Investigator (Sponsor):
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Title:
A phase I/II clinical trial of nivolumab and plinabulin for patients with advanced stage non-small cell lung cancer that have progressed through first line platinum doublet chemotherapy.

1.0 SUMMARY.

Title:
A phase I/II clinical trial of nivolumab and plinabulin for patients with advanced stage non-small cell lung cancer that have progressed through first line platinum doublet chemotherapy.

Indication:
Patients with advanced stage non-small cell lung cancer who have received a platinum doublet chemotherapy regimen.

Hypothesis:
In patients with advanced stage non-small cell lung cancer who have received platinum doublet chemotherapy, the addition of plinabulin to nivolumab therapy will increase response rate compared to historical controls treated with nivolumab alone.

Objectives:

Primary Objective

- For the initial phase. To determine the safety and tolerability of the combination of nivolumab and plinabulin.
- For the subsequent phase. To determine the overall response rate (ORR) of treatment with nivolumab in combination with plinabulin for the treatment of advanced stage non-small cell lung cancer in the second line setting.

Secondary Objectives

- To determine the progression free survival (PFS), disease control rate (DCR), Duration of Response (DOR) and overall survival (OS) of patients treated with nivolumab in combination with plinabulin.
- To establish the safety and tolerability of nivolumab in combination with plinabulin.

Clinical trial design:
The trial will be conducted in two phases: the first phase will be a phase 1 dose-escalation to determine the safety of the combination and phase two will be an open label, phase II Simon Minimax 2-stage design of the combination of plinabulin and nivolumab in the treatment of patients with metastatic non-small cell lung cancer.

2.0 BACKGROUND

Lung cancer remains and most common cause of cancer death in both men and woman in the United States and worldwide. In 2015 it is estimated to cause 158,040 deaths in the United States.[1] The majority of these patients have non-small cell lung cancer, where despite advances in medical therapy, the response rate of patients treated with chemotherapy in the second line setting is below 10%. Recent studies have established that inhibition of the programmed death-1 (PD-1) checkpoint leads to a response rate of 15-20% in both squamous and non-squamous NSCLC. These results have led to a paradigm change, the FDA approved nivolumab for the treatment of metastatic squamous lung cancer and the National Comprehensive Cancer Network (NCCN) guidelines has recommended nivolumab as a second line therapy in all histologies. Although, undeniably, a great advance still the majority of patients do not respond to immune checkpoint inhibition and improving the response rate is of paramount importance.

With the hypothesis that treatment with a combination of plinabulin and nivolumab will increase the overall response rate of patients with metastatic non-small cell lung cancer compared to historical controls treated with nivolumab alone, we have designed the phase II study presented in this protocol.

Plinabulin

Plinabulin is a synthetic analog of the diketopiperazine phenylahistin discovered from an Aspergillus species. Plinabulin exerts its effect against cancer growth with several mechanisms. It inhibits Ras-JNK pathway and disrupts tubulin polymerization which causes direct cytotoxic action in a variety of cancer cell lines such as the human HT-29 (colon), PC 3 (prostate), MDA-MB-231 (breast), RPMI-8226 (multiple myeloma), and NCI-H292 (NSCLC) tumor cell lines.[2, 3] It disrupts the cytoskeleton and tubulin network of endothelial cells, thereby inhibiting angiogenesis and causing vascular disruption and subsequent tumor cell death.[2, 3].

A total of 141 patients with advanced cancers have received plinabulin in two clinical studies, a phase 1 monotherapy study and a phase 1/2 combination study with docetaxel.

As monotherapy plinabulin was generally well tolerated. Adverse events (AEs) that occurred in ≥ 20% of patients included nausea (61%), vomiting (47%), diarrhea and fatigue (34% each), constipation, pyrexia, and headache (26% each), anorexia (24%), and anemia (21%). AE of all grades that were possibly related to plinabulin included nausea (53%), vomiting (37%), diarrhea (29%), and fatigue (21%). Overall, 16 (42%) patients experienced serious adverse events (SAEs), the majority of which were considered not related to plinabulin. No cardiac or neurologic side effects were observed. DLTs were not observed in patients treated at 30mg/m2 in the dose-escalation portion of the study, however RP2D was determined to be 30 mg/m2

based on mechanism-based adverse events and the presence of a biological response on tumor blood flow. While no patient achieved a CR or PR, the proportion of patients with stable disease appeared to increase with increasing plinabulin dose (7/18 (47%) evaluable patients achieved stable disease at the RP2D level).

In the phase 1/2, open label study to evaluate plinabulin in combination with docetaxel in patients with advanced NSCLC that had progressed after treatment with at least 1 chemotherapy regimen (2014 ASCO abstract. J Clin Oncol 32:5s, 2014 (suppl; abstr 8054). On the phase II portion of the study patients were randomized to receive plinabulin (30 mg on days 1 and 8) and docetaxel (75 mg on day 1) vs docetaxel alone. A total of 90 patients received plinabulin plus docetaxel and 73 received docetaxel alone. Plinabulin was found to be generally well tolerated in combination with docetaxel and appeared to have a similar AE profile as single agent docetaxel, although surprisingly the combination had a significantly lower rate of neutropenia (all events and events ≥ Grade 3) than patients treated with docetaxel alone ($p \leq 0.01$). Furthermore, the proportion of patients who required G-CSF and the median cycles of G-CSF received, as well as the rate of docetaxel dose reduction, was lower with the combination than with docetaxel alone.

The most common AEs without regard to causality in the Phase 2 portion of the Phase 1/2 protocol at 30 mg/m2 (RP2D) were diarrhea (58%), fatigue (52%), nausea (48%), constipation (36%), vomiting (34%), anorexia (34%), and transient hypertension (32%). Plinabulin did not cause a bleeding side effect, which is commonly seen with other anti-angiogenesis agents, such as bevacizumab or ramucirumab. There was no significant improvement in efficacy in patients who received plinabulin in addition to docetaxel, although the duration of response was significantly ($p<0.05$) longer for those responded to plinabulin 30 mg/m2 plus docetaxel (12.7 months) than docetaxel alone (1.5 months).

Nivolumab

Nivolumab is a fully human IgG4 programmed death 1 (PD-1) immune-checkpoint–inhibitor antibody. CheckMate-017 was a randomized phase III trial that randomly assigned 272 patients with metastatic squamous lung cancer to receive nivolumab, at a dose of 3 mg per kilogram of body weight every 2 weeks, or docetaxel, at a dose of 75 mg per square meter of body-surface area every 3 weeks. Overall survival for patients treated with nivolumab was 9.2 months compared to 6 months in patients treated with docetaxel alone (HR, 0.59; 95% CI, 0.44 to 0.79; $P<0.001$). The response rate was 20% with nivolumab versus 9% with docetaxel ($P=0.008$). These results led to the approval of nivolumab for the patients with metastatic squamous lung cancer.[4]

CheckMate-057 used the same study design but focused on patients win non-squamous lung cancer, here OS was also improved for patients treated with nivolumab compared to those treated with docetaxel (12.2 months vs. 9.4 months, HR= 73; 95% CI, 0.59 to 0.89; p=0.0012). The NCCN guideline has recommended consideration for this agent in the second line setting and it has been granted approval by the FDA for patients who have progressed through a first lne platinum doublet chemotherapy.

Nivolumab treatment is well tolerated with a low incidence of severe adverse events. In checkmate 0/17 treatment-related adverse events of grade 3 or 4 were reported in 7% of the patients in the nivolumab group as compared with 55% of those in the docetaxel group. The most common side effects seen with nivolumab are fatigue (16%), decreased appetite (11%), asthenia (10%), nausea (9%), diarrhea (8%), arthralgia (5%), pyrexia (5%) and pneumonitis (5%).[4]

Microtubule depolymerizing agents as immunomodulators.

Microtubule depolymerizing agents (MDA) have been shown to promote dendritic cell maturation[5-7]. Ansamitocin P3 is a MDA that induces DC maturation, production of pro-inflammatory cytokines and when used in combination with immune check point inhibitors, potentiated the antibody-mediated blockade and produced a more pronounced anti-tumor effect in a colon cancer murine model.[5] Dolastatin, another animal derived MDA, has been shown in murine models to promote antigen uptake and migration of tumor-resident DCs to tumor draining lymph nodes. In the same models, blockade of the PD-1 pathway induces therapeutic synergy when combined with dolastatin. Brentuximab vedotin, an antigen drug conjugate, that uses an antimitotic agent monomethyl auristatin E as its chemotherapy partner has been shown to induce of dentritic cell homing and activation of the cellular antitumor immune responses in patients.[7] Plinabulin has been shown to have activity in inducing DC maturation in a dose dependent manner, similar to that of Ansamitocin P3 (unpublished data). Plinabulin has also been shown in an immune- competent mice model MC38 model that it adds efficacy synergy in tumor inhibition of PD-1 antibody in a statistical significant manner (unpublished data).

3.0 TRIAL OBJECTIVES

Primary Objective.

- For the initial phase. To determine the safety and tolerability of the combination of nivolumab and plinabulin.
- For the subsequent phase. To determine the overall response rate (ORR) of treatment with nivolumab with the addition of plinabulin in the treatment of advanced stage non-small cell lung cancer in the second line setting.

Secondary Objectives.

- To determine the progression free survival (PFS), disease control rate (DCR), Duration of Response (DOR) and overall survival (OS) of patients treated with nivolumab in combination with plinabulin.
- To determine the safety and tolerability of the combination of plinabulin and nivolumab.

Translational objectives.

- Patients who have pre-treatment and/or post cycle one biopsy will have flow cytometry of their tissue to identify infiltration of immune cells, rates of expression of PD-1, PD-2 and PDL1. In partnership with Dr. McGarry Houghton, we will correlate the percentage and characteristics of immune cells with response to therapy.

4.0 STUDY POPULATION AND SELECTION OF SUBJECTS.

Inclusion criteria

Patients must meet the following criteria to be eligible to participate in the current study:

1. Subjects must have histologically or cytologically-documented stage IIIB or stage IV, recurrent, or metastatic NSCLC.
2. Subjects must have received prior platinum doublet based treatment.
    - Up to 2 lines of prior systemic therapy for metastatic disease are permitted.
    - Adjuvant chemotherapy or concurrent chemoradiation for early stage disease does not count as prior therapy unless subject progressed within 6 months of completion of regimen
    - Patients with known activating mutations in EGFR, or known translocation in ALK or ROS-1 are eligible provided they have progressed on or were intolerant to FDA approved targeted therapy.
3. Subjects must be ≥18 years of age.
4. Subjects must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2.
5. Subjects, including those in the dose-escalation portion of the study, must have measurable disease per RECIST 1.1 criteria. Imagining must be within 28 days of trial enrollment.
    - Target Lesions may be located in a previously irradiated field if there is documented (radiographic) disease progression in that site prior to trial enrollment
6. Subjects must have organ and marrow function as follows:
    - Absolute neutrophil count (ANC) ≥1000/mm3,

- Platelets ≥75,000/dL,
- Hemoglobin ≥9 g/dL,
- Total bilirubin ≤1.5 x ULN (except subjects with Gilbert Syndrome who can have total bilirubin ≤ 3.0 mg/dL),
- Serum creatinine ≤1.5 mg/dL or creatinine clearance ≥60 mL/min,
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5 times the upper limit of normal if no liver involvement or ≤5 times the upper limit of normal with liver involvement

7. For women of child bearing potential, documented negative pregnancy test within two weeks of study entry and agreement to acceptable birth control throughout the trial starting with the screening visit through 120 days after the last dose of study medication
   - Abstinence is an acceptable method of birth control
8. Male subjects with a female partner(s) of child-bearing potential must agree to use acceptable birth control throughout the trial starting with the screening visit through 120 days after the last dose of study medication.
9. Capability to understand and comply with the protocol requirements as and signed informed consent documents.

Exclusion criteria

1. Systemic anticancer therapy within 21 days of the first dose of study drug.
   - All adverse events from prior systemic therapy must have either stabilized or returned to baseline.
2. Prior treatment with nivolumab or any other PD1/PDL1 checkpoint inhibitor.
3. Major medical conditions that might affect study participation (e.g. uncontrolled pulmonary, renal, or hepatic dysfunction, uncontrolled serious infection, cardiac disease).
4. Significant cardiac history:
   - History of myocardial infarction or ischemic heart disease within 1 year before first study drug administration;
   - Uncontrolled arrhythmia;
   - History of congenital QT prolongation;
   - New York Heart Association Class III or IV cardiac disease;
   - Uncontrolled hypertension: blood pressure consistently greater than 150 mm Hg systolic and 100 mm Hg diastolic in spite of antihypertensive medication.
5. History of hemorrhagic diarrhea, inflammatory bowel disease or active uncontrolled peptic ulcer disease. (Concomitant therapy with ranitidine or its equivalent and/or omeprazole or its equivalent is acceptable). History of ileus or other significant gastrointestinal disorder known to predispose to ileus or chronic bowel hypomotility.

6. Subjects with untreated symptomatic CNS metastases are excluded.
    - Subjects are eligible if symptomatic CNS metastases are treated and subjects have neurologically returned to baseline (except for residual signs and symptoms related to CNS treatment) for at least 7 days prior to first dose of study treatment.
        - Subjects must be off corticosteroids for at least 7 days prior to first dose of study treatment.
7. Subjects with leptomeningeal disease are excluded.
8. Subjects with planned radiation therapy to a target lesion will be excluded.
9. Radiation therapy within 14 days of the first dose of study drug.
10. Subjects who are pregnant or breastfeeding are excluded.
11. Subjects who are unable or unwilling to abide by the study protocol or cooperate fully with the investigator or designee are excluded.
12. Pulmonary conditions such as sarcoidosis, silicosis, idiopathic pulmonary fibrosis, or hypersensitivity pneumonitis are excluded.
13. Subject who have active non-infectious pneumonitis.
14. Subjects who have a diagnosis of immunodeficiency or are receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study treatment.
15. Subjects with any active, known, or suspected autoimmune disease. Subjects with type I diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.
    - Subjects with asthma that require intermittent use of bronchodilators, inhaled steroids, or local steroid injections would not be excluded from the study.
    - Subjects on chronic systemic steroids for any reason would be excluded from the study. The use of topical steroids is allowable.
16. Any known additional malignancy (with exception of non-melanoma skin cancer, in-situ breast cancer or a malignancy diagnosed ≥3 years ago and with no evidence of requiring active treatment).
17. Patients with known active Hepatitis B, or Hepatitis C will be excluded.
18. Patients with risk factors for bowel obstruction or bowel perforation (e.g., acute diverticulitis) will be excluded.
19. Has any serious or uncontrolled active infection.

5.0 TRIAL DESIGN AND TREATMENT PLAN.

Trial design

The trial will be conducted in two phases an initial phase 1 portion and a subsequent phase 2 portion which will be a single center, two stage Simon,

phase 2 open label study of patients with advanced stage lung cancer treated in the second or third line setting with plinabulin and nivolumab.

Endpoints

5.1.1 Primary endpoints.

- For the initial phase. To determine the safety and tolerability of the combination of nivolumab and plinabulin.
- For the subsequent phase, To evaluate the overall response rate, defined as the sum of complete and partial responses according to RECIST 1.1 criteria, of patients treated with a combination of plinabulin and nivolumab.

5.1.2 Secondary endpoints.

- Evaluate disease control rate, defined as the proportion of patients with CR, PR and SD for more than 8 weeks while on combination plinabulin and nivolumab.
- Evaluate duration of response (DOR).), Progression Free Survival (PFS) and Overall Survival (OS)
- Determine the safety and tolerability of the combination of nivolumab and plinabulin.

Dose rationale

Plinabulin has been studied as a single agent in a dose escalation study, the maximum tolerated dose was not reached and the recommended phase 2 dose was 30mg/m2[8]. A subsequent phase 1 study using plinabulin with docetaxel showed that plinabulin did not contribute to the toxicity of docetaxel[9]. In this study the initial phase will start with a dose of plinabulin of 20 mg/m2 and nivolumab at a flat dose of 240mg. If no dose limiting toxicities (DLTs) are seen the dose of plinabulin will be escalated to 30 mg/m2

Treatment plan

5.1.3 Phase one

The phase one portion of this trial will be a phase-1 dose escalation to determine the safety of plinabulin in combination with nivolumab. For the initial portion of this phase, 3 patients will be enrolled and will receve plinabulin at a dose of 20 mg/m2 as well as nivolumab at 240 mg for 1 cycle. Cycles will consist of plinabulin and nivolumab on days 1 and 15 of a 28 day cycle. After these patients receive at least 1 cycle of treatment, and if no DLTs are seen, another cohort of 3 patients will enroll, and be treated with plinabulin at a dose of 30mg/m2 and nivolumab at a dose of 240 mg. If there are no DLTs after the first cycle of treatment, the trial will continue with the second phase of the study. Alternatively, if

one or more patients in either cohort experience a DLT the cohort in question will expand to include a total of 6 patients. If no other DLT are seen in these 6 patients, then the next phase of the study will continue. If two or more (2/6) patients in this cohort experienced a DLT, then will stop and an alternative dose level may be considered after further discussion with the sponsor and analysis of the DLT by the study team. If this were to be the case the protocol will be amended. If a delayed DLT occurs (i.e. a DLT that occurs during cycle 2 of treatment) the DLT evaluation period will be extended to include the first 2 cycles of treatment.For definitions of DLT please see section 7.1

5.1.4 Second Phase

In the second phase of the study patients will receive plinabulin at the MTD identified previously and nivolumab 240 mg on the same day and repeated every 2 weeks. Re-staging studies will be conducted every 8 (+/-1) weeks, after cycle seven scans will be done every 12 (+/-1) weeks. Those who achieve a complete response, partial response, or stable disease will continue on study. Will use RECIST 1.1 criteria to determine response to treatment. Patients with progressive symptomatology and progressive disease seen in imaging studies will be taken off study. To account for pseudo-progression, in the case of progressive disease in the absence of worsening symptomatology, at the discretion of the treating physician, a patient will be allowed to continue receiving treatment for 2 more cycles followed by restaging studies. If progressive disease is confirmed the patient will be taken off study.

6.0 STUDY CONDUCT AND TRIAL PROCEDURES

Prior to undergoing any study-specific procedure, patients must read and sign the current Institutional Review Board (IRB)-approved informed consent form. Procedures conducted as part of the subject's routine clinical management (e.g., blood count, imaging study) and obtained prior to signing of informed consent may be utilized for screening or baseline purposes provided these procedures are conducted as specified in the protocol.

Screening
Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent. Documentation of the informed consent will be maintained in the patient's chart.

Studies or procedures that were performed per standard of care prior to signing the consent form may be used for baseline values if they fall within the screening specified window (see table 6.10).

The screening visit will occur up to 28 days prior to initiating study treatment. The following should be obtained, see table 6.10.

- Medical History
  - Histologic type of lung cancer diagnosis
  - Prior NSCLC treatment history
  - Medical History
  - Subject demographics
- Physical examination
  - Examination of major organ systems
  - ECOG performance status
  - Height, weight, and vital signs (temperature, blood pressure, heart rate, and respiratory rate),
- Laboratory Evaluations should include:
  - CBC with differential
  - Serum chemistry (sodium, potassium, chloride, carbon dioxide, creatinine, BUN, glucose, calcium, albumin, total bilirubin, AST, ALT, total protein, and alkaline phosphatase)
  - Thyroid function (TSH, T3, Free T4)
  - Pregnancy test (serum or urine)for all women of childbearing potential
- CT scan of known sites of involvement (a prior CT scan that is no more than 28 days before the initiation of treatment may be used).
- Brain MRI or CT (prior MRI or CT scan that is no more than 28 days before the initiation of treatment may be used).

Biopsy

All subjects who sign consent to participate in this trial will undergo a mandatory biopsy prior to the start of treatment and after 1 cycle of treatment. The biopsy is mandatory but the treating physician must deem the biopsy feasible and safe; if the physician feels that the biopsy is not feasible or it is not safe the subject will still be allowed to enroll in trial. The biopsy will take place at baseline after eligibility criteria has been confirmed and after the first cycle. Tumor tissue samples consisting of core needle biopsies of deep tumor tissue or lymph nodes or excisional, incisional, punch, or forceps biopsies of cutaneous, subcutaneous, or mucosal lesions will be obtained. Fine needle aspirates, cell pellets from effusions or bone biopsies are not permitted. Target lesions considered for core needle biopsies should be deemed suitable for retrieval of at least three cores (minimum diameter 18- gauge). The biopsy will be delivered to the laboratory of Dr. McGarry Houghton.

Treatment on Days 1 and 15 of each Cycle.

Cycles are defined as 28 days.

- On Days 1 and 15 of each cycle (+/- 2 days), the following will be performed prior to therapy administration:
  - History and physical examination that includes examination of major organ systems, ECOG performance status (Days 1 only), weight (Days 1 only), vital signs, adverse event evaluation
  - Laboratory evaluations (Days 1 only) including:
    - CBC with differential
    - Serum Chemistry (sodium, potassium, chloride, carbon dioxide, creatinine, BUN, glucose, calcium, albumin, bilirubin, AST, ALT, total protein, and alkaline phosphatase)
    - Thyroid function (TSH, T3, Free T4) will be performed on Day 1 of every ODD cycle

Drug Administration

6.1.1 Dose calculation.

Plinabulin doses will be determined by the patient's actual weight on Day 1 of each cycle, Nivolumab doses are fixed at a flat dose of 240mg.

6.1.2 Schedule of administration.

Patients will receive therapy on days 1 and 15 of a 28 day cycle. Therapy will consist of plinabulin administered via intravenous infusion (IV) over 30 minutes (+/- 5 minutes) followed by nivolumab administered via intravenous infusion (IV) over 60 minutes (+/- 10 minutes).

- Cycles one and two require a 1-hr observation period between plinabulin and nivolumab administration. No observation period will be required in subsequent cycles if infusions were tolerated without any infusion reactions.

- Patients will be observed during each infusion and vital signs (blood pressure, heart, and respiratory rate) will be measured prior to (+/- 5 minutes) and at the end of every infusion (+/- 5 minutes).

6.5 Medications

6.5.1 Plinabulin

Plinabulin is an investigational drug that will be supplied by the BeyondSpring.

The clinical formulation of plinabulin will be supplied as a clear solution in amber vials containing 80 mg of drug in 20 mL (4

mg/mL). The correct volume of plinabulin (at a concentration of 4 mg/mL in the vial) is diluted between 1:20 and 1:200 with dextrose 5% in water (D5W) dependent on dose and patient BSA. Prepared dose (approximately 500 mL in DEHP-free bag) will be administered via IV, using standard SCCA infusion tubing[11], with an in-line filter peripherally or centrally within 8 hours of dilution. Product must be protected from light at all times (storage, prior to, during and after dilution). Infusion time may be increased as clinically indicated at the direction of the Sponsor.

Source and Product Accountability of Plinabulin
Plinabulin must be stored at room temperature protected from light. Each vial is designated for single use. Vials should be stored upright. The Investigator or designated pharmacist will acknowledge receipt of the shipment and note content and condition of the shipment on the clinical material shipping form. BeyondSpring or its representatives will supply the appropriate forms.

All partially used or empty vial drug counts should be verified by the sponsor's monitor. The site will contact and discuss with the sponsor the method of study drug destruction to determine whether the study drug will be shipped to a designated facility contracted by the sponsor or destroyed at the study center according to the site's Standard Operating Procedures (SOP). If it is determined that the study drug will be destroyed on site, written confirmation of vial destruction and a copy of the institutional SOP must be provided to the Sponsor or its representative.

At the end of the study, all expired or unused medication will be returned to the contract repository with an inventory of returned clinical materials or destroyed on site according to site procedures. BeyondSpring will be notified prior to shipment or destruction.

The pharmacist or person responsible for dispensing the study drug at the site will maintain an accurate and current record of all drug supplies received from the repository and dispensed to the patients. The dispensing record should contain the protocol number and information regarding the amount dispensed; date dispensed, lot #, patient identifier number, patient initials, and the initials of the person dispensing the medication.

6.5.2 **Nivolumab**

Nivolumab is commercially available. Should be obtained from the institutional pharmacy and prepared per institutional protocol. Please see prescribing information provided by the manufacturer for further details about nivolumab.

6.6 Tumor Evaluations

CT scan of all known areas of disease with tumor evaluation using RECIST 1.1 and IrRC criteria will be performed every 8 (+/-1) weeks. After 7 cycles of therapy, imaging studies will be performed every 12 (+/- 1) weeks.

- Patients who have a CR, PR, or SD may continue on study per study protocol.
- If progressive disease is documented the patient may remain on study provided:
  - The patient does not have clinical signs or symptoms of tumor progression
  - No decline in ECOG performance status
  - Absence of progressive tumor at critical anatomic sites (i.e. spinal cord compression).
  - Treating physician believes that the patient continues to derive clinical benefit from study treatment
  - Repeat imaging scans should be repeated $\geq$ 4 weeks (+/- 7 days) after documented progression. If progression is confirmed the subject will be removed from study.

Off Study Visit.

Patients will undergo the following assessments 28 days (+/- 14 days) after last dose of study treatment

- History and physical examination that includes examination of major organ systems, ECOG performance status, weight, vital signs, adverse events assessment
- Laboratory evaluations including:
  - CBC with differential
  - Blood chemistry (sodium, potassium, chloride, carbon dioxide, creatinine, BUN, glucose, calcium, albumin, bilirubin, AST, ALT, total protein, and alkaline phosphatase)

Permitted medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the

investigator in keeping with the community standards of medical care.
If an increase in systolic blood pressure to > 160 mmHg is observed after administration of plinabulin, oral amlodipine 10 mg or an equivalent calcium channel blocker should be administered before each subsequent dose. Increases in systolic blood pressure above 200 mmHg should be managed with nitroprusside or similar regimen per institutional practice.

Dexamethasone may be used as either a pre-medication or as a rescue medication for patients that experience plinabulin-related nausea and vomiting that is not controlled adequately with a 5HT3 agonist (e.g. ondansetron) alone

6.9 **Prohibited medications**

Patients are prohibited from receiving the following therapies during the treatment phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy not part of this protocol
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than those listed in this protocol
- Radiation therapy.
  - Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with the principal investigator.
  - Radiation to target lesions is prohibited.

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology except for:
  - Dermal, inhaled or intranasal corticosteroids (with minimal systemic absorption) are permitted if the subject is on a stable dose.

- Non-absorbed intra-articular steroid injections are permitted.
- The use of steroids as prophylactic treatment for subjects with contrast allergies to diagnostic imaging contrast dyes will be permitted.
- Dexamethasone is permitted as either a pre-medication or as a rescue medication for nausea related to Plinabulin in patients whose nausea is not controlled with a 5HT3 inhibitor alone.

- The Exclusion Criteria describes other medications which are prohibited in this trial.

End of study treatment / Withdrawal procedures

Patients will have completed their participation in the study in the case of:

- Disease progression, except as described in assessment section.
- Unacceptable toxicity
- Need for anticancer therapy not specified in the protocol
- Patient noncompliance with the protocol guidelines.
- Patient lost to follow-up
- Patient choice to withdraw from treatment or withdrawal consent their own request.
- Study closure by Sponsor or Investigator.
- Patients may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.

Replacement of patients

Patients who discontinue treatment without receiving study medication will be replaced. No other patients will be replaced

Schedule of events

	Screening[1]	Cycle 1		Cycle 2+		Off Study[12]	Survival follow up
		Day 1 +/- 2 days	Day 15 +/- 2 days	Day 1 +/- 2 days	Day 15 +/- 2 days		
Informed consent[2]	X						
Demographics	X						
Medical history	X						
Concurrent meds	X	X	X	X	X	X	
Physical exam	X	X	X	X	X	X	
Adverse event evaluation		X	X	X	X	X	X[13]
Vital signs[3]	X	X[14]	X[14]	X[14]	X[14]	X	
Height, weight[4]	X	X		X		X	
Performance status	X	X		X		X	
Biopsy[5]	X			X			
CBC w/differential	X	X		X		X	
Serum chemistry[6]	X	X		X		X	
Thyroid Function[7]	X	X		X[7]			
Serum pregnancy test	X[8]						
Disease assessment (CT scan)[9]	X			8 (±1) weeks, starting in C7 12 (±1) weeks[10]		X	
Brain CT or MRI	X[11]	As clinically indicated					
Survival assessment							X
Plinabulin		X	X	X	X		
Nivolumab		X	X	X	X		

1: screening evaluations must be performed within 28 days of Cycle 1 Day 1
2: consent must be performed prior to initiation of any screening or study-specific procedures

3: vital signs should include: temperature, blood pressure, heart rate, and respiratory rate
4: height is needed at screening only
5: biopsy is mandatory for all subjects if treating physician deems it to be safe and feasible. If treating physician deems biopsy not safe and/or not feasible the subject may still be enrolled if they meet all other inclusion criteria.
6: sodium, potassium, chloride, carbon dioxide, creatinine, BUN, glucose, calcium, albumin, total bilirubin, AST, ALT, total protein, and alkaline phosphatase
7: TSH, T3, Free T4 will be performed at screening and Day 1 of every ODD cycle
8: serum pregnancy test (for women of childbearing potential only). Subjects must use an acceptable method of birth control.
9: CT scan scan of known sites of involvement. A prior CT scan that is no more than 28 days before the initiation of treatment may be used.
10: disease will be assessed 8 (±1) weeks, after cycle 7 patients will be reimaged every 12 (±1) weeks.
11: brain CT or MRI does not need to be repeated if scans were performed within 28 days of C1D1
12: off-study evaluation will be performed within 28 days (+/- 7 days) after last dose of study treatment
13: adverse events are to be recorded from first administration of investigational drug until 30 days after last dose or until another anti-cancer therapy is initiated.
14: vital signs (temperature, blood pressure, heart rate, and respiratory rate) needed prior to (+/- 5 mins) and at the end of every infusion (+/- 5 mins).

7.0 ADVERSE EVENTS

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting the Cancer Consortium IRB to any event that seems unusual in accordance with IRB policy. The investigator is responsible for the appropriate medical care of patients during the study. The investigator remains responsible to follow, through an appropriate health care option, adverse events that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event resolves or stabilizes. Frequency of follow-up is left to the discretion of the investigator.

Safety measurements that will be used in the study include physical examinations and clinical laboratory tests (hematology and blood chemistries). The adverse event will be graded for toxicity using the NCI CTC version 4.0.Toxicity assessment will occur at each visit. Any adverse events leading to a treatment interruption or dose reduction along with all adverse events that are grade 2 or higher related to either study medication,will be recorded.

Before initiation of subsequent cycles, each patient will be evaluated for possible toxicities that may have occurred after the previous treatment. All Grade 3 or 4 toxicities related to study medication should resolve to Grade 1, tolerable, non-clinically significant Grade 2, or baseline before re-initiation of study treatment. Dose modification will be made based on the toxicity with the greatest severity.

7.1 Definition of Dose-LimitingToxicities (DLT)

A DLT is defined as one of the following adverse events reported during the DLT observation period, if considered to be definitely, probably, or possibly related to either study regimen by the investigator; and fulfills any one of the following criterion using NCI CTCAE Version 4.0:

1.Nonhematologic toxicity as follows:

a) Grade 4 nonlaboratory toxicity

b) Grade 3 nonlaboratory toxicity (for example, nausea, vomiting, and diarrhea) lasting >3 days despite optimal supportive care.

c) Any Grade 3 or Grade 4 laboratory value if:

i) Medical intervention is required to treat the patient, or ii) The abnormality persists for >1 week.

Note: Liver function abnormality: For patients with liver metastasis who begin treatment with Grade 2 aspartate aminotransferase (AST) or alanine amino transferase (ALT), if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 7 days

2. Hematologic toxicity, as follows:

a) Grade 4 thrombocytopenia and Grade 4 anemia.

b) Other grade 4 toxicities lasting ≥ 7 days, or

c) Grade 3 thrombocytopenia if associated with bleeding and requires platelet transfusion, or

d) Febrile neutropenia Grade 3 or Grade 4

3. Any grade 5 toxicity

4. Any other significant toxicity deemed by the primary investigator and the sponsor to be dose limiting, for example:

a) Any toxicity that is possibly related to study treatment that requires the withdrawal of the patient from the study

b) A delay of > 14 days due to persistent Grade ≥ 2 toxicities in initiating the subsequent cycle, with the exception of Grade 2 fatigue

Any infusion or hypersensitivity reactions occurring during the infusion of the drug are not considered dose-related and therefore will NOT be considered to be a DLT.

Attribution of adverse events.

Unrelated: This relationship suggests that there is no association between the study drug and the reported event.

Unlikely: This relationship suggests that the association between the study drug and the reported event is unlikely.

Possible: This relationship suggests that treatment with the study drug caused or contributed to the AE, ie, the event follows a reasonable temporal

sequence from the time of drug administration or follows a known response pattern to the study drug, but could also have been produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with drug administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the study drug seems likely. The event disappears or decreases on cessation or reduction of the dose of study drug.

Definite: This relationship suggests that a definite causal relationship exists between drug administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

Nivolumab

There will be no dose reductions for nivolumab, dose delays will be discussed below. If an immune related adverse event (irAE) is suspected, a thorough evaluation should be conducted in an effort to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to diagnosing an irAE. Serological, immunological, and histological (biopsy) data should be considered to support the diagnosis of an immune-related toxicity. Systemic corticosteroids (1-2mg/kg/day) are indicated for all grade 3 or grade 4 irAEs if not otherwise specified as below. Steroid should be tapered once symptoms improve to Grade 1 or less and tapered over at least 1 month. Nivolumab should be permanently discontinued for

- Life-threatening or non-hematologic grade 4 toxicity
- Any severe or Grade 3 treatment-related adverse reaction that recurs.
- Inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent/day within 12 weeks
- Persistent Grade 2 or 3 treatment-related adverse reactions that do not recover to Grade 1 or resolve within 12 weeks after last dose of nivolumab.

Table 1: Dose modification guidelines for toxicities related to Nivolumab

Adverse reaction	Severity	Dose modification*
Pneumonitis	Grade 2	Withhold dose
	Grade 3 or 4	Permanently discontinue
Hepatitis	Aspartate aminotransferase (AST)/or alanine aminotransferase (ALT)	Withhold dose

	more than 3 and up to 5 times the upper limit of normal or total bilirubin more than 1.5 and up to 3 times the upper limit of normal	
	AST or ALT more than 5 times the upper limit of normal or total bilirubin more than 3 times the upper limit of normal	Permanently discontinue
Hypophysitis Adrenal Insufficiency Hyperglycemia Rash Colitis or Diarrhea	Grade 2 or 3	Withhold dose
	Grade 4	Permanently discontinue
Nephritis and renal dysfunction	Serum creatinine more than 1.5 and up to 6 times the upper limit of normal	Withhold dose
	Serum creatinine more than 6 times the upper limit of normal	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose
	Immune-mediated encephalitis	Permanently discontinue
Other	Grade 3 first occurence	Withhold dose
	Recurrence of same grade 3 AE	Permanently discontinue
	Grade 4	Permanently discontinue
* if treatment is withheld, it should be resumed once the adverse events return to grade 0 or 1. If this does not happen in >12 weeks then treatment should be discontinued and the patient should be taken off study		

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Please refer to “Immune mediated adverse reaction management guide” published by Bristol-Myers Squibb for additional recommendations regarding irAE management.

7.4 Plinabulin

Dose delay and/or dose reduction of plinabulin should be considered for significant tumor pain, hypertension, or constipation (including ileus). As a single agent, plinabulin has not been associated with severe hematological toxicity. If a patient experiences a Grade 3 or 4 non-hematologic AE related to plinabulin or the combination of plinabulin with nivolumab, OR requires more than a 14-day delay of scheduled treatment due to toxicity related to plinabulin, regardless of severity, the dose of plinabulin will be reduced per Table 2.

If the same toxicity with same or higher severity recurs despite dose reduction, plinabulin will be discontinued and the patient will continue receiving nivolumab alone. Only one dose reduction for plinabulin is permitted. See Table 2 Dose Modification for Plinabulin.
Treatment with plinabulin will be discontinued permanently for a concomitant elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels of more than 3 times the institutional upper limit of normal and bilirubin levels of more than 2 times the institutional upper limit of normal.

Table 2. Dose Modification for Plinabulin.

Condition	Action
First Occurrence of grade 3 or 4 non-hematologic AE assesed as possibly, probably or definetly related to plinabulin	Reduce plinabulin by 10 mg/m^2 on subsequent administrations
Second occurrence of Grade 3 or 4 non-hematologic AE assessed as as possibly, probably or definetly related to plinabulin	Discontinue plinabulin (patients may continue on nivolumab alone)
AST and/or ALT > 3 x ULN AND total bilirubin > 2 x ULN assessed as as possibly, probably or definetly related to plinabulin	Discontinue plinabulin (patients may continue on nivolumab alone)

8.0 SAFETY PARAMETERS AND DEFINITIONS:

8.1 Adverse Events:

Adverse events will be collected after the patient has taken the first dose of study drug. After discontinuation from treatment, patients must be followed for all existing AEs for 28 calendar days after the last dose of study drug or until another anti-cancer therapy is initiated.

Prior to beginning study treatment, study site personnel will note the occurrence and nature of each patient's medical condition(s). During the study, site personnel will again note any change in the condition(s) and/or the occurrence and nature of any adverse events. Toxicities are to be graded according to the NCI CTC version 4.0.

A description of the event, including its date of onset and resolution, any action taken should be provided along with the investigator's assessment of causality. An event that is due to unequivocal progression of disease should not be reported as an AE. Any adverse events leading to a treatment interruption or dose reduction along with all grades 2 and higher adverse events related to study medication must be recorded on a case report form (CRF).

8.2 Serious Adverse Events:
(Immediately Reportable to the Sponsor and BeyondSpring)

An adverse event or suspected adverse reaction is considered "serious" (SAE) if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. In the event of an SAE, regardless of suspected causality, occurring after the patient has initiated study therapy and until 28 days after the patient has stopped study treatment must be reported to the sponsor and BeyondSpring within 24 hours of learning of its occurrence. Any SAEs experienced after this 28-day period should only be reported to the sponsor if the investigator suspects a causal relationship to the study drug exists.
Deaths attributed to disease progression do not need to be reported as an SAE regardless of when they occur.

IND safety reports will be submitted to the FDA in compliance with 21 CFR 312.32.

8.3 Data and Safety Monitoring Plan

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the FHCRC/UW Cancer Consortium Scientific Review Committee and Institutional Review Board. The PI will ensure that the monitoring plan is followed, that all data required for oversight

of monitoring are accurately reported to the FHCRC/UW Cancer Consortium Data and Safety Monitoring Committee (DSMC), that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported.

Under the provisions of the FHCRC/UW Cancer Consortium Data and Safety Monitoring Plan (DSMP), Cancer Consortium Clinical Research Support (CRS) provides monitoring for quality process and compliance by qualified monitors unaffiliated with the conduct of the study. Monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of the previous visit as described in the Cancer Consortium DSMP.

9.0 STATISTICAL METHODS AND DATA ANALYSIS.

9.1 Sample size determination for the second phase of the study.

For all patients treated at a dose of plinabluin of 30 mg/m2 or at the MTD the study will follow a Simon 2 two-stage Minimax design.[10] The null hypothesis that the true response rate is 20% will be tested against the one-sided alternative of a response rate of more than 40%. In the first stage, once 18 patients are accrued the study will stop. If there are 4 or fewer responses in these 18 patients, the study will be terminated. Otherwise the study will reopen and 15 additional patients will be accrued. The null hypothesis will be rejected if 11 or more responses are observed in these evaluable 33 patients. This design yields a type I error rate of .05 and a power of 0.8 when the true response rate is 40%. If the true response rate is 20% the probability of ending the trial during the first stage is 0.72. If the true response is 0.4 the probability that the trial will be stopped in the first stage is 0.09.
During the initial phase 1 portion of the study 3 or 6 patients will be enrolled for a total sample size of 39 patients.

9.2 Efficacy Analysis.

The study population for analyses will include all patients enrolled in the study who receive treatment at the MTD and receive at least one dose of study medication.The primary endpoint will be overall response rate. As noted above, 11 or more observed responses among 33 patients would be considered potentially efficacious and would justify further study. Responses include both complete and partial responses, as defined by RECIST 1.1 criteria. The disease control rate (DCR), defined as the stable disease + partial response rate will be calculated. Toxicity rates will be described as the overall percentage of patients experiencing Grade 3 or higher toxicity.

9.3 Time to event definitions.

Progression free survival is defined as the time from registration until objective tumor progression or death due to any cause, whichever comes first. The date of disease progression will be defined as the earliest date of radiological disease progression as assessed by the investigator using RECIST 1.1 criteria, or clinical disease progression. For patients who have not progressed or died at the time of the analysis, censoring will be performed using the date of the last adequate disease assessment.

Overall survival is defined as the time from registration until the date of death due to any cause. Patients last known to be alive at the time of the analysis will be censored at the date of last contact.

Duration of response is defined as the time between receipt of first study drug and until the date of progression.

9.4 Analysis of other endpoints

Demographic characteristics such as patient age, gender, tumor histology, and ECOG performance status will be tabulated. All continuous data will be summarized using descriptive statistics (mean, standard deviation, median, minimum and maximum values). All categorical data will be summarized using frequencies and percentages.

Study drug administration will be described in terms of the total number of months administered, the median (range) of months administered, dose intensity, dose modifications, and reasons for the deviations from planned therapy.

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