing hormone-releasing hormone agonists (LHRH) and somatostatin analogues. Palliative radiotherapy may be used for treatment of pain in the site of bony metastases that were present at baseline providing the investigator does not feel that these are indicative of clinical disease progression during the study period. Corticosteroids are not permitted, except as indicated in exclusion criteria number 1.

- The use of any herbal/natural products or other "folk remedies" should be discouraged.

### 7 GENERAL EVALUATIONS

#### 7.1 Biological activity.

Biological activity comprises:

- Induction of tumor necrosis/apoptosis
- Induction of innate and/or an adaptive, specific anti-tumor T cell immune response in the injected tumor lesion and also systemically.

#### 7.2 Immune response.

Tumoral tissue, serum will be obtained at different time points. The following correlative research studies will be carried out:

<table>
<thead>
<tr>
<th>Table 2: Evaluation of immune response</th>
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<tbody>
<tr>
<td><strong>Type of study</strong></td>
</tr>
<tr>
<td>Immunohistochemistry: CD8 and CD4 T cells, PDL-1, PDL-2, PD1, GranzymeB, and apoptotic markers (possibility of multiplex immunofluorescence)</td>
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<tr>
<td>Type of study</td>
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<td>------------------------------------------------------------------------------</td>
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<tr>
<td>possible, otherwise from lesion to be injected. Only one biopsy is requested.</td>
</tr>
</tbody>
</table>
| Plasma concentration: IFN; other cytokines will be assessed                  | Blood                                    | **Part 1**: Day 0, day 2\(^{(a)}\) and day 8 post last injection (+/- 2 days)  
(a) For 2\(^{\text{nd}}\) and 3\(^{\text{rd}}\) administrations: The sample will be obtained the day of the administration. Timepoint 240’ (+/-30 min)  
**Part 2**: D1 (pre-dose), D2 (approx. 24 h +/- 2 h post dose of D1), AND  
- pembrolizumab combination: D15, D36, D57 (all pre-BO-112 dose),  
- nivolumab combination: D8, D36, D50 (all pre-BO-112 dose) |
| Phenotyping of blood cell populations.                                       | Blood                                    | **Part 1**: Day 0, day 2\(^{(b)}\) and day 8 post last injection (+/- 2 days)  
(b) For 2\(^{\text{nd}}\) and 3\(^{\text{rd}}\) administrations: The sample will be obtained the day of the administration. Timepoint 240’ (+/-30 min).  
**Part 2**: D1 (pre-dose), D2 (approx. 24 h +/- 2 h post dose of D1), AND |
<table>
<thead>
<tr>
<th>Type of study</th>
<th>Tumoral tissue/Blood (1)</th>
<th>Time*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- pembrolizumab combination: D15, D36, D57 (all pre-BO-112 dose), - nivolumab combination: D8, D36, D50 (all pre-BO-112 dose)</td>
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<tr>
<td>Pharmacokinetics</td>
<td>Blood</td>
<td><strong>Part 1</strong> Day 1 (c): time points 0-15'-30' (+/-5min) - 240' (+/-30min) -24h (+/-1h) post injection. (Day 2). (c) For 2nd and 3rd administration time points will be: 0-15'-30' and 240'. <strong>Part 2</strong>: D1 30' pre-BO-112 +/- 15' and 4h post-BO-112 +/- 30'</td>
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* Part 1: In the event a patient receives two or three administrations, determinations described on Day 8 will occur 7 days after the last administration (+7 days window). Except for tumor tissue post-injection that could occur 7 days after any of the planned administrations (+7 days window).

(1) Part 1: In the event a patient receives two or three administrations, blood and tumor tissue determinations will occur as described in table 2. Pharmacokinetics for second and third administrations will be on 0-15'-30' and 240' time points. For patients that have received BO-112 in a single-dose and will be included in the multi-dose scheme cohorts, no new tumor tissue will be obtained.

### 7.3 Pharmacokinetics/pharmacodynamics.

Pharmacokinetics evaluation of BO-112 will be performed using a ligand-binding assay technique (ELISA) using two specific antibodies against the active ingredient of BO-112, Poly I:C, previously extracted from plasma. Blood aliquots will be collected and processed from patients at specific time points (0'-15'-30'-4h and 24h post-administration in Part 1; and at 30' pre-administration and 4h post-administration of BO-112 for Part 2) and kinetic parameters (Cmax, AUC) will be obtained from Poly I:C levels to have a clear indication of BO-112 dynamics in blood.

### 7.4 Clinical trial calendar and procedures.

Subjects are considered to have entered the study on the date that the Sponsor confirms registration of the subject by approving and signing the registration form.
Baseline

The following assessments and procedures should be performed within 14-28 days prior to first dose of study treatment for Part 1 and between 1-28 days for Part 2. For details of the schedule and nature of the assessments, see Table 3 for Part 1 and Tables 4 and 5 for Part 2.

- Signed informed consent
- Date of birth and race
- Menopausal status; serum pregnancy test for women of childbearing potential (preferably within 7 days prior to treatment start)
- Clinical history
- Current and concomitant medications including previous cancer therapies (if applicable)
- Physical examination, ECOG performance status, vital signs (blood pressure and pulse rate, body temperature)
- 12-lead ECG
- Height and Weight
- In-and exclusion criteria verification

Laboratory assessments:
  - Hematology: hemoglobin, red blood cell count (RBC), platelet count, white blood cells, differential white cell count, absolute neutrophil count, activated partial thromboplastin time (APTT), and international normalised ratio (INR)
  - Clinical chemistry: urea, creatinine, ALT, AST, ALP, lactate dehydrogenase, albumin, total bilirubin, direct bilirubin, total protein, sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium
  - Urinalysis: blood, protein and glucose

- Tumour assessment: all biopsy-accessible lesions will be assessed in order to select the lesion to be injected. The longest lesion dimension will be measured (target lesion).
- Adverse events & concomitant medications

Part 2: extension cohort of BO-112 in combination with pembrolizumab or nivolumab

- Baseline CT scan for assessment of tumor burden (CT scans performed within 28 days of 1st dose may be used as baseline and do not need to be repeated, unless clinically indicated).
- Tumor burden will be assessed according to RECIST v1.1 criteria. It is acceptable that there are no measurable lesions as per these criteria. It is also acceptable that there is only one lesion present.
On trial assessments

The following assessments will be performed at time points specified in the study plan (Table 3 for Part 1 and Tables 4 and 5 for Part 2).

Part 1: Single agent dose escalation and multiple dose
- Physical examination including ECOG performance status and vital signs
- Hematology, clinical chemistry and urinalysis
- PK samples
- Adverse events (AEs) & concomitant medications
- Target lesion assessment: the baseline assessment method will be repeated at day 8 after injection (a window period of +7 days is allowed)

Part 2: Extension cohort of BO-112 in combination with pembrolizumab or nivolumab

Pre-dose on day 1:
- Vital signs, weight, ECOG (not to be repeated if assessed within 3 days before dosing)
- Physical examination (not to be repeated if assessed within 3 days before dosing)
- Hematology, Coagulation, Chemistry and urinalysis (not to be repeated if assessed within 3 days before dosing)
- ECG: pre-dose (not to be repeated if assessed within 3 days before dosing) and 2 hours (+/- 30 min) post-dose
- Serum pregnancy test
- PK assessment: pre-dose and 4 hours (+/- 30 min) post-dose
- Immune response assessment
- Biopsy: a sample of accessible (visible, palpable or imaging-guided) tumor tissue should be taken using a fine needle aspiration technique or with a core biopsy. The biopsy should be taken from the lesion to be injected with BO-112, before IT administration (see Table 2).
- Adverse events and concomitant medication

Other visits when BO-112 is administered (for more details of on-study assessments during other visits, see Tables 4 and 5):
- Vital signs, weight, ECOG
- Physical examination
- Hematology, Coagulation, Chemistry
- Adverse events and concomitant medication
Response assessment: After Weeks 8 or 9 and before Week 12, tumor response will be assessed by physical examination and CT scan. RECIST v1.1 will be used for the response assessment. The CT scan should be done using IV administration of contrast and should cover the same anatomical regions as the CT scan done prior to study entry (used for screening), as well as any additional regions if clinically indicated. Slice thickness of 5 mm or less is recommended.

Subsequently, for patients who have objective response or stable disease and who continue study treatment, tumor response assessment will be performed every 10 - 14 weeks counting from the previous scan/assessment, or earlier if clinically indicated and according to the routine practice of the site, using RECIST v1.1.

Other assessments are indicated in the tables 4 and 5.

End of treatment/study

In general, subjects will return for an end of study visit within 30 days after the last administration of the study drug. If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone after 2 attempts, a certified letter should be sent to the subject (or the subject’s legally authorized representative, if appropriate) requesting contact with the investigator. This information should be recorded in the study records.

Where possible, a follow up medical examination should be performed 30 days after the last dose of BO-112 for Part 1. This will consist of a 12-lead ECG, a physical examination, hematology, clinical chemistry, urinalysis assessments and a review of AEs and concomitant medication. Any new findings or any deterioration in existing abnormalities should be recorded as adverse events.

For Part 2, the end of study visit will be done when disease progression is observed. See Tables 4 and 5 below for assessments to be done.

Any serious and/or non-serious AEs ongoing at the time of the withdrawal visit or which have occurred during the defined 30-day follow-up period must be followed-up. Appropriate safety evaluations should be repeated and/or additional tests performed at any time when clinically indicated, or at the discretion of the investigator, until resolution, unless, in the investigator’s opinion, the condition is unlikely to resolve due to the patient's underlying disease. If the patient is lost to follow-up, then this should be noted in the CRF.
Table 3: Part 1 single agent dose escalation and multiple dose: Calendar and procedures

<table>
<thead>
<tr>
<th>Assessments</th>
<th>Screening</th>
<th>Treatment</th>
<th>End of study visit</th>
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<td>Within 28 days</td>
<td>Within 14 days</td>
<td>Pre-dose</td>
<td>Post-dose</td>
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<td>Selection criteria</td>
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<td>Demographics</td>
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<td>Vital signs, height (^1), weight, ECOG PS</td>
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<td>Immunological studies(^g)</td>
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<tr>
<td>Target lesion assessment &amp; biopsy</td>
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<tr>
<td>Adverse events monitoring</td>
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**Clinical Trial Protocol**

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<td>Pathological tumor evaluation</td>
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</tbody>
</table>

* a. In the event a patient receives two or three administrations, determinations described on Day 1 will occur each day of the administration (ie. Day 1, Day 8 (+/- 1 day) and Day 15, +/- 1 day window). See Table 2 for specific determinations.

* b. In the event a patient receives two or three administrations, Day 2 visit won’t occur and determinations described on Table 2 will occur as mentioned in the own table.

* c. In the event a patient receives two or three administrations, determinations described on Day 8 will occur 7 days after the last administration (+7 day window). See Table 2 for specific determinations.

**Abbreviations:**
- ECGs = electrocardiograms; ECOG PS = Eastern Cooperative Oncology Group Performance Status.

1. **Height** is only needed at screening (with shoes off).
2. **The assessment does not need to be repeated** if it was done within 3 days before the first dose.
3. **Complete physical examination** at screening, day 1 pre-dose, at day 8, and at the end of study (EOS) visit. Symptom-directed physical exams may be conducted at each administration, and at the EOS visit.
4. **Two serial 12-lead ECGs will be done** at screening (preferably within 7 days prior to treatment start), at pre-dose and 2 hours (+ 30 min) post-dose on day 1 and at end of study visit.
5. **Hematology:** complete blood count with differential, hematocrit, platelet count, and hemoglobin.
6. **Chemistry:** urea, creatinine, ALT, AST, alkaline phosphatase, lactate dehydrogenase, albumin, total bilirubin, direct bilirubin, total protein, sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium. Urinalysis: blood, protein and glucose.
7. **Only for female subjects with child-bearing potential.** Serum pregnancy test will be done at screening (preferably within 7 days prior to treatment start), pre-dose on day 1 and at the EOS visit.
8. **Plasma pharmacokinetic samples** will be obtained at pre-defined time points pre- and post-BO-112 dosing (15'-30' -4h and 24h post-administration).
9. **Plasma and fresh tumor tissue biopsies** will be obtained at pre-defined time points pre- and post-BO-112 dosing.
10. **Concomitant medications** will be collected from 30 days before the first dose until termination.
11. **Tumor biopsy** will be performed at screening within 7 days pre-dose (it is permitted to obtain on Day 1 prior BO-112 administration); a new biopsy will be performed 7 days post- first treatment (7 day window). In the event a patient receives two or three administrations, tumor biopsy post-injection could occur 7 days after any of the planned administrations (+7 days window).
Table 4: Part 2: extension cohort of BO-112 in combination with PEMBROLIZUMAB
First cycle (up to end of week 11)

<table>
<thead>
<tr>
<th>Assessments/Procedure</th>
<th>Treatment</th>
<th>If PD</th>
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</thead>
<tbody>
<tr>
<td><strong>Week</strong></td>
<td><strong>Week 1</strong></td>
<td><strong>Week 2</strong></td>
</tr>
<tr>
<td>Visit</td>
<td>Screening</td>
<td>V1</td>
</tr>
<tr>
<td>Day</td>
<td>- 28 days to - 1 day</td>
<td>Day 1</td>
</tr>
<tr>
<td>Informed Consent, Medical history, Selection criteria, Demographics</td>
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<td></td>
</tr>
<tr>
<td>BO-112 IT administration</td>
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<td>X</td>
</tr>
<tr>
<td>Pembrolizumab IV administration</td>
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Notes:
1. Height is only needed at screening (with shoes off).
2. The assessment does not need to be repeated if it was done within 3 days before the first dose.
3. Complete physical examination at screening, Visit 1, Visit 3, Visit 6 and at the end of study (EOS) visit. Symptom-directed physical exams at each other visit.
4. Two serial 12-lead ECGs will be done at screening, at pre-dose and 2 hours (± 30 min) post-dose on day 1 and at end of study visit.
5. Hematology: complete blood count with differential, hematocrit, platelet count, and hemoglobin
7. Only for female subjects with child-bearing potential. Serum pregnancy test will be done at screening, pre-dose on day 1 (if screening test > 3 days before) and at the EOS visit.
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10. The visit for the response assessment will be an unscheduled visit.
# Subsequent 12-week cycles

<table>
<thead>
<tr>
<th>Assessments/Procedure</th>
<th>If CR, PR or SD at 1st &amp; subsequent response assessments:</th>
<th>If PD</th>
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<tr>
<td><strong>Week</strong></td>
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<td>Week 15&lt;br&gt;Week 27&lt;br&gt;Week 39&lt;br&gt;Week 51</td>
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<td>Serum pregnancy test&lt;sup&gt;7&lt;/sup&gt;</td>
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<td>Urinalysis</td>
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<tr>
<td>Biopsy</td>
<td>Week 15&lt;br&gt;pre-BO112</td>
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<td><strong>CT scan/response assessment</strong></td>
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<td>Every 10-14 weeks from previous scan (or earlier if clinically indicated)</td>
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<td>Adverse events monitoring</td>
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<td>Concomitant medications&lt;sup&gt;9&lt;/sup&gt;</td>
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</tbody>
</table>

**Notes**

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2. The assessment does not need to be repeated if it was done within 3 days before the first dose.
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10. The visit for the response assessment will be an unscheduled visit.
Table 5: Part 2: extension cohort of BO-112 in combination with NIVOLUMAB First cycle (up to end of week 11)

<table>
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<tr>
<th>Assessments/Procedure</th>
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<td>- 28 days to - 1 day</td>
<td>Day 1</td>
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<td>Nivolumab IV administration</td>
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<td>Pharmacokinetic timing</td>
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<td>Concomitant medications7</td>
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</table>

Notes

1 Height is only needed at screening (with shoes off).
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4 Two serial 12-lead ECGs will be done at screening, at pre-dose and 2 hours (± 30 min) post-dose on day 1 and end of study visit.
5 Hematology: complete blood count with differential, hematocrit, platelet count, and hemoglobin
6 Chemistry: urea, creatinine, ALT, AST, alkaline phosphatase, lactate dehydrogenase, albumin, total bilirubin, direct bilirubin, total protein, sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium. Urinalysis: blood, protein and glucose.
7 Only for female subjects with child-bearing potential. Serum pregnancy test will be done at screening, pre-dose on day 1 (if screening test > 3 days before) and at the EOS visit.
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## Subsequent 12-week cycles

<table>
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</tbody>
</table>

**Notes**

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8 SAFETY EVALUATION

8.1 Description of safety parameters.

The number and percentage of adverse effects observed and their severity will be reported. Each patient will be monitored regularly to detect possible adverse effects. All AEs will be graded using the NCI-CTCAE version 4.0, and will be coded according to the MedDRA. Those AEs that cannot be graded using this system will be classified according to the MedDRA method:

- Mild (asymptomatic).
- Moderate (symptomatic but does not interfere significantly with function).
- Severe (interferes significantly with function).
- Life-threatening.

8.2 Documentation, notification and follow-up of adverse event

An AE is any unfavorable medical occurrence in a patient or human subject participating in clinical research in which a medicinal product has been administered. The AE does not necessarily have a causal relation with the treatment. Therefore, an AE can be any disease, symptom or untoward or unfavorable sign (including an abnormal laboratory finding) associated in time to the use of a medicinal product in investigation, whether or not it is considered related to the medicinal product.

Adverse events associated to the use of a medicinal product in human beings, whether or not related to the product, include:
- An AE appears during the use of medicinal product in professional practice
- An AE derived from an overdose, whether accidental or deliberate
- An AE derived from abuse of a drug
- An AE derived from withdrawal of a drug
- When a reasonable possibility exists that the AE occurred merely because of the participation of the patient in the study (e.g., AE caused by discontinuing antihypertensives during the washout phase), it will also be reported as an AE, even if it does not have any relation to the product under investigation.

The clinical manifestation of the failure of the expected pharmacologic action (progression) will not be recorded as an AE if it has already been recorded among the data in the CRF. However, if the episode meets any criteria of a serious adverse event (SAE), it must be recorded and reported as such.

**Serious adverse event**

A SAE is any unfavorable medical occurrence that, at any dose:
- Results in death
- Is life threatening
• Requires hospitalization or prolongs an existing hospitalization
• Results in persistent or significant disability/incapacity
• Results in a congenital anomaly/birth defect, or
• Any other AE that may jeopardize the subject’s health.

Life threatening: The term "life-threatening" used in the definition of "serious" refers to an AE that resulted in the subject running a risk of dying when it occurred. It does not refer to an AE that hypothetically could have caused death if it had been more intense.

Hospitalization: Any AE that results in or prolongs hospitalization will be considered serious, EXCEPT for the following exceptions:

• Admission involves a hospital stay of less than 24 hours. or
• The admission was planned previously (e.g., surgery scheduled before initiation of the study).
  or
• The admission is not related to an AE (e.g., a social hospitalization to allow the caretaker to rest).

Nevertheless, it should be noted that invasive treatment applied during any hospitalization can meet the criteria of "medically important" and, as such, must be reported as a SAE depending on clinical discretion. In addition, when local health authorities specifically require a stricter definition, local law will have preference.

Disability means substantial alteration of a person’s capacity to perform normal vital functions.

Important medical event: An AE can be considered serious if it puts the subject in danger and requires intervention to avoid another serious disorder. The WHO publication "WHO Adverse Reaction Terminology – Critical Terms List" serves as guide to assess important medical occurrences. The terms listed refer to or may indicate a serious morbid condition. These episodes require special attention because they may be associated to a serious disease and give rise to more forceful measures than the notifications made using other terms.

Reporting of serious adverse events

All SAEs that occur during the period of study treatment or in the 30 days after the last infusion of drug study must be communicated following the procedure described below. For any late SAE (which is manifested after this 30-day period) that is possibly or probably related with the study drug, the same notification procedure should be followed. Progression of an underlying disease of the patient that gives rise to one of the events described previously should be reported as a serious adverse experience (but expected) that (a) is not related with the study medication or (b) is produced by failure of the anticipated therapeutic effect of the study drug.
The investigator will notify the Sponsor by telephone or fax, all serious adverse events that occur during the study within 24 h of having knowledge of the adverse event or on the next work day. In the case of SAEs that are life-threatening or result in death, the investigator must notify the Monitor immediately:

PIVOTAL, SL
Gobelas, 19, La Florida
28023 Madrid, Spain
Tel: +34 91 708 1250
Fax: +34 91 708 1301

All reports of SAE by telephone must be followed by the respective notification report in writing in the following 48 hours. For notifications by fax the investigator will collect the information on the SAE on the corresponding form.

The minimum initial information for the notification of an adverse event must include the following:
- Description of the adverse event and date of onset of the same.
- Sex and age (or date of birth) of patient.
- Information on the treatment received.
- Name and address of the notifying physician.
- Whether or not a relation of causality with the study drugs exists.

When the SAE is notified by telephone or fax with the minimum initial information, the complete SAE notification form with all the information will be sent in the next 2 work days.

The investigator and people in charge of the patient’s care should initiate any complementary investigation of the serious adverse events based on their clinical opinion of the possible causal factors. This procedure may include the need to seek the opinion of a specialist in the field of the adverse event.

If the death of a participating subject has been communicated, the investigator will provide the Sponsor and Clinical Research Ethics Committees (CRECs) involved all the complementary information that they request.
In accordance with the dispositions of this protocol, the investigator has to assume these responsibilities.

**Pregnancy**

While pregnancy is not considered to be a SAE, pregnancies that occur during a clinical trial must be notified in the same time periods as a SAE. The pregnancies of patients enrolled in the clinical trial or their partners will be notified. The outcome of the pregnancy will be monitored closely and any abnormality experienced by the mother or child will be reported. The same is applicable to pregnancies resulting from the sexual relations of fathers who have received the product under investigation.

**Unexpected adverse event**

An unexpected AE is any adverse reaction to the medicinal product whose nature or severity is not consistent to those indicated in the Investigator Brochure/Summary of Product Characteristics in effect. Reports that add significant information about the specificity or intensity of a known and documented AE constitute unexpected AEs. For example, a more specific or more intense AE than is described in the investigator’s brochure must be considered “unexpected.” Specific examples are:

- Acute renal insufficiency notified as an AE followed by another notification later of interstitial nephritis and
- Hepatitis with a first notification of fulminant hepatitis.

The Sponsor is responsible for notifying all suspected unexpected serious adverse reactions (SUSAR) in accordance with the RD 1090/2015 from 4th December 2015.

**Relation between the AE and the product in investigation**

The assessment of the relation between an AE and the administration of the study drug is a clinical decision based on all the information available when the case report form is completed. A “negative” assessment implies:

- The existence of a clear alternative explanation, for example, mechanical bleeding in the surgical area.
  or
- Lack of probability; for example, if the subject is run over and there are no indications that the drug caused disorientation that might have lead to the accident or if a cancer appears days after the first administration of the drug.

A “positive” assessment indicates that reasonable suspicions exist that the AE is associated with the use of the drug in investigation.

The factors that must be taken into account when the relation between the AE and the study drug is assessed are:
• Temporal sequence with respect to administration of the study drug: the episode must occur after the drug is used. The time from exposure to the drug to occurrence of the episode will be evaluated within the clinical context.

• Recovery occurs with interruption (withdrawal of exposure) and recurs with resumption (renewed exposure): the subject’s response to interruption of the drug (withdrawal of exposure) or resumption of the drug (renewed exposure) will be assessed in light of the usual clinical course of the episode in question.

• Underlying, concomitant, and intercurrent diseases: any notification will be evaluated considering the natural history and evolution of the disease treated and any other disease that the subject may have.

• Concomitant medicinal products or treatment: the other drugs that the subject is taking or treatment that the subject is receiving must be examined to ascertain whether one of them could have caused the episode in question.

• Pharmacology and pharmacokinetics of the study drug: the pharmacokinetics properties (absorption, distribution, metabolism and excretion) of study drugs will be evaluate, in addition to the subject’s pharmacodynamics.

**Intensity of the adverse event**

The intensity of AEs will be graded according to the toxicity criteria of the NCI-CTCAE version 4.0:

- Grade 1: Mild AE
- Grade 2: Moderate AE
- Grade 3: SAE
- Grade 4: Life-threatening or potentially disabling AE.
- Grade 5: Death related to the AE.

**Documentation of adverse events**

All AEs that occur during the study and all SAEs that appear after the subject has signed the informed consent form, whether or not causally related with the study drug, must be recorded in detail in the subject’s CRF.

The documentation must be supported by an annotation in the subject’s file. Any clinically relevant abnormality in an analysis, such as abnormalities that force the subject to withdraw from the study, require treatment, cause evident clinical manifestations, or are considered relevant by the investigator, must be notified as an AE. Each event will be described in detail, including the date of onset and conclusion, the intensity, relation with the product under investigation, measures taken, and outcome.

**Follow-up of unresolved adverse events**

Any AE that is unresolved at the patient’s last AE assessment in the study should be followed up by the investigator for as long as medically indicated, but without further recording in the
CRF. The Sponsor will retain the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

**Follow-up of adverse events of special interest: immune-related select adverse events**

Adverse events of special interest such as immune hepatitis, hormone deficiency, pneumonitis and immune diarrhoea will be specifically monitored, in order to study its relationship with the study drug. Appendix 5 shows the management guidelines for immune-related select adverse events.

### 9 Statistical Procedures

**9.1 Sample size. Justification**

**Part 1 single agent dose escalation and multiple dose**: The total number of patients finally included will range between 9-18. The possibility of an amendment to the current protocol that include 2-3 injections will be considered and has been implemented. A total number of 16 subjects were included: 7 in the 0.6 mg single dose cohort, 3 in the 0.6 mg multiple dose cohort and 6 in the 1 mg multiple dose cohort.

**Part 2 extension cohort of BO-112 in combination with pembrolizumab or nivolumab**: the number of subjects to be included initially is 12. If an acceptable safety profile is observed upon review of data available for the 8-9 week period of the first 10 subjects (and any available data of subjects 11 and 12), then the total sample size will be increased to at most 30 subjects. If 20 subjects with NSCLC are enrolled before reaching the total sample size of 30 subjects, further enrollment will be stopped.

The extension of Part 2 to a larger number of patients has two purposes:

1. Generate more safety information on the combination of BO-112 with nivolumab or pembrolizumab in a variety of tumor types (approved indications for pembrolizumab or nivolumab) to provide support for different indications for potential future development;
2. Generate clinical activity data in a specific indication (NSCLC) with a sample size that is expected to provide a more robust signal of potential efficacy.

**9.2 Statistical analysis**

Descriptive statistics, tabulation and graphic representation will be used, due to the study purposes. There is no pre-defined statistical hypothesis testing and no sample size or power calculation for this Phase I study.

All patients taking at least one dose of study drug will be included in the safety and efficacy primary analysis according to the principle of intention to treat.
Part 1 and Part 2 will be analyzed separately. A formal interim analysis will be performed for Part 1 which will be reported on. For Part 2, an interim analysis primarily for safety and to support the increase in sample size, will be performed when approximately 10 subjects have completed the treatment period up to the first on-study response assessment. All available data at that time will be analysed. When the recruitment has completed for Part 2, an additional interim analysis may be performed of all available data at the point in time when the last subject enrolled has completed the first on-study response assessment. The final analysis for Part 2 will be done when all patients have completed the study. The final study report will include the results of both Part 1 and Part 2. A statistical analysis plan describing in detail the planned analyses will be prepared and updated prior to each analysis.

10 DIRECT ACCESS TO DATA/SOURCE DOCUMENTS

The CRFs and all source data must be easily available for review during scheduled visits by the monitor.

11 CONTROL AND QUALITY ASSURANCE

In order to verify compliance with the protocol, the representatives designated by the Sponsor will be allowed to visit all the study centers periodically to check data integrity and to validate the quality and veracity of the study. The study documents will be reviewed in the center, compared with the source documents (e.g., clinical histories), and discussed with the investigator. The suitability of the facilities will be evaluated continuously.

The study may be evaluated by the Sponsor's internal auditors or authorized representatives and the inspectors designated by health authorities. They will be allowed access to the CRFs, source documents, and other study files. The audit reports will be kept confidential.

The investigator must notify the Sponsor immediately of any inspection by the Health Authorities and provide a copy of the resulting reports.

12 ETHICS

The study will be conducted according to the guidelines specified in the Declaration of Helsinki, Good Clinical Practice, ICH (International Conference on Harmonisation) directives, and the legislation in effect.

The study will be conducted according to protocol requirements. Before beginning the study, the protocol and any relevant amendment, as well as the patient information sheet and informed consent form must receive the approval/favorable opinion of the CREC and the AEMPS. Minor amendments will be approved according to the procedures indicated in the legislation in effect.
The informed consent form of each subject must be freely granted and obtained in writing before the subject may participate in the clinical trial.

The rights, safety, and well-being of the clinical trial subjects have prevalence over the interests of science and society.

The study personnel involved in conducting this clinical trial will be qualified to carry out the assigned tasks. Personnel who have been sanctioned and/or suspended for scientific fraud or clinical malpractice may not participate.

**Patient information sheet and informed consent**

Informed consent will be obtained in accordance with the legislation in effect and the Declaration of Helsinki and its modifications.

The patient must be properly informed by the investigator before he or she may be included in the clinical trial. The patient will give his or her consent after having received all the pertinent information adapted to his or her level of understanding. This will documented in the patient information sheet and informed consent form. Consent should reflect the subject’s presumptive wishes and may be withdrawn at any time without prejudice to the patient.

The Investigator must give the subject the time to ask about the details of the study. When ready, the subject will sign and date the informed consent personally. The Investigator will provide the subject with a copy of the consent form and patient information sheet.

The informed consent and any other information given to the subjects must be revised whenever relevant new information becomes available that may affect the subject’s voluntary participation. These documents will have to receive the approval/favorable opinion of the CREC before they may be used. The Investigator, or a person designated by the Investigator, will have to inform the subject fully about all relevant aspects of the study and about any new information that may affect the subject’s willingness to continue participating in the study. This communication must be documented.

The subject participating in the clinical trial may withdraw consent at any time, without having to give any explanation and without this resulting in any responsibility or prejudice.

**Clinical Research Ethics Committee**

The protocol and informed consent form will be reviewed by a duly constituted CREC. The decision of the CREC regarding the development of the study will be delivered to the investigator in writing; a copy of this decision will be sent to the sponsor.

Any modification to the protocol must be documented in writing as an amendment. Amendments will be properly identified by a number corresponding to their chronological order and will be dated and signed by the Sponsor and Investigator.

All protocol amendments will be reported to the CRECs involved in the clinical trial, the AEMPS, and the respective Autonomous Communities (if applicable) before they are applied. Authorization by the CRECs involved and the AEMPS is necessary for relevant modifications.
The Sponsor will submit the required progress reports on the study to the CREC. Suspected unexpected serious adverse reactions (SUSARs) will also be reported to the CREC. The sponsor will inform the CREC of the termination of the study.

**Subject data protection**

The informed consent form will incorporate wording that complies with relevant data protection and privacy legislation.

**13 DATA MANAGEMENT AND ARCHIVING DATA**

The Sponsor will provide electronic CRFs. The clinical trial monitor will verify the eCRFs by comparing them with the source data recorded (and work logs, if necessary).

**Archives and reports**

The Investigator will prepare and maintain clinical histories, recording all relevant examinations and data for the study of each subject treated with the medicinal product in investigation. The data recorded in the CRF from the source documents must coincide with these original documents; any discrepancies must be explained.

The confidentiality of any documents that can identify the subjects must be protected to respect their privacy and the rules of confidentiality in accordance with the applicable regulatory requirements.

The investigator will maintain a Signature Page to document everyone authorized to make entries and/or corrections in the CRFs. Corrections should be made by crossing out the incorrect entry and writing the correct information in the adjacent space. The correction should be initialed by the person who makes the correction, who will date the correction and add an explanation (if applicable). The original entry should remain legible.

The completed CRF will be reviewed, signed and dated immediately by a qualified physician who is either an investigator or collaborator. The Investigator will conserve a copy of the CRFs with the changes and corrections.

**Conservation of archives**

The Sponsor is responsible for the archive of clinical trial documentation. The investigator will conserve the authentication codes of the patients for at least 15 years the conclusion or termination of the clinical trial. The patients’ clinical histories and other original data will be conserved for the maximum time period allowed by the hospital, institution, or clinic where the clinical trial is conducted.

The Investigator must contact the Sponsor before destroying any records associated with the study. The Sponsor will notify the Investigator when it no longer is necessary to conserve of the clinical trial archives.

If the investigator leaves the study, the records will be transferred to another person designated by mutual agreement. The Sponsor will communicate this transfer to the CREC and AEMPS.
14 INSURANCE POLICY

An insurance policy for the clinical trial has been contracted with the insurance company HDI Gerling: Policy number 08061187-14005.

15 PUBLICATION CONDITIONS

The Sponsor will review all requests for publication of the results of the present study. The Sponsor recognizes the importance of disseminating the results and agrees to allow the principal investigators to publish part or all of these results, as long as the manuscript has been previously approved by the Sponsor. The Sponsor may require changes considered necessary to ensure quality and protect intellectual property rights. The principal investigator understands and accepts that under certain conditions the publication of results may be delayed to guarantee the scientific quality and integrity of the data, for instance, until the results from a representative number of study centers are available. The principal investigator understands and accepts the Sponsor’s authority to choose the proper moment for disseminating the data. By signing the present protocol, the principal investigator accepts the terms of the Sponsor’s publication policy and agrees to abide by them.

16 REFERENCES


Appendix 1. Investigator Brochure of BO-112

See attached document.
Appendix 2. National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0

See attached document (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).
## Appendix 3. Performance status

<table>
<thead>
<tr>
<th>Description</th>
<th>ECOG Grade</th>
<th>Karnofsky Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
<td>0</td>
<td>100 Normal, no complaints; no evidence of disease.</td>
</tr>
<tr>
<td>Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature ie, light housework, office work</td>
<td>1</td>
<td>90 Able to carry on normal activity; minor signs or symptoms of disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 Normal activity with effort; some signs or symptoms of disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 Cares for self but unable to carry on normal activity or to do work.</td>
</tr>
<tr>
<td>Ambulatory and capable of self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.</td>
<td>2</td>
<td>60 Requires occasional assistance but is able to care for most of personal needs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 Requires considerable assistance and frequent medical care.</td>
</tr>
<tr>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.</td>
<td>3</td>
<td>40 Disabled; requires special care and assistance.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 Severely disabled; hospitalisation is indicated although death not imminent.</td>
</tr>
<tr>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.</td>
<td>4</td>
<td>20 Very ill; hospitalisation and active supportive care necessary.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 Moribund.</td>
</tr>
</tbody>
</table>
Appendix 4: Acceptable birth control methods

BO-112 is regarded as a compound with medium/high foetal risk.

Patients of childbearing potential and their partners, who are sexually active, must agree to the use of two highly effective forms of contraception throughout their participation in the study and for 3 months after last dose of study drug (washout period).

Acceptable non-hormonal birth control methods include:

• Total sexual abstinence, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception. Abstinence must be for the total duration of the trial and the drug washout period

• Vasectomised sexual partner plus male condom. With participant assurance that partner received post-vasectomy confirmation of azoospermia

• Tubal occlusion plus male condom with spermicide

• Intrauterine device plus male condom + spermicide. Provided coils are copper-banded.

Acceptable hormonal methods:

• Etonogestrel implants (e.g. Implanon, Norplan) + male condom with spermicide

• Normal and low dose combined oral pills + male condom with spermicide

• Norelgestromin/EE transdermal system + male condom with spermicide

• Intravaginal device + male condom with spermicide (e.g., EE and etonogestrel)

• Cerazette (desogestrel) + male condom with spermicide. Cerazette is currently the only high efficacious progesterone based pill.
Appendix 5: Management guidelines for immune-related select adverse events


A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

Corticosteroids are a primary therapy for immune-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended.

The frequency and severity of the related adverse events covered by these algorithms will depend on the immune-oncology agent or regimen being used.
GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-0 therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/Colitis (NCI CTCAE v4)

**Grade 1**
- Diarrhea: 4 stools/day over baseline; Colitis: asymptomatic

**Management**
- Continue I-0 therapy per protocol
- Symptomatic treatment

**Follow-up**
- Close monitoring for worsening symptoms.
- Educate patient to report worsening immediately
  - If worsens:
    - Treat as Grade 2 or 3/4

**Grade 2**
- Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hrs; not interfering with ADL
- Colitis: abdominal pain; blood in stool

**Management**
- Delay I-0 therapy per protocol
- Symptomatic treatment

**Follow-up**
- If improves to grade 1:
  - Resume I-0 therapy per protocol
  - If persists > 5-7 days or recur:
    - 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent
    - When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-0 therapy per protocol.
    - If worsens or persists > 3-5 days with oral steroids:
      - Treat as grade 3/4

**Grade 3-4**
- Diarrhea (≥3): ≥7 stools per day over baseline; Incontinence; IV fluids ≥24 hrs; interfering with ADL
- Colitis (≥3): severe abdominal pain, medical intervention indicated, peritoneal signs, G4: life-threatening, perforation

**Management**
- Discontinue I-0 therapy per protocol
- 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent
- Add prophylactic antibiotics for opportunistic infections
- Consider lower endoscopy

**Follow-up**
- If improves:
  - Continue steroids until grade 1, then taper over at least 1 month
  - If persists > 3-5 days, or recur after improvement:
    - Add infliximab 5 mg/kg (if no contraindication).
    - Note: Infliximab should not be used in cases of perforation or sepsis.

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

<table>
<thead>
<tr>
<th>Grade of Creatinine Elevation</th>
<th>Management</th>
<th>Follow-up</th>
</tr>
</thead>
</table>
| **Grade 1** Creatinine > ULN and > 1.5x baseline | • Continue I-O therapy per protocol  
• Monitor creatinine weekly | If returns to baseline:  
• Resume routine creatinine monitoring per protocol  
If worsens:  
• Treat as Grade 2 or 3/4 |
| **Grade 2-3** Creatinine > 1.5x baseline to ≤ 6x ULN | • Delay I-O therapy per protocol  
• Monitor creatinine every 2-3 days  
• 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent  
• Consider renal biopsy | If returns to Grade 3:  
• Taper steroids over at least 1 month; consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol  
If elevations persist > 7 days or worsen:  
• Treat as Grade 4 |
| **Grade 4** Creatinine > 6x ULN | • Discontinue I-O therapy per protocol  
• Monitor creatinine daily  
• 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent  
• Consult nephrologist  
• Consider renal biopsy | If returns to Grade 1:  
• Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections |

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g., prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade 1
Radiographic changes only

- Consider delay of I-O therapy
- Monitor for symptoms every 2-3 days
- Consider Pulmonary and ID consults

Management

Follow-up

- Re-image at least every 3 weeks
- If worsens:
  - Treat as Grade 2 or 3-4

Grade 2
Mild to moderate new symptoms

- Delay I-O therapy per protocol
- Pulmonary and ID consults
- Monitor symptoms daily, consider hospitalization
- 1.0 mg/kg/day methylprednisolone IV or oral equivalent
- Consider bronchoscopy, lung biopsy

- Re-image every 1-3 days
- If improves:
  - When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics
  - If not improving after 2 weeks or worsening:
    - Treat as Grade 3-4

Grade 3-4
Severe new symptoms: New/worsening hypoxia; Life-threatening

- Discontinue I-O therapy per protocol
- Hospitalize
- Pulmonary and ID consults
- 2-4 mg/kg/day methylprednisolone IV or IV equivalent
- Add prophylactic antibiotics for opportunistic infections
- Consider bronchoscopy, lung biopsy

- IF improves to baseline:
  - Taper steroids over at least 6 weeks
  - IF not improving after 48 hours or worsening:
    - Add additional immunosuppression (e.g., infliximab, cyclophosphamide, IVIG, or mycophenolate mofetil)

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g., prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)

Grade 1
AST or ALT > ULN to 3.0 x ULN and/or T. bill > ULN - 1.5 x ULN

- Continue I-O therapy per protocol

Grade 2
AST or ALT > 5.0 to ≤ 5 x ULN and/or T. bill > 1.5 to ≤ 3 x ULN

- Delay I-O therapy per protocol
- Increase frequency of monitoring to every 3 days

Grade 3-4
AST or ALT > 5 x ULN and/or T. bill > 3 x ULN

- Discontinue I-O therapy*
- Increase frequency of monitoring to every 1-2 days
- 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent**
- Add prophylactic antibiotics for opportunistic infections
- Consult gastroenterologist

Management

Follow-up

- Continue LFT monitoring per protocol
- If worsening:
  - Treat as Grade 2 or 3-4

If returns to baseline:
- Resume routine monitoring, resume I-O therapy per protocol

If elevations persist > 5-7 days or worsen:
- 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol

If returns to grade 2:
- Taper steroids over at least 1 month

If does not improve in >3-5 days, worsens or rebounds:
- Add mycophenolate mofetil 1 g BID
- If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T. bill ≤ 5 x ULN.

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.
Endocrinopathy Management Algorithm

**Asymptomatic TSH elevation**
- Continue I-O therapy per protocol
- If TSH < 0.5 x LLOQ or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult

**Symptomatic endocrinopathy**
- Evaluate endocrine function
- Consider pituitary scan
- Symptomatic with abnormal lab/pituitary scan:
  - Delay I-O therapy per protocol
  - 1-2 mg/kg/day methylprednisolone IV or PO equivalent
  - Initiate appropriate hormone therapy
- No abnormal lab/pituitary MRI scan but symptoms persist:
  - Repeat labs in 1-3 weeks / MRI in 3 month

**Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness)**
- Delay or discontinue I-O therapy per protocol
- Rule out sepsis
- Stress dose of IV steroids with mineralocorticoid activity
- IV fluids
- Consult endocrinologist
- If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

**Grade of Rash**
- Grade 1-2: Covering ≤ 30% BSA
- Grade 3-4: Covering >30% BSA; life threatening consequences

**Management**
- Symptomatic therapy (e.g., antihistamines, topical steroids)
- Continue I-O therapy per protocol
- Delay or discontinue I-O therapy per protocol
- Consider skin biopsy
- Dermatology consult
- 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent

**Follow-up**
- If persists > 1-2 weeks or recurs:
  - Consider skin biopsy
  - Delay I-O therapy per protocol
  - Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
- If worsens:
  - Treat as Grade 3-4
- If improves to Grade 1:
  - Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections
  - Resume I-O therapy per protocol

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g., prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v4 for term-specific grading criteria.*
Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

**Grade of Neurological Toxicity (NCI CTC v4)**

**Grade 1**
- Asymptomatic or mild symptoms; intervention not indicated
  - Continue I-O therapy per protocol

**Grade 2**
- Moderate symptoms; Limiting Instrumental ADL
  - Delay I-O therapy per protocol
  - Treat symptoms per local guidelines
  - Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent

**Grade 3-4**
- Severe symptoms; Limiting self-care ADL; Life-threatening
  - Discontinue I-O therapy per protocol
  - Obtain neurology consult
  - Treat symptoms per local guidelines
  - 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent
  - Add prophylactic antibiotics for opportunistic infections

**Management**

**Follow-up**

Continue to monitor the patient.
- If worsens:
  - Treat as Grade 2 or 3-4

If improves to baseline:
- Resume I-O therapy per protocol when improved to baseline
- If worsens:
  - Treat as Grade 3-4

If improves to Grade 2:
- Taper steroids over at least 1 month
- If worsens or atypical presentation:
  - Consider IVIG or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g., prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Appendix 6: Summary of Product Characteristics of pembrolizumab

See attached document.
Appendix 7: Summary of Product Characteristics of nivolumab

See attached document.
Appendix 8: Investigator’s agreement. Protocol signature page by principal investigator

I have read the preceding protocol:

“An exploratory first in human Phase I clinical and pharmacokinetic study of intratumoral administration of BO-112 in adult patients with aggressive solid tumors, with an extension cohort in combination with anti-PD1 treatment.”

and agree that it contains all necessary details for conducting this study.

I will conduct the study as outlined therein and will attempt to complete the planned enrolment of patients within 24 months of the receipt of clinical supplies. I will provide copies of the protocol and all drug information relating to the preclinical and prior clinical experience, furnished to me by the Sponsor, to all relevant staff/members. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the study. I agree to keep accurate records on all patient information (Case Report Forms and patient informed consent statement), drug transportation and return forms, and all other information collected during the study for a minimum period of 25 years.

I agree not to publish all or any part of the results of the study carried out under this protocol, without the prior written consent of the Sponsor.

All parties agree to ensure direct access to examine, analyze, verify and reproduce source data/documents, and reports from all trial related sites for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign Regulatory Authorities.

Investigator Name:

Printed __________________________ Signature __________________________ Date (dd/mmm/yyyy)

Sponsor

Name and Title __________________________ Signature __________________________ Date (dd/mm/yyyy)