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STUDY INFORMATION

Title of Project:
A Phase I Study to Evaluate the Safety of Trigriluzole (FC-4157/BHV-4157) in Combination with PD-1 Blocking Antibodies

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1.0 Research Introduction

1.1 Purpose/Specific Aims

1.1.1 Objectives:

Primary Objectives

- The primary objective of this study is to determine the safety of trigriluzole in combination with PD-1 inhibiting antibodies, and to define a maximum tolerated dose (MTD) of trigriluzole in combination therapy.

Secondary Objectives

- To characterize the efficacy of the combination therapy.
- To identify markers of response to trigriluzole in the tumor microenvironment.

1.1.2 Hypotheses:

Primary Hypotheses

- Combination treatment with trigriluzole + PD-1 inhibitor will have a tolerable adverse event profile.

Secondary Hypothesis/Hypotheses

- The combination therapy will induce tumor regression.
- Features in the tumor microenvironment (TME) may predict response to therapy.

1.1.3 Endpoints:

Primary Endpoint

- The primary endpoint is the MTD/RP2D. Data on the adverse event type, severity and frequency will be recorded.

Secondary Endpoints

- objective response rate (ORR)
- adverse event type, severity and frequency

- survival time (OS), landmark survival rates at 1 and 2 years
- duration of response for responding patients
- time to progressive disease (PFS)
- time to treatment failure
- time to next therapy or death (TTNTD)
- freedom from new metastases

Correlative science endpoints: changes in the tumor microenvironment and peripheral blood in the following categories:

- TILs and PD-L1 expression
- Immune cell phenotypes and gene expression
- Angiogenesis markers
- Metabolic effector molecules
- Exosomal formation

1.2 Research Significance

GRM1 is a metabotropic glutamate receptor, which is expressed on 60-80% of melanomas and numerous other cancer types[1,2]. GRM1 signal transduction results in a number of downstream effects mainly through activation of the MAPK and PI3k/AKT pathways[1,2]. These effects include increased tumor growth, increased tumor angiogenesis, increase in tumor-derived exosome production, and decreased tumor apoptosis[3-6]. We have confirmed these findings using specimens from patients with advanced melanoma who participated on clinical trials using the GRM1-blocking agent riluzole[7].

Recently, we found that another effect of GRM1 signal transduction is suppression of tumor in the microenvironment. GRM1 signal transduction increases the expression of CCL4 and M-CSF in the tumor microenvironment *in vivo*, an effect that is likely mediated through tumor-derived exosome biogenesis and release[6,8]. Conversely, inhibiting GRM1 signal transduction results in a decrease in the numbers of M2-type (suppressive) tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (T regs), and an increase in the numbers of tumor-infiltrating lymphocytes (TILs).

Response to immune checkpoint agents is more frequent in patients with tumors containing TILs and other markers of a so-called “hot” tumor. Agents such as riluzole or its pro-drug trigriluzole could therefore be used to convert a “cold” tumor into a “hot” tumor, priming the

tumor to respond to anti-PD-1 immune checkpoint antibodies. The primary objective of this study is therefore to determine the safety of trigriluzole in combination with PD-1 inhibiting antibodies, and to define a MTD of this agent in combination with PD-1 inhibiting antibodies for use in future combinatorial studies.

1.3 Research Design and Methods

All visits are relative to the treatment start date, Day -14. If a treatment visit is delayed, the next visit will still occur on time relative to the initial start date (e.g. If Week 3 occurs 7 days late, Week 5 will still occur at Week 5).

1.3.1 Overview

In the first portion of this study, cohorts of subjects will be treated with increasing doses of trigriluzole in combination with nivolumab. After the MTD of trigriluzole is identified, it will be tested in combination with pembrolizumab. The MTD of trigriluzole with nivolumab is likely to be safe and tolerable with pembrolizumab. The sequential design is justified because nivolumab and pembrolizumab are both PD-1-blocking antibodies and there are no discernible differences in the safety profiles. Thus, the data obtained from the pembrolizumab group are not independent of the data in the nivolumab treatment group. The sequential design limits the size and the cost of the trial.

1.3.2 Duration of Study

Subjects will remain on the active treatment phase of the study until confirmed disease progression, unacceptable toxicity, or withdrawal of consent, up to a maximum treatment duration of 109 weeks. The entire study is expected to take 2 years to complete. Patients who have derived clinical benefit from study treatment may have access to trigriluzole through a separate continuation trial (rollover trial).

1.3.3 Screening period/Baseline

Screening evaluations must be performed no more than 28 days prior to registration. The subject must begin study treatment no more than 14 days after registration. Baseline research blood and tumor samples may be performed anytime prior to the start of therapy. Ideally, the research blood samples should be collected 3-14 days prior to the tumor biopsy, but it is also acceptable to collect them on the same day.

1.3.4 Active phase, lead-in period

During the 14-day lead-in period, subjects will take trigriluzole monotherapy. The 14-day period is: Week -2 and Week -1.

1.3.5 Active phase, combination treatment

After Week 2, regular study visits will occur in the outpatient clinic at an interval of 14 or 21 days (depending on whether treatment includes nivolumab or pembrolizumab) as per the Study Calendar.

Subjects will receive treatment up to 109 weeks. Subjects may elect to discontinue therapy at any time for any reason. Subjects will remain on the active treatment phase of the study until confirmed disease progression, unacceptable toxicity, or withdrawal of consent, up to a maximum treatment duration of 109 weeks. Once the subject discontinues active treatment, the subject will enter the follow-up phase.

1.3.6 Off treatment procedures (up to 1 year)

If a subject has discontinued active treatment due to reasons other than PD, the subject will continue to have toxicity assessments (H&P and bloodwork) and tumor assessments (CT scans, etc.) Q12 weeks (window can be widened to ± 28 days) for up to 1 year from the start of treatment.

Subjects should be strongly encouraged to adhere to in-person toxicity assessments and tumor assessments per protocol for the 1-year active phase of the protocol. However, subjects who are unable or unwilling adhere to in-person assessments (but do not withdraw consent to be followed) should be contacted Q12 weeks to assess for adverse events. Safety labs and tumor assessments should be obtained at outside facilities if possible. This practice is allowable because it is preferable over removal of the patient from study entirely, and it will not be considered to be a protocol deviation.

If cancer return/progression is observed within the 1-year treatment period (in the opinion of the treating investigator, no strict criteria) and the risk/benefit ratio of restarting study therapy (combination therapy or trigriluzole monotherapy) is acceptable to the treating investigator, the patient may be restarted on therapy with the approval of the PI for up to 12 additional months.

1.3.7 Long term Follow-up phase

Subjects will remain in the follow-up phase of the trial for up to 3 years until start of another therapy, withdrawal of consent, or death. Subjects or their caretakers will be contacted by a member of the study team every 12 weeks (± 28 days) to collect post-study survival status (alive or deceased) and receipt of subsequent anticancer therapy (yes or no). If available, start date and type of subsequent anticancer therapy should be documented in the medical record or research shadow chart. Any method of data collection is acceptable for long-term follow-up (medical records, phone, electronic means, social security death index website, etc.) The source of the information should be noted in the medical record or research shadow chart.

1.3.8 Study Population

Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. No special recruitment will be performed based on gender or minority status.

Contraception for Men and Women of child-bearing potential (WOCBP)

Women of child-bearing potential (WOCBP) are defined as women who have the potential to become pregnant. Peri-menopausal women are considered WOCBP and must use effective contraception. Women who are menopausal (defined as no menses in >1 year) are excluded. WOCBP must have a negative serum pregnancy test prior to the first dose. During study treatment, pregnancy testing may be performed as indicated at the treating physician's discretion. WOCBP may opt out of pregnancy testing if they provide an acceptable reason with their treating provider (such as 100% abstinence from heterosexual intercourse).

WOCBP and men must agree to use effective contraception during the study participation, and for 5 months after the last dose of the drug. Effective contraception options include: 100% abstinence from heterosexual intercourse, surgical (tubal sterilization or partner's vasectomy), intrauterine device, and combination hormonal contraceptives including birth control pills, skin patches, shots, under-the-skin implants, or vaginal rings. Barrier methods of birth control such as condoms are not adequate as primary method of contraception, but may be used as a secondary method.

Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

Participation of Children

Children under the age of 18 will be excluded from this study. trigriluzole and pembrolizumab have not been adequately tested in children and the impact on fertility and learning disabilities are not known.

Sources or Methods of Recruitment

Patients with advanced unresectable or metastatic cancer will be recruited from the surgical oncology and medical oncology clinics at the RCINJ or satellite site. The treating physician will determine if the patient is a potential candidate for the study and discuss the protocol with the patient. The patient will also meet with the research nurse clinician to review each page of the informed consent document.

Investigators may contact referring doctors to tell them about the study by phone or email. Patients will not be recruited directly, as the information about the study will be shared with them through their treating physician. The study team will contact the patient only after the treating physician has determined that the patient is interested in learning more about the study.

Patients may also learn of the study through websites such as clinicaltrials.gov and cancer patient chatrooms and blogs. No patient recruiting materials will be posted by the study team members without IRB approval.

No payment will be offered to recruit subjects. Patients may be reimbursed if funds are available for expenses such as parking and hotel rooms.

Study Enrollment Procedures

A copy of the institution's IRB-approved informed consent document and written justification for any changes made to the informed consent for this protocol must be on file at the Cancer Institute of New Jersey's Office of Human Research Services (OHRS) before any participating institution may enter patients.

To register eligible patients on this study, each site will contact the Cancer Institute of New Jersey's OHRS Registration Desk (732) 235-8990 and fax (732) 235-9399 the signed and dated eligibility checklist, completed signature page of the consent form and additional source documents if requested by OHRS. Once the OHRS Registration Desk verifies eligibility, a unique patient study number will be issued. The patient will not be

identified by name. This is the point that the patient is considered on study. Patients must not start protocol treatment prior to registration.

1.4 Background and Preliminary Data

1.4.1 Glutamate Signaling in Cancer

The glutamatergic system plays a key role in tumor biology. The metabotropic glutamate receptors mediate angiogenesis and cell proliferation, thus promoting cancer progression. The growth inhibition of various human tumors including melanoma, colon adenocarcinomas, breast cancers, gliomas, and non-small cell lung cancers by specific antagonists to ionotropic glutamate receptors has been reported.

Metabotropic glutamate receptors (GRM) are members of the seven-transmembrane domain G-protein-coupled receptor (GPCR) family. GRMs bind glutamate as their natural ligand and are divided into three groups based on sequence homology, agonist selectivity, and effector coupling. Metabotropic glutamate receptor 1 (GRM1) and GRM5 comprise Group I and are mainly involved in excitatory responses induced by strong presynaptic stimulation. Group I GRMs are coupled to a Getabot protein and stimulate phospholipase C beta (PLCβ[9-11]. Ectopic expression or constitutive activation of a variety of different GPCRs has been implicated in neoplastic transformation including the FSH receptor in ovarian cancer, CCK2 receptor in colorectal cancer 14, and the neuromedin B receptor in small-cell lung cancer[12,13]. GRM1 activation results in activation of PLCβ diacylglycerol (DAG). DAG activates protein kinase C (PKC) that in turn can activate the MAPK and PI3K/AKT pathways[1,10]. PKC plays a key role in a multitude of cellular processes including apoptosis, malignant transformation, and metastasis. Activation of the MAPK pathway also appears important for cell migration and cancer cell invasiveness and PI3K/AKT pathway activation appears important for tumor cell survival, epithelial-mesenchymal transition (EMT), and angiogenesis[14].

The majority of human melanomas (>60%) express the GRM1[1,2]. Western blot examination of over 175 different human melanoma samples representing all phases of progression demonstrated that 68% expressed GRM1[1-3]. A tissue microarray made up of melanoma samples from 100 individual patients with advanced disease was stained with anti-hGRM1 antibodies and 80% were found to express GRM1. Skin and melanocytes from these same patients fail to express this receptor. Eighteen of 20 melanoma cell lines tested express GRM1 and stimulation of this receptor resulted in activation of the MAPK and PI3K/AKT pathways as evidenced by the upregulation of the activated forms of AKT and ERK. GRM1

has been deemed both necessary and sufficient for melanocyte transformation[9]. Metabotropic glutamate receptor 1 (GRM1) has been implicated in melanomagenesis and has become a new promising target for melanoma therapy[4,7,15].

In addition to cancer, glutamate abnormalities have been implicated in the pathophysiology of a number of disorders including Amyotropic Lateral Sclerosis (ALS), spinocerebellar ataxia (SCA), Alzheimer's disease, Rett syndrome, dementia, tinnitus, anxiety, depression, and pain. Glutamate is the natural ligand for glutamate receptors, but a large number of agonists and antagonists of these receptors have been developed. Riluzole (RILUTEK) is an FDA-approved agent used to treat ALS, or Lou Gehrig's disease. Riluzole depletes glutamate by the inhibition of its release[16]. The exact mechanism of action of riluzole is not known, but it is a potent inhibitor of glutamate release from neurons, likely disrupting the autocrine loops responsible for the excitotoxicity thought to be the cause of ALS.

1.4.2 BHV-4157, a Pro-drug of Riluzole

Prodrug Development

Biohaven Pharmaceuticals Holding Company Limited [Biohaven] is developing a new glutamate modulating drug, trigriluzole (BHV-4157), as a potential therapy for the treatment of neurodegenerative, neuropsychiatric and other disorders associated with glutamate dysfunction. Trigriluzole is a novel tripeptide prodrug of the glutamate modulating agent riluzole.

Trigriluzole was designed for improved bioavailability, optimized pharmacokinetics and broad dosing options. Riluzole tablets have 60% bioavailabilty, attributed to high first-pass metabolism in the liver. This is thought to be related to metabolism by the heterogeneously expressed CYP1A2 enzyme, which is also attributable to the high PK variability associated with riluzole[17-19]. In addition, riluzole is associated with reduced exposure when taken with meals (i.e., a negative food effect), resulting in the guidance to take riluzole within a three hour fast (one hour before or two hours after a meal). Riluzole is also dosed twice a day, has dose-dependent effects on liver function tests and the drug substance itself has other intrinsic limitations including: very low solubility in water, poor oral palatability, pH dependent chemical stability and intense oral numbness if administered directly to the oral mucosa.

In an effort to mitigate the aforementioned limitations of riluzole, several classes of prodrugs were designed, synthesized, and evaluated in multiple in vitro stability assays to predict in vivo drug levels[20]. Trigriluzole is a third generation of prodrug development

representing multiple years of chemistry effort with optimized in vivo and in vitro features based on stability while transiting the digestive system, enhanced gastrointestinal absorption, avoidance of first pass metabolism, favorable safety pharmacology, metabolic cleavage in the plasma, enhanced pharmacokinetic properties and good oral palatability.

Trigriluzole offers an optimized pro-drug approach to exploit the active moiety, riluzole, with potentially better oral bioavailability, enhanced safety and tolerability profile, reduced PK variance, and once-daily administration without regards to meals.

Clinical Safety Summary

Trigriluzole: Study BHV4157-101 is a phase 1 randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability and pharmacokinetics of single and multiple ascending doses of trigriluzole in normal healthy volunteers. In this study, the initial safety and tolerability of trigriluzole at single doses ranging from 9.5 mg to 200 mg and multiple doses ranging from 35 mg to 200 mg were assessed. Approximately 58 healthy volunteers have been dosed with trigriluzole and 20 have been dosed with placebo. Dosing of all cohorts has completed. Based on preliminary data, both single and multiple doses up to 200 mg have been well tolerated without evidence of novel, clinically significant safety signals or lab abnormalities. There is no apparent dose response regarding the frequency or severity of AEs. In the blinded group, including subjects treated with both placebo and trigriluzole, the most common AEs were headache (five subjects, two with moderate severity and three with mild severity) and constipation (two subjects). No pattern of AEs or lab abnormalities has become apparent to provide specific cautions or to suggest cautions beyond what is appropriate for the active metabolite, riluzole. Based on this Phase 1 experience, the maker of trigriluzole, Biohaven, has advanced trigriluzole into an ongoing Phase 2/3, double-blind placebo controlled trial of trigriluzole in 120 subjects with spinocerebellar ataxia, dosed with 140 trigriluzole mg daily.

Riluzole: The clinical experience of riluzole is relevant given the intention of dosing trigriluzole to yield riluzole exposures within the range already well described for riluzole. Riluzole is approved for treatment in ALS and US Prescribing Information reflects data from placebo-controlled trials and long-term follow-up. Riluzole doses of up to 200 mg per day were considered well-tolerated and studied in randomized controlled trials. Among all chronic randomized controlled trials the discontinuation rate due to AEs was approximately 14%.

No AEs occurred greater than 5% in the riluzole group and twice that of placebo. The AEs occurring greater than 5% and at least 2% more than placebo included asthenia (18% vs. 12% placebo) and nausea (14% vs. 9%).

The most commonly observed AEs associated with the use of riluzole tablets more frequently than placebo treated patients were: asthenia nausea; dizziness; decreased lung function diarrhea; abdominal pain; pneumonia vomiting; vertigo; circumoral paresthesia; anorexia; and somnolence.

Rare cases of neutropenia (3 out of 4000 subjects in USPI) and interstitial lung disease have been reported rarely.

Phase 1 studies of riluzole safety explored much higher doses[21]. Twelve subjects each were administered single oral doses of 150mg, 200 mg, 250 mg and/or 300 mg. At doses of 250 mg and 300 mg, riluzole administration was associated with dose-dependent dizziness/vertigo and buccofacial or manual paresthesia.

Lab abnormalities associated with riluzole consist of elevated transaminases that typically are below 5x upper limit of normal (ULN) and often resolve while on treatment. Experience with incidents of alanine aminotransferase (ALT) increasing >5xULN are limited, insofar as the USPI recommends immediate drug discontinuation. Effects on transaminases show a dose response[22]. Importantly, total daily doses of 50 mg were not associated with increased rates of marked ALT elevations (>5xULN) compared to placebo (1.3% vs. 2.1%, respectively) and such occurrences had a later onset than placebo (median 254 vs. 219 days). Total daily doses of 100 mg and 200 mg had greater rates of marked ALT elevations than placebo, and the median occurrence was within 60 days. Trigriluzole is anticipated to have lesser effects on LFTs, as lower molar doses of trigriluzole will be expected to yield the riluzole exposures achieved with oral RILUTEK – by virtue of bypassing first-pass metabolism. In addition, peak liver concentrations are expected to be reduced via use of trigriluzole as compared to riluzole, which may reduce propensity for LFT changes.

1.4.3 Trigriluzole Preliminary PK Data

Study BHV4157-101 is a phase 1 randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of trigriluzole in normal healthy volunteers. Based on this study, the Phase 2/3 dose of trigriluzole was determined to be 140 mg daily. A Clinical Study Report (CSR) is not yet complete for this trial; PK from this study is presented below.

The study design consisted of the administration of single doses of trigriluzole (sequential cohorts with 17.5, 35, 70, 140 and 200 mg, with the last cohort undergoing repeat dosing under conditions of a high-fat meal) and 5 days of multiple dose (sequential cohorts administered doses of 17.5, 35, 70, and 100 mg BID followed by 200 mg qD). To date of this writing, preliminary results are available (single doses up to 200 mg fasted and fed; multiple doses of 100 mg BID mg daily). Overall, single doses up to 200 mg and multiple doses up to 200 mg daily have been well tolerated without evidence of novel, clinically significant safety signals or lab abnormalities. In addition, PK suggests minimal accumulation with daily dosing.

Under single dose fasting conditions, riluzole concentrations at 24 hours after dosing average under 5% of peak concentrations. PK appears linear. No food effect was observed based on AUC; however, Cmax concentrations were reduced for both trigriluzole and riluzole. A single dose of 140 mg trigriluzole yields exposures (e.g., AUC_{inf} 1,014.7 ng*h/mL) that are expected to be similar to that achieved in clinical trials using riluzole tablets at 50 mg BID (e.g., approximately 1254 ng*h/mL, based on Chandu et al 2010), a dose which was previously studied in ataxia (Romano et al 2015; Ristori et al 2010).

Performance in healthy volunteers has been similar to what has been observed in preclinical species, insofar as the concentrations of trigriluzole have been negligible compared to active metabolite and time to peak concentration of the active metabolite have been delayed. That is, the AUC of the active metabolite riluzole is shown to be over 350-fold greater than trigriluzole

Table 1).

In addition, the time to peak concentration of the active metabolite (Tmax) is delayed compared to trigriluzole and longer than seen with Rilutek tablets (Chandu 2010). This PK pattern of delayed time to peak riluzole concentration is consistent with diminished first pass liver metabolism. Inspection of multiple dose PK does not suggest substantial accumulation of trigriluzole or its metabolite (Table 2).

Table 1. Preliminary PK results, Study BHV4157-101: Single Dose Phase

	Dose of BHV-4157					
	17.5 mg	**35 mg**	**70 mg**	**140 mg**	**200 mg**	**200 mg FED State#**
n	6	5	5	6	6	6

PK attributes of BHV-4157						
Cmax (ng/mL) [CV%]*	…	0.360 [29.1%]	1.81 [46.7%]	2.92 [42.9%]	3.78 [123.8%]	0.79 [65.8%]
Tmax (h) [range]	…	0.6 [0.33-0.67]	0.38 [0.2 – 0.57]	0.67 [0.33-0.67]	0.44 [0.28-0.69]	1.58 [0.67-2.5]
AUC_{inf} (ng*h/mL) [CV%]*	…	…	1.50 [132%]	2.61 [42.5%]	4.49 [114.3%]	3.19 [74.6%]
PK attributes of active metabolite (riluzole)						
Cmax (ng/mL) [CV%]*	24.9 [23.8%]	44.1 [26.3%]	107.7 [20%]	210.2 [37.0%]	307.3 [55.4%]	191.2 [50.0%]
Tmax (h) [range]	1.67 [1.33 – 3.0]	2.03 [1.33-3.0]	2.07 [1.33 – 3.0]	1.53 [1.0-2.5]	2.64 [1.67-4.0]	2.83 [2.0-6.0]
AUC_{inf} (ng*h/mL) [CV%]*	119.2 [37.6%]	294.5 [73%]	583.8 [30.3%]	1,014.7 [33.9%]	1,770 [47.0%]	1,595 [38.0%]

*, geometric mean
#, 200 mg dose administered in crossover manner to one cohort. Ratio of AUC(inf) for fed over fasted conditions was 90.1% for riluzole and 71% for BHV-4157.
n/a, not available
(…) Due to small sample size or limited quantifiable values some parameters were not calculated

Table 2. Preliminary PK results, Study BHV4157-101: Multiple Dose Phase

	Dose of BHV-4157				
	17.5 mg BID	**35 mg BID**	**70 mg BID**	**100 mg BID**	**200 mg qD**
n	6	6	6	6	6
BHV-4157, Day 1					
Cmax (ng/mL) [CV%]*	…	…	0.95 [43.9%]	2.86 [31.6%]	n/a
Tmax (h) [range]	…	…	0.67 [0.33-1.33]	0.75 [0.67-1.0]	n/a
BHV-4157, Day 5					
Cmax (ng/mL) [CV%]*	…	…	1.13 [56.8%]	2.73 [70.7%]	n/a
Tmax (h) [range]	…	…	0.89 [1.0 – 1.33]	0.72 [0.33-1.33]	n/a
AUC_{0-24} (ng*h/mL) [CV%]*	…	…	0.90 [39.3%]	2.42 [138.2%]	n/a
Riluzole, Day 1					
Cmax (ng/mL) [CV%]*	18.8 [38.9%]	44.5 [32.0%]	122.9 [48.2%]	138.1 [29.4%]	n/a
Tmax (h) [range]	1.45 [1.0-1.67]	1.44 [0.67-2.0]	1.33 [1.0-1.67]	1.72 [0.67-2.5]	n/a
Riluzole, Day 5					
Cmax (ng/mL) [CV%]*	24.5 [39.2%]	55.6 [38.2%]	115.9 [37.7%]	153.4 [28.8%]	n/a
Tmax (h) [range]	1.67 [1.0 – 3.0]	1.31 [0.67-2.5]	1.58 [1.0-2.5]	1.67 [1.0-4.0]	n/a
AUC_{0-24} (ng*h/mL) [CV%]*	129.2 [65.2%]	298.1 [32.6%]	717.9 [40.4%]	962.3 [20.5%]	n/a

*, geometric mean
#, 200 mg dose administered in crossover manner to one cohort. Ratio of AUC(inf) for fed over fasted conditions was 90.1% for riluzole and 71% for BHV-4157.
n/a, not available
(…) Due to small sample size or limited quantifiable values some parameters were not calculated

1.4.4 Potential for Drug-Drug Interactions

Clinical drug interaction studies for trigriluzole have not been conducted yet. Trigriluzole, itself, is not expected to interfere with drug metabolism and its cleavage via plasma peptidases render it unlikely to be affected significantly by liver cytochrome P450 inhibitors.

Trigriluzole is not an inhibitor of CYP3A4, CYP1A2, or CYP2D6. In CYP induction studies, the estimated EC50 and Emax for CYP1A2 mRNA was 1.44 µM and 3.47-fold induction, respectively. The estimated EC50 and Emax for CYP2B6 mRNA was 12.6 µM and 27.0-fold induction, respectively. Trigriluzole did not increase CYP3A4 mRNA at doses up to 30 µM.

The active metabolite, riluzole, is metabolized by CYP1A2. Subjects should be strongly discouraged from using potent CYP1A2 inhibitors and inducers that could affect the metabolism of trigriluzole. Further guidance is listed in the section "Concomitant Medications."

1.4.5 Potential Risk to Fetal Development.

Trigriluzole has not yet been assessed in fertility and fetal development studies. Riluzole has been assessed previously and has been characterized as a Category C drug. As described in the USPI, oral administration of riluzole to pregnant animals during the period of organogenesis caused embryotoxicity in rats and rabbits at doses of 27 mg/kg and 60 mg/kg, respectively, or 2.6 and 11.5 times, respectively, the recommended maximum human daily dose on a mg/m^2 basis. Evidence of maternal toxicity was also observed at these doses. When administered to rats prior to and during mating (males and females) and throughout gestation and lactation (females), riluzole produced adverse effects on pregnancy (decreased implantations, increased intrauterine death) and offspring viability and growth at an oral dose of 15 mg/kg or 1.5 times the maximum daily dose on a mg/m^2 basis. There are no adequate and well-controlled studies in pregnant women. Riluzole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

1.4.6 Pharmacokinetics and Dose Selection Rationale

Clinical pharmacokinetics is pending and will be available via an amendment to the IB. The starting dose of 140 mg of trigriluzole is the molar equivalent of 70 mg riluzole, which is below the well-tolerated riluzole dose of 100 mg per day that has been administered chronically to patients with ALS and SCA. This dose of trigriluzole is selected to provide riluzole exposure that is comparable to that achieved in the two positive randomized controlled trials of riluzole in SCA.

1.4.7 Immune Checkpoint Inhibitor Therapy

Pembrolizumab and nivolumab are antibodies that target the immune checkpoint inhibitor programmed death-1 (PD-1). These agents have been used in the clinic since 2014. PD-1 and PD-L1 blocking antibodies have achieved regulatory approval in melanoma, renal cell carcinoma, non-small cell lung cancer, Hodgkin's lymphoma, head and neck squamous cell carcinoma, and urothelial cancers. In addition, recent studies and reports demonstrate activity in Merkel cell carcinoma, as well as microsatellite-instable colorectal cancer, and other gastrointestinal and endometrial cancers associated with Lynch syndrome or Hereditary Non-polyposis Colorectal Cancer (HNPCC). Defects in DNA damage repair genes such as POLE[23] and tumor mutation burden[24,25] are emerging as predictive markers of response to checkpoint blockade. There are a great deal of trials currently investigating the use of checkpoint inhibiting antibodies in a variety of cancers, and the use of PD-1 inhibitors is rapidly expanding in clinical care.

Nivolumab has demonstrated activity in multiple tumor types at a dose of 3mg/kg IV every two weeks or 240 mg flat dose IV every two weeks. Nivolumab treatment resulted in a 31% response rate and a 1 year survival rate of 62% in patients with previously treated metastatic melanoma[26], and in the previously untreated population, the response rate was 40% and the 1-year overall survival rate was 73%[27]. Nivolumab is also effective in renal cell carcinoma with a response rate of 20%[28]. In a randomized study of nivolumab versus docetaxel in patients with previously-treated metastatic squamous cell carcinoma of the lung, patients receiving nivolumab had an improved overall survival[29].

Pembrolizumab has been studied at weight-based doses of 2 and 10 mg/kg every 2-3 weeks and a flat dose of 200 mg every 3 weeks. In melanoma patients previously treated with ipilimumab, the response rate was 29%. In the previously untreated population, the response rate was 45% and the 1-year overall survival rate was 73%[30]. In patients with non-small cell lung cancer whose tumor cells have high PD-L1 expression (defined as 50% or more), pembrolizumab treatment was associated with median overall survival that was 7-9 months longer than standard chemotherapy[31]. In October 2016, the FDA approval for pembrolizumab was extended to include first-line treatment of patients whose tumors have high PD-L1 expression[32].

Overall, across these cancers, the anti-PD-1 agents have response rates of 20-45% [33], meaning that the majority of cancer patients do not achieve an objective response to single-agent treatment. Patients whose tumors lack immune cell infiltration more often fail to respond to anti-PD-1 and anti-CTLA-4 therapies, which highlights the importance of the

tumor-immune system interaction in the tumor microenvironment as a target for therapeutic manipulation in combination strategies.

1.4.8 Clinical Safety Summary

Nivolumab and pembrolizumab have been administered to tens of thousands of patients, and are generally well-tolerated, but serious side effects related to auto-immunity can occur. The most common adverse reactions are fatigue, rash, musculoskeletal pain, pruritus, diarrhea, nausea, asthenia, cough, dyspnea, constipation, decreased appetite, back pain, arthralgia, upper respiratory tract infection, pyrexia, vitiligo, and headache. The most common laboratory abnormalities are hyponatremia and liver function test abnormalities.

Refer to the United States Prescribing Information (USPI) for a full description of the clinical safety of each agent.

1.4.9 Supporting Data for Combination Study

Riluzole for cancer therapy

Riluzole decreases migration, invasion and proliferation of melanoma cells in xenograft models[6]. Based on this finding, we evaluated riluzole, for potential anti-cancer activity in a preclinical setting and in a Phase 0 clinical trial for advanced stage melanoma[7]. We tested the efficacy of riluzole in melanoma patients with a poor prognosis and severely limited treatment options. Twelve subjects with Stage III/IV melanoma were treated with riluzole for two weeks[7]. Four subjects demonstrated reduction MAPK or PI3K/AKT signaling pathway and involution of tumors. Two other subjects demonstrated a clinical response as on radiologic examination. Objective response was observed in 34% in patients after only 14 days of treatment (riluzole 100 mg every 12 hours).

GRM1 signal transduction up-regulates factors that suppress tumor immunity

Further studies on the downstream effects of GRM1 signal transduction suggest that another effect of GRM1 signal transduction is suppression of tumor in the microenvironment. In order to study downstream signaling along the GRM1 pathway we designed a set of cell lines where the parental lines had low or absent GRM1 expression and the modified lines had enhanced expression of this cell surface receptor[6]. We previously reported that enhanced GRM1 expression resulted in enhanced tumor angiogenesis and in vivo tumor growth[4,6,7]. Using the same cell lines we have now demonstrated that xenografts produced from melanoma cell lines with enhanced GRM1 expression have a more pro-tumor microenvironment as compared to xenografts

produced from parental lines. Enhanced GRM1 expression results in up-regulated expression of CCL2 and M-CSF in these melanoma lines. Over-expression of M-CSF and its receptor, CSF-1R, in tumors has been associated with a poor prognosis and M-CSF and CCL4 have also been implicated in mobilization of tumor-associated macrophages into the tumor microenvironment[8,34,35].

Xenografts produced from melanoma cell lines with enhanced GRM1 expression have a more pro-tumor microenvironment as compared to xenografts produced from parental lines

To see if our *in vitro* findings of up-regulated expression of CCL2 and M-CSF with enhanced GRM1 expression would translate to a more tumor-permissive microenvironment *in vivo*, we produced xenografts with the parental and enhanced GRM1 expressing cell lines. We found an increased number of macrophages in the spleens and peripheral blood in the xenografts produced from the enhanced GRM1 expressing cell lines as compared to the parental lines. The GRM1 enhanced tumors also showed greater amounts of angiogenesis[6] and an overall decrease in infiltrating lymphocytes as compared to the parental tumors.

Eliminating macrophages inhibits tumor growth in a GRM1 driven transgenic mouse melanoma model

To further test our hypothesis that GRM1 signal transduction results in suppression of tumor immunity through an increase in M2 suppressive macrophages secondary to up-regulation of M-CSF, we treated a small group of melanoma-prone transgenic mice[1,2] with liposome-encapsulated clodronate. The transgenic line ectopically expresses GRM1 and this drives the development of melanocytic lesions, indistinguishable from melanoma, with a 100% penetrance. Liposome-encapsulated clodronate is a macrophage "suicide" approach, which is used to deplete macrophages in vivo[36]. The liposome-encapsulated clondronate can be taken up by phagocytic cells but not excreted. This results in the death of these cells. We treated a cohort of these animals with Liposome-encapsulated clodronate, and confirmed that we were suppressing macrophages in these animals. An untreated cohort was used as controls. We found that both treated and untreated animals developed pigmented lesions early on, but in the treated group these lesions did not continue to grow and the animals remained healthy for up to 19 months. The untreated controls had to be sacrificed at 3 months because of overgrowth of tumor (Figure 1).

Figure 1. Eliminating macrophages inhibits tumor growth in a GRM1 driven transgenic mouse melanoma model

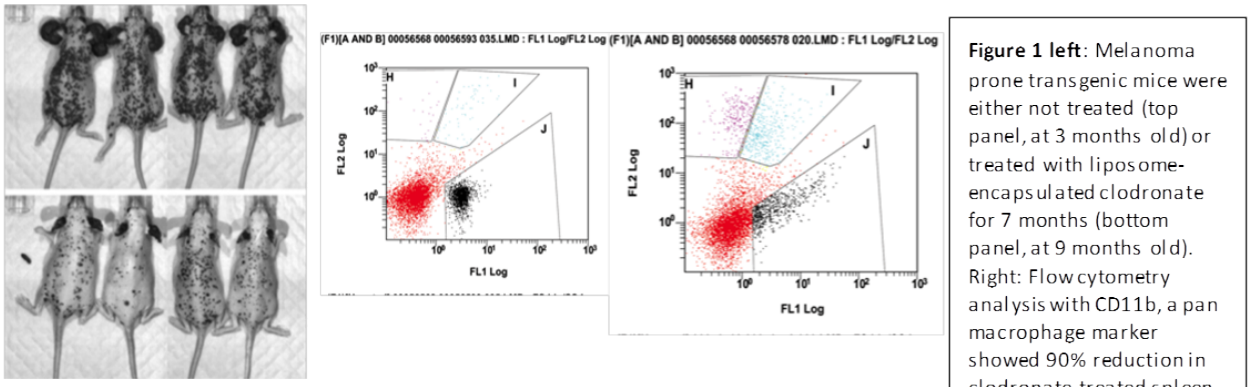


Figure 1 left: Melanoma prone transgenic mice were either not treated (top panel, at 3 months old) or treated with liposome-encapsulated clodronate for 7 months (bottom panel, at 9 months old). Right: Flow cytometry analysis with CD11b, a pan macrophage marker showed 90% reduction in clodronate-treated spleen.

A subset of the treated animals was taken off of liposome-encapsulated clodronate and within 2 weeks they once again began to show rapid tumor growth and had to be sacrificed one month later. Necropsy demonstrated a resurgence of macrophages populations within this subset of animals. This demonstrates that in a transgenic model of melanoma driven by GRM1 expression, depletion of macrophages will suppress tumor growth and reconstitution of macrophages results in a rapid return of tumor growth.

1.4.10 Summary of Supportive Data

This body of work demonstrates that GRM1 signal transduction appears to suppress tumor immunity, likely through the up-regulation of tumor associated M2 macrophages. Blocking the pathway with riluzole or trigriluzole could reduce or reverse this immunosuppressive tumor microenvironment in patients with cancer and increase TILs. It would therefore be logical to combine an agent such as trigriluzole, with an immune-based therapy such as an immune checkpoint inhibitor, in patients with advanced cancer. This forms the rationale for the phase I trial that we propose in this application.

While many combinations with a PD-1 blockade backbone are under investigation, there is the potential for toxicity with overlapping side effects. Dual checkpoint blockade with nivolumab in combination with ipilimumab, is effective in treating patients with metastatic melanoma[37,38] and potentially other cancers[28,39]. Treatment results in a response rate of 55%, but due to overlapping toxicity of these two, grade 3-4 immune-related adverse

events occur in 55% of subjects[37]. Because riluzole and trigriluzole have few adverse events and the toxicity does not overlap with the toxicity of the checkpoint inhibitors, we hope that the combination of PD-1 blockade and trigriluzole in this clinical study will be tolerable and effective.

1.5 Sample Size Justification

Four dosing cohorts are planned in order to test a dose of trigriluzole up to 280 mg PO BID. We do not anticipate testing higher doses. With approximately 3 subjects per dosing cohort, the required sample size is 9 - 21 subjects for testing trigriluzole with nivolumab. The MTD will then be tested with pembrolizumab and an additional 3-6 subjects will be required. The total sample size will be 12 - 27 subjects (9 – 21 in the nivolumab group, plus 3 – 6 in the pembrolizumab group).

1.6 Study Variables

- **Independent Variables, Interventions, or Predictor Variables**
  - Treatment variable: Dose of trigriluzole
  - Treatment variable: pembrolizumab or nivolumab
- **Dependent Variables or Outcome Measures**
  - Adverse events
  - Tumor response
  - Survival time

1.7 Drugs/Devices/Biologics

1.7.1 Drug/Device Accountability And Storage Location

Investigational drug will be stored in the RCINJ Research Pharmacy, a shared resourced. The investigational pharmacists will be responsible for preparation, dispensing, disposal, and drug accountability.

1.7.2 Drug Accountability

The investigator is required to maintain adequate records of receipt, dispensing and final disposition of study drug. This responsibility has been delegated to the pharmacy. Include on receipt record (e.g. packing slip) from and to whom study drug was shipped, date, quantity, and batch or lot number. On dispensing record, note quantities and dates study drug was dispensed to and returned by each subject. It is permitted to dispense supply that will last until the next scheduled visit plus 1-3 days "emergency" supply to ensure patient has access to drug in the event that the visit is unexpectedly delayed.

1.7.3 Drug Destruction/Disposal

Empty and partially empty containers of study drug will be disposed of in accordance with institutional policies and procedures. Unused containers of study drug will be returned to the study sponsor following completion of the study.

1.7.4 Pharmaceutical Information

Investigational Agent: Trigriluzole (BHV-4157/FC-4157)
The investigational drug will be supplied by and Biohaven Pharmaceuticals. Refer to the Investigational Drug Brochure (IDB)

- Product description: The dosage form for the intended clinical trials is Capsules for oral administration, consisting of a common blend formulation of the drug substance with standard excipients filled in size 1 hard gelatin capsule shells at a strength of 140 mg. The manufacturing and packaging of BHV-4157 capsules in bottles (35-count, 75 cc) is by QS Pharma, and the labeling of clinical supplies is by The Coghlan Group.
  - Appearance: Size 1 White Opaque Capsules filled with white to off-white powder.
  - Storage and Handling: Controlled Room Temperature (68° - 77°F or 20° - 25°C).
  - Stability: The common blend capsules are expected to be stable for at least 1 year. Stability studies are ongoing and are expected to extended the retest period of clinical supplies.
- Preparation (how the dose is to be prepared): n/a
- Route of administration: oral
  - Drug Interactions: Clinical drug interaction studies for trigriluzole have not been conducted yet. Trigriluzole, itself, is not expected to interfere with drug metabolism and its cleavage via plasma peptides render it unlikely to be affected significantly by liver cytochrome P450 inhibitors.

Commercial Agent: Pembrolizumab (KEYTRUDA)
Refer to the package insert for agent information.

http://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf

Reconstitution of KEYTRUDA for Injection (Lyophilized Powder)

- Add 2.3 mL of Sterile Water for Injection, USP by injecting the water along the walls of the vial and not directly on the lyophilized powder (resulting concentration 25 mg/mL).
- Slowly swirl the vial. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial.

Preparation for Intravenous Infusion

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.
- Dilute KEYTRUDA injection (solution) or reconstituted lyophilized powder prior to intravenous administration.
- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.
- Discard any unused portion left in the vial.

Storage of Reconstituted and Diluted Solutions

The product does not contain a preservative. Store the reconstituted and diluted solution from the KEYTRUDA 50 mg vial either:

- At room temperature for no more than 6 hours from the time of reconstitution. This includes room temperature storage of reconstituted vials, storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of reconstitution. If refrigerated, allow the diluted solution to come to room temperature prior to administration. Store the diluted solution from the KEYTRUDA 100 mg/4 mL vial either:

- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration. Do not freeze.

Administration

- Administer infusion solution intravenously over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.
- Do not co-administer other drugs through the same infusion line.

Commercial Agent: Nivolumab (OPDIVO)

Refer to the package insert for agent information.

http://packageinserts.bms.com/pi/pi_opdivo.pdf

Preparation and Administration

Visually inspect drug product solution for particulate matter and discoloration prior to administration. OPDIVO is a clear to opalescent, colorless to pale-yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

- Withdraw the required volume of OPDIVO and transfer into an intravenous container.
- Dilute OPDIVO with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL.
- Mix diluted solution by gentle inversion. Do not shake
- Discard partially used vials or empty vials of OPDIVO.

Storage of Infusion

The product does not contain a preservative. After preparation, store the OPDIVO infusion either:

- at room temperature for no more than 4 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or
- under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation. Do not freeze.

Administration

- Administer the infusion over 60 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer).
- Do not co-administer other drugs through the same intravenous line. Flush the intravenous line at end of infusion.

1.7.5 TREATMENT PLAN

1.7.5.1 Study Agents

Trigriluzole

All patients will receive trigriluzole, which will be self-administered as continuous oral daily to BID dosing, depending on the dosing cohort (see Table 3). Subjects will begin therapy with the prescribed dose of trigriluzole for a run-in period of 14 days. After the lead-in period, subjects will continue trigriluzole and begin therapy with pembrolizumab or nivolumab. The first dose of trigriluzole will be administered in the clinic. The patient will have PK and EKG done after the first dose.

Anti-PD-1 antibody (pembrolizumab or nivolumab)

PD-1 inhibitor treatment will consist of either nivolumab or pembrolizumab. PD-1 inhibitor will begin at Week 1 (following 14 day lead-in with trigriluzole). Subsequent PD-1 inhibitor treatments will occur at 2-3-week intervals, depending on the schedule of the PD-1 inhibitor as per the Study Calendar.

Treatment Assignment

Dose escalation of trigriluzole with nivolumab will proceed up to a maximum of 280 mg PO BID to identify the MTD. Subjects will be enrolled on the trial starting with Cohort 1 (Table 3) in a starting cohort size of 3 subjects. Subjects will be followed for 4 weeks to observe for dose-limiting toxicity (DLT). The decision to escalate or deescalate to a new dosing cohort or

to expand a dosing cohort will be made using the procedure described in the statistical analysis section.

Table 3. Dosing cohorts for Trigriluzole + Nivolumab

Escalation cohort	Nivolumab	Trigriluzole
Cohort -1	240mg IV Q 2 weeks	140 mg PO every other day (QOD)
Cohort 1	240mg IV Q 2 weeks	140 mg PO daily
Cohort 2	240mg IV Q 2 weeks	140 mg PO BID
Cohort 3	240mg IV Q 2 weeks	140 mg PO QAM+ 280 mg PO QHS
Cohort 4	240mg IV Q 2 weeks	280 mg PO BID

After the MTD of trigriluzole with nivolumab is identified, the MTD of trigriluzole will be tested in combination with pembrolizumab in 3-6 patients in Cohort P1. If the MTD is not tolerable in Cohort P1, the dose will be decreased by 1 level.

Table 4. Dosing cohort for Trigriluzole + Pembrolizumab

Escalation cohort	Pembrolizumab	Trigriluzole
Cohort P -1	200 mg IV Q3 weeks	MTD -1 level
Cohort P1	200mg IV Q3 weeks	MTD

1.7.6 Treatment Administration

Trigriluzole Administration

Trigriluzole will be self-administered by the patient. Trigriluzole will be first administered in the clinic setting. Trigriluzole can be taken without regards to meals (on a full stomach or an empty stomach). Morning or evening administration is acceptable for once daily dosing. No weight-based dosing calculation is necessary as trigriluzole is administered using flat dosing.

Lead-in period**.** Subjects will begin therapy with continuous oral dosing of trigriluzole for a lead-in period of 14 days (Week -2, Day -14 to Week -1, Day -1). Day -1 is followed by Day 1 (There is no Day 0 or Week 0).

Combination treatment. At the start of Week 1, subjects will continue oral dosing of trigriluzole and begin therapy with PD-1 inhibitor. On the days of IV treatment visits, there is no window for administration of trigriluzole with respect to the PD-1 inhibitor.

Compliance/adherence assessment. Subjects will be *strongly encouraged* to use the Pill Diaries that are provided by the study. If a patient reports compliance <80% of days, they should be re-educated by the research nurse and/or treating investigator on instructions for dosing trigriluzole at home.

Nivolumab or Pembrolizumab Administration

Nivolumab or pembrolizumab will be administered in the outpatient setting.

Nivolumab will be administered at the FDA-approved dose and schedule of 240 mg IV Q2 weeks over 60 minutes.

Pembrolizumab will be administered at the FDA-approved dose and schedule of 200 mg IV Q3 weeks over 30 minutes.

1.7.7 Dose Modifications and Withholding

As toxicity of these agents is largely non-overlapping, management of toxicity is dependent on which agent is more likely to have resulted in the toxicity. Vertigo, somnolence, and circumoral paresthesia should be attributed to trigriluzole. Immune-related adverse events should be attributed to the anti- PD-1 antibody. Refer to agent-specific management instructions below for dose modifications and holdings.

Overlapping toxicities of trigriluzole and anti- PD-1 antibody include fatigue, asthenia, nausea, dizziness, diarrhea, anorexia and liver injury. When these toxicities occur, both drugs should be held until toxicity resolves to grade 0-1.

Study therapy (both agents) may be held for up to 6 consecutive weeks. Subjects who are not able to resume at least 1 study drug within a period of 6 weeks should be removed from the active treatment phase of the study and begin follow-up procedures.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Missed doses should be omitted, not delayed. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the study PI or co-PI. The reason for interruption should be documented in the patient's study record. Tumor assessments should continue per the study calendar even if study medication was missed.

Delays due to holidays, weekends and bad weather will be permitted and will not be counted as a protocol violation, and efforts will be made to get the patient back on the original schedule so that assessments are uniform across subjects. If correlative samples are collected and the scheduled treatment that day is delayed/held for any reason, there is no need to re-collect correlative samples.

Trigriluzole

Grade 3-4 Toxicity. Any Grade 3 or 4 adverse event thought to be related (possibly, probably, or definitely) to trigriluzole will require the dose to be held until the event recovers to Grade 1 or less. When therapy resumes, the dose must be reduced by one level. If the adverse event does not improve to Grade 0-1 within 4 weeks of holding trigriluzole, the patient will be removed from the study.

Grade 1-2 Toxicity. Grade 1-2 adverse events do not require dose adjustment, except if the patient/physician considers them to be serious or intolerable—in this case, the dose may be reduced by one level as per the table below at the discretion of the treating investigator. An example is grade 1 dizziness that the patient finds intolerable.

Table 5. Dose Modifications of Trigriluzole

Escalation cohort	Trigriluzole dose	Dose Reduction #1	Dose Reduction #2	Dose Reduction #3
Cohort -1	140 mg PO every other day (QOD)	Discontinue Trigriluzole	Discontinue Trigriluzole	Discontinue Trigriluzole
Cohort 1	140 mg PO daily	140 mg PO every other day (QOD)	Discontinue Trigriluzole	Discontinue Trigriluzole
Cohort 2	140 mg PO BID	140 mg PO daily	140 mg PO every other day (QOD)	Discontinue Trigriluzole
Cohort 3	140 mg PO QAM + 280 mg PO QHS	140 mg PO BID	140 mg PO daily	Discontinue Trigriluzole
Cohort 4	280 mg PO BID	140 mg PO QAM + 280 mg PO QHS	140 mg PO BID	Discontinue Trigriluzole

Pembrolizumab and Nivolumab Dose Modifications

No dose reductions of pembrolizumab or nivolumab are allowed. PD-1 inhibitor must be withheld (not delayed) for drug-related toxicities

Adverse events (both non-serious and serious) associated with PD-1 inhibitor exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. For suspected immune-mediated adverse reactions, ensure adequate evaluation to confirm etiology or exclude other causes.

Grade 3-4 toxicity. For Grade 3-4 immune-related adverse event (irAE) related to PD-1 inhibitor, withhold PD-1 inhibitor and if indicated, administer corticosteroids as per immune related adverse event guidelines. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 4 weeks. Resume PD-1 inhibitor when the adverse reaction remains at Grade 1 or less following corticosteroid taper. Permanently discontinue PD-1 inhibitor for any Grade 3 immune-mediated adverse reaction that recurs and for any life-threatening immune-mediated adverse reaction. If steroids are indicated longer than 8 weeks, the subject must discontinue treatment with PD-1 inhibitor. Upon improvement to Grade 1 or less, the subject may resume treatment. The PD-1 inhibitor dose should be skipped (not delayed) and treatment should resume at the next scheduled timepoint.

If the toxicity is liver-related, trigriluzole must also be held (see above). Otherwise, PD-1 inhibitor therapy may be continued at the discretion of the treating investigator.

Grade 3-4 toxicity. For Grade 3-4 toxicity that is treatment-emergent and but not an irAE, (examples: fatigue, nausea), PD-1 inhibitor will be held until improvement of toxicity to a Grade 0-1.

Grade 1-2 Toxicity. Grade 1-2 adverse events do not require dose adjustment, except if the patient/physician considers them to be serious or intolerable—in this case, the PD-1 inhibitor may be held at the discretion of the treating investigator. An example is grade 2 fatigue.

1.7.8 Concomitant Medications

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Prohibited Medications. Systemic steroids (other than those used for replacement of adrenal insufficiency) or treatment of irAE are prohibited. If systemic steroids are indicated longer than 8 weeks, the subject must discontinue study treatment. Prophylactic use of Colony stimulating factors (CSFs) is not permitted. They may be used for supportive care and should be discontinued when the event resolves/improves. If needed longer than 4 weeks, the subject must discontinue study treatment.

The following medicines are inhibitors of CYP1A2 and are prohibited (they may increase blood levels of the active form of the drug, riluzole):

- fluvoxamine
- cimetidine
- amiodarone
- efavirenz
- fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin, ofloxacin or norfloxacin)
- fluvoxamine
- furafylline
- interferon
- methoxsalen
- mibefradil
- ticlopidine

Subjects who are taking one of these medications at screening will be excluded if it cannot be discontinued. Subjects who are prescribed one of these medications (e.g. fluoroquinolone antibiotic for an infection) while on-study should be switched to another antibiotic as soon as the prescription is noted.

Strongly Discouraged Concomitant Foods and Medications. Subjects should be strongly discouraged from using potent CYP1A2 inhibitors and inducers that could affect the metabolism of trigriluzole.

The following medicines are CYP1A2 inducers, which may increase the rate of elimination of the active metabolite riluzole, thus decreasing blood levels. The drugs should be discontinued if possible or another medicine may be substituted in its place:

- Insulin
- Omeprazole
- Carbamazepine
- Modafanil
- Rifampin
- Tobacco and nicotine products

Other common medications given for gastroesophageal reflux disease, such as antacids, esomeprazole, pantoprozole, lansoprazole, famotidine, ranitidine are acceptable to take while on study.

Of note, chronic use of nicotine has modest effects on riluzole exposure (i.e., approximately 30 – 35% reduction in exposure). This effect would not compromise subject safety (as it represents a decrease in exposure). Subjects should be encouraged to consider smoking cessation or reduction, which is the standard of care.

Caffeine is an inhibitor of CYP1A2, however caffeine is considered a prototypical CYP1A2 substrate itself. There is theoretical potential for competitive inhibition; however, caffeine is not typically avoided with other drugs which are reliant on CYP1A2 metabolism. Therefore, subjects may use caffeine without any restriction due to drug interactions.

Acceptable Concomitant 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. Inhaled, topical, and intra-articular steroids are permitted. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. Selected ones include:

- IVIG
- Erythropoietin
- Hormone replacement therapy

Vaccinations/Immunizations. Routine vaccinations are prohibited until Week 13, but there are no restrictions on vaccinations after Week 13. If the treating investigator feels that a vaccination is unsafe to delay, an exception may be granted by the PI. It is encouraged to administer the seasonal influenza vaccination (flu shot) prior to study entry.

1.7.9 Supportive Care Guidelines

Patients are allowed to receive full supportive care therapies concomitantly during the study. Supportive medications may be used to treat fever, rash, diarrhea and other common adverse events.

Management of infusion reactions should follow the site's SOPs for infusion or hypersensitivity reactions. Premedications are not required but are allowed for patients with previous infusion reaction.

Radiation therapy may be delivered to a non-target lesion and the patient may remain on-study. For example, radiation therapy may be given to patients with isolated CNS progression.

Surgery or radiation therapy may be performed to palliate a tumor if it is not a target lesion for tumor assessment. If a target lesion requires surgery, the patient should be removed from active treatment due to clinical progressive disease.

1.7.10 Adherence/Compliance and Pill Diary

At each clinic visit each subject will be given a bottle containing capsules of study drug and instructed to return all unused capsules to the investigator. Patients will be given a Pill Diary and instructed to record the number of capsules taken and the time taken.

There are 3 different pill diaries for different portions of the protocol:

1. Trigriluzole Lead-in Period

2. Trigriluzole + Nivolumab
3. Trigriluzole + Pembrolizumab

Study staff should select the appropriate Pill Diary to give to the subject. Subjects will be strongly encouraged (but not required) to keep a pill diary to assess adherence as accurately as possible.

At the end of each cycle of treatment, the actual amount of unused drug will be compared to the amount taken as recorded on the subject's medication diary/verbal report. The desired level of compliance is ≥ 75%. Patients who cannot maintain this level of compliance may be removed from the study. If a subject is not compliant, the treating investigator should reeducate the subject, assess barriers to compliance, and address correctable barriers.

1.7.11 TOXICITY MONITORING AND ADVERSE EVENT REPORTING

All patients who receive one dose of protocol therapy will be evaluable for assessment of toxicity. Prior to each cycle the treating physician will fully assess the patient's condition with respect to possible treatment related toxicities. All adverse events, whether observed by the physician or reported by the patient, occurring during the active portion of therapy, or up to 30 days after the last dose of treatment will be graded by a numerical score according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0 (http://ctep.cancer.gov/reporting/ctc.html) and recorded in the patient's medical record. For the purposes of reporting laboratory abnormalities, only Grade 3-4 adverse events will be recorded on the adverse event CRF pages. Grade 1-2 laboratory abnormalities will not be recorded on the adverse event CRF pages, unless the investigator judges it to be clinically significant and wishes to report it. Information entered on the adverse event CRF pages will include:

- Specific type and duration of reaction (i.e., start and stop dates, resolution).
- Severity/grade.
- Relationship to study drug (causality, attribution).
- Management of the event, if treated with medication and other actions taken to alleviate the clinical event.
- Whether or not it was considered a SAE.

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Adverse Event Reporting Requirements

An adverse experience is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to the study therapy, or it has worsened in intensity or frequency following exposure to the study therapy.

SAE Reporting Period

The SAE reporting period starts at start of treatment and lasts through 30 days after the patient's last dose or start of subsequent therapy, whichever comes sooner. SAE's that during screening period prior to the start of therapy are presumed to be unrelated to the study and need not be reported as SAEs.

SAE Reporting Timeline

All "unexpected" (defined below) and/or "serious" (defined below) adverse events related to pembrolizumab/nivolumab or the combination of pembrolizumab/nivolumab + trigriluzole and occurring during the active portion of therapy, or up to 30 days after the last dose of treatment, will be reported to the Office of Human Research Services at (732) 235-7577 or (732) 235-8675. Events will be promptly reported, in writing, to the local IRB in accordance with IRB policy. If a death occurs the IRB will be notified **within 24-hours** of initial receipt of information. All other SAEs must be reported to the IRB **within 3 to 10 days** of initial receipt of information. Written follow-up reports are required when additional information is needed to fully characterize the event. Copies of each report sent to the IRB will be kept in the study regulatory file.

Collaborating institutions will report all SAEs to RCINJ Office of Human Research Services (OHRS) and RCINJ OHRS will be responsible for forwarding SAE reports to the IRB, FDA, Fox Chase Chemical Diversity Center, Inc. and Biohaven Pharmaceuticals.

In addition to reporting to the local IRB, reporting to external bodies such as industry and/or the FDA may be necessary.

Reporting SAEs using commercially available drugs

In addition, any unexpected (not listed in the package insert) and serious adverse events that are related (definitely, probably or possibly related) with the use of pembrolizumab/nivolumab, it must be reported to the FDA within 10 business days using a FDA Form MedWatch 3500 form http://www.fda.gov/medwatch/safety/3500.pdf (fax # 1-800-FDA-0178).

Reporting SAEs for Investigational Agent Trigriluzole

The PI shall notify the FDA of any adverse experience related to the use of trigriluzole or the combination of pembrolizumab/nivolumab + trigriluzole that is both serious and unexpected, as soon as possible and in no event later than 15 calendar days after the PI's discovery of the event. Each written notification may be submitted on FDA Form MedWatch 3500A http://www.fda.gov/medwatch/safety/3500a.pdf (fax # 1-800-FDA-0178).

The PI shall also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experiences associated with the use of trigriluzole or the or the combination of pembrolizumab/nivolumab + trigriluzole, as soon as possible but no later than 7 calendar days from the PI's discovery of the event information.

Definitions

Definition of SAE

A serious adverse event (SAE) is one occurring at any dose level that results in any of the following outcomes:

- Death
- Life-threatening- immediate risk of death from the reaction.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.
- Requires intervention to prevent one of the outcomes listed in this definition.

The definition of serious adverse event (experience) also includes *important medical events*. Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These events will usually be considered serious. Examples of such events

are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

Definition of Related

There is a reasonable possibility that the drug caused the adverse experience. That is, the event is judged by the investigator to be possibly, probably or definitely related to the treatment.

Definition of Unexpected

Any adverse drug experience and/or specificity, that is not included in the current investigator's brochure and/or package insert.

1.7.12 TREATMENT EVALUATION/CRITERIA FOR RESPONSE

RECIST and Modified RECIST Definition

RECIST

Response and progression will be evaluated in this study using the revised international criteria called RECISTv1.1 proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee[40]. RECIST v1.1 criteria will be **modified** for this protocol to include a stipulation that PD must be confirmed to account for pseudoprogression (tumor flare phenomenon). The first instance of PD must be confirmed by either:

1) confirmation of disease progression by repeat assessment 4-12 weeks later OR
2) association with clinical deterioration (such as a significant decline in performance status).

Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Timing of Assessments

For the purposes of this study, patients should be evaluated at baseline and then reevaluated for response at Week 7, Week 13, and then every 12 weeks thereafter per the Study Calendar. Confirmatory scans should be obtained not less than 4 weeks following

initial documentation of objective response or progressive disease (PD). If a subject has discontinued active treatment due to reasons other than PD, the subject will continue to have CT scans Q12 weeks until Week 49 (window can be widened to ±28 days).

Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-Measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response. There may be occasions when progressive disease is suspected but cannot be fully characterized. In these cases the treating physician may decide to continue treatment for one or two cycles before reassessment, if he/she feels it is in the patients' best interest and the patient agrees to continue treatment.

Non-Target Lesions

All other lesions (or sites of disease) will be identified as non-target lesions and will be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each will be noted throughout follow-up.

Guidelines for Evaluation of Measurable Disease

All measurements will be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique will be used whenever possible to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment. When necessary, the tumor assessment method may be a combination two methods (clinical lesion measurement for a dermal metastasis + CT scan for a liver metastasis), if the investigator feels this is the most accurate way to assess the tumors.

CT scans with contrast are preferred over PET/CT scans, but PET/CT scans are acceptable in patients who have relative contraindications to IV contrast, at the discretion of the treating investigator.

Conventional CT and MRI- These techniques will be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT will be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis.

Clinical lesions- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion.

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

Ultrasound (US)- US will not be used to measure tumor lesions. US might be used, at the discretion of the investigator, to confirm the complete disappearance of superficial lesions assessed by clinical examination.

Tumor markers- Tumor markers (e.g. LDH) alone will not be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, histology- These techniques may be used to differentiate between partial responses (PR) and complete responses (CR) if necessary and determined by the investigator. Cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for

response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

RECIST Response Categories

Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD.
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
Non-CR/Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD):	Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).
Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the investigator will prevail.	
*Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.	

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Notes:

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort will be made to document the objective progression.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, the residual lesion will be investigated (fine needle aspirate/biopsy if possible) before confirming the complete response status.

Modified RECIST

Note on modified RECIST: In this particular study, RECIST criteria are modified with a stipulation that PD must be confirmed to account for pseudoprogression (tumor flare phenomenon). The first instance of PD must be confirmed by either:

1) confirmation of disease progression by repeat assessment 4-12 weeks later, and/or
2) association with clinical deterioration (such as a significant decline in performance status).

Confirmatory Measurement/Duration of Response

Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that will be performed no less than 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of not less than 6 weeks.

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

1.7.13 REMOVAL OF PATIENTS FROM STUDY/OFF STUDY CRITERIA

In the absence of treatment delays due to adverse events, treatment may continue up to 109 weeks until one or more of the following criteria applies:

a. Disease progression/relapse during active treatment, defined per modified RECIST v1.1 criteria
b. Intercurrent illness that prevents further administration of treatment
c. Unacceptable adverse event(s)
d. In the event of any drug-related life-threatening toxicity or laboratory abnormality the patient will be withdrawn from further treatment
e. Patient decides to withdraw from the study
f. Non-compliance with treatment plan as per investigator discretion
g. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
h. Protocol violation - any patient found to have entered this study in violation of the protocol might be discontinued from the study at the discretion of the Principal Investigator.

1.8 Primary Specimen Collection

Collection of Blood and Tumor Tissue for Laboratory Correlative Studies

Tumor biopsies and blood samples will be performed for correlative analyses to measure changes in the tumor microenvironment and peripheral blood including, but not limited to, the following categories:

- Immune cell phenotypes and gene expression
- Angiogenesis markers
- Metabolic effector molecules
- Exosome biogenesis
- Pharmacokinetic (PK)

Please refer to Lab Manual for analysis plan for laboratory correlative assays.

If correlative samples are collected and the scheduled treatment that day is delayed/held for any reason, there is no need to re-collect correlative samples.

Tumor Sampling

- Archival tissue (a block or 6-10 unstained slides) will be obtained for all subjects, when available.
- Tumor biopsies for research purposes are optional, but strongly encouraged, at Baseline/Week -2 (anytime pre-treatment, untimed sample), Week 1 (pre-infusion, untimed sample) and Week 7 (pre-infusion, untimed sample).
- Regarding the method of biopsy, excisional biopsy or punch biopsy is preferred over core needle biopsy for purposes of tissue sampling for correlative studies. An optimal size is 15 x 15 mm, but 5 mm x 5 mm is also useful, and there is no minimum size that is required. If a core needle biopsy is performed, 1-3 cm of core is required. If the lesion is < 2 cm in longest diameter and it is a RECIST target lesion, care should be taken to ensure that the overall size of the target lesion is not affected by the tissue sampling. In this case, a small excisional biopsy or core biopsy should be performed.
- Refer to the Lab Manual for more details on collection, processing and storage.

Blood sampling for correlative studies

- Research blood collection for correlative studies is to be performed at Baseline/Week -2 (anytime pre-treatment), Week 1 (pre-infusion, untimed sample), Week 7 (pre-infusion, untimed sample), and Week 13 (pre-infusion, untimed sample).
- At Baseline, 1 green top tube and 1 red top tube will be collected.
- At Week 1, 1 green top tube and 1 red top tube will be collected.
- At Week 7, 1 green top tube and 1 red top tube will be collected.
- At Week 13, 1 green top tube and 1 red top tube will be collected.
- Refer to the Lab Manual for details on collection, processing and storage.

Blood sampling for PK analysis

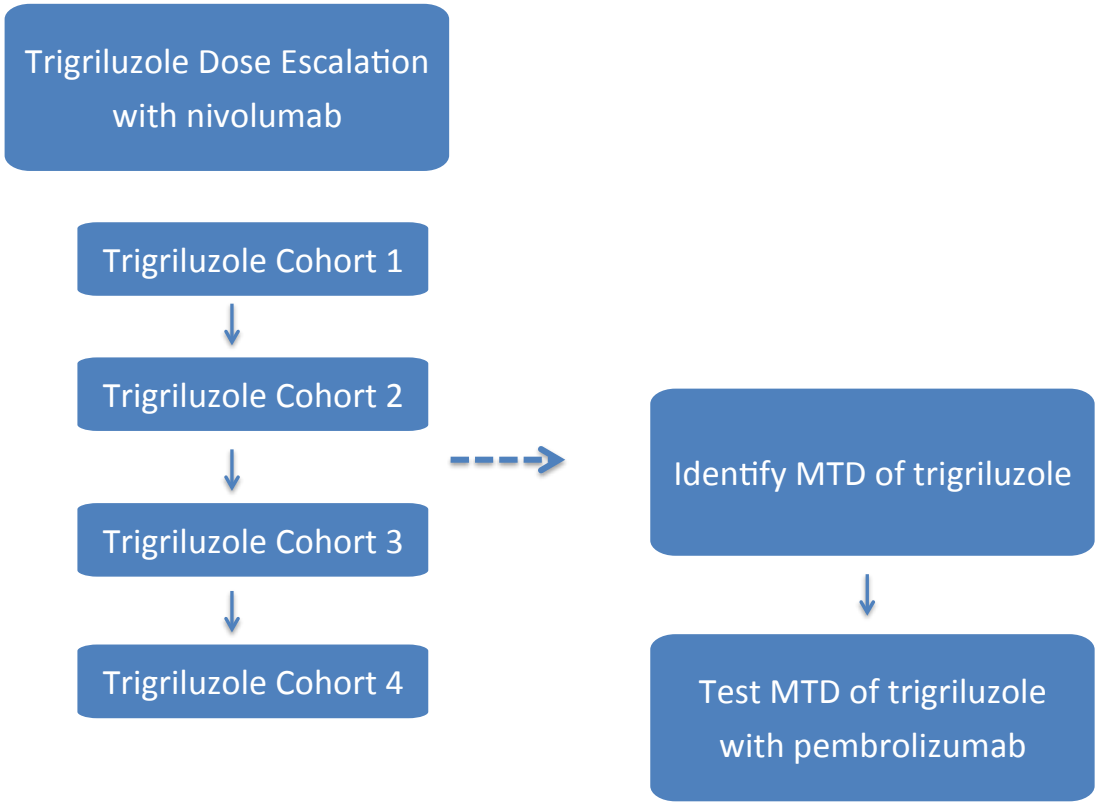
- Research blood collection for PK analysis is to be performed at the following treatment visits: a) Week -2 at pre-treatment, and at 2 hours and 4 hours post trigriluzole (window ± 30 minutes), b) Week 1 (untimed sample pre-infusion), and c) Week 7 (untimed sample pre-infusion).
- Approximately 2 mL of blood will be collected in a lavender top EDTA tube, which should be immediately placed on ice.

- Refer to the Lab Appendix for details on collection, processing and shipping.

1.9 Interviews, Focus Groups, or Surveys

N/A

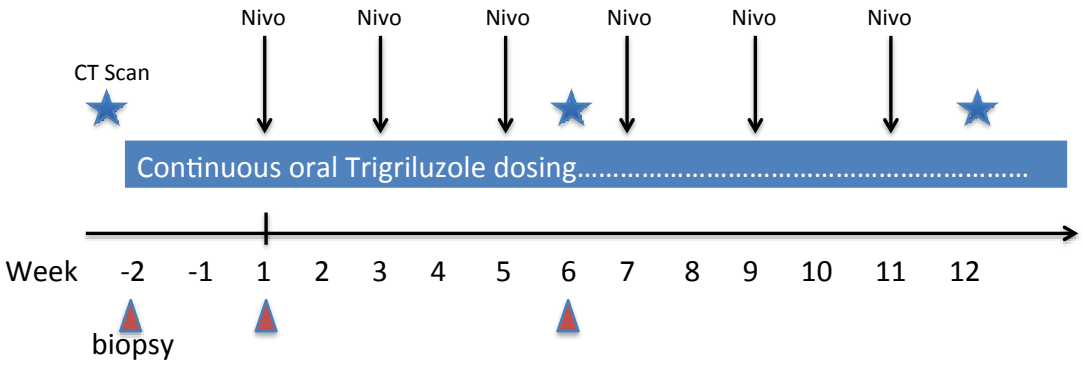
- **Study Design Flow Chart**



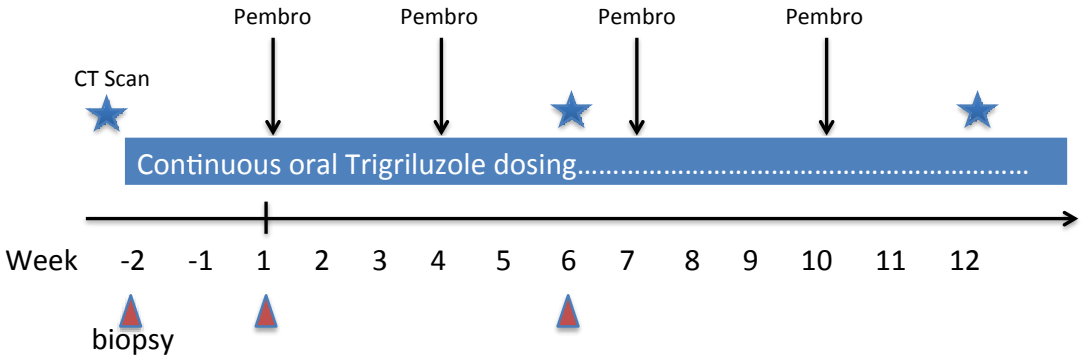
1.10 Timetable/Schedule of Events

Schema

Trigriluzole + Nivolumab



Trigriluzole + Pembrolizumab



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Trigriluzole + Nivolumab Study Calendar

	Screening/ Baseline[1]	Treatment Week									
		Week -2	Week -1	Week 1	Week 3	Week 5	Week 7	Week 9	Q2 weeks (Weeks 11 – 109)	Q4 weeks[8] (Weeks 13 – 109)	Weeks 13, 25, 37, 49
Scheduling Window (Days):		Within 14 d	±3	±1	±7	±7	±7	±7	±7	±7	±7
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Demographics and Medical History, including BRAF status	X										
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X
Assess Baseline Symptoms/Adverse Events and Compliance	X	X	X	X	X	X	X	X	X	X	X
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Status, Physical Examination	X	X		X	X	X	X	X		X	X
Routine Safety Laboratory Studies[2]	X	X[2]		X	X	X	X	X		X	X
Serum GGT	X			X			X				X
ECG[3]		X	X	X			X				X[3]
Trigriluzole Administration (PO continuous dosing)		X	X	X	X	X	X	X	X	X	X
Nivolumab Administration				X	X	X	X	X	X	X	X
Research Blood Collection for PK[4]		X		X			X				
Research Blood Collection for correlative science[5]	X			X			X				X[5]
Tumor Tissue Collection[6]	X			X			X				
Tumor Assessment[7]	X						X				X

Footnotes

1. Screening evaluations must be performed no more than 28 days prior to registration. The subject must begin study treatment no more than 14 days after registration.
2. Routine safety labs: CBC with Differential, Comprehensive Metabolic Panel, Amylase, Lipase, TSH, Free T4, LDH. Routine safety labs do not need to be repeated on Week -2 if performed in the past 14 days. More frequent labs may be performed as clinically necessary, as determined by treating investigator. WOCBP must have a negative serum beta-HCG pregnancy test prior to the first dose. During study treatment, pregnancy testing may be performed as indicated at the treating physician's discretion.
3. ECG monitoring is to be performed at the following treatment visits: a) at baseline (prior to dose on start date Week -2), and at expected C_{max} 4 hours post trigriluzole (window ± 30 minutes), b) at steady state on Week -1 and c) at steady state at Week 1 (untimed), d) at steady state at weeks 7, 13, 25, 37, and 49. If trigriluzole has been discontinued, EKG monitoring may be discontinued.
4. Research blood collection for PK analysis is to be performed at the following treatment visits: a) start date (Week -2) at pre-treatment, and at 2 hours and 4 hours post trigriluzole (window ± 30 minutes), b) Week 1 (untimed sample, pre-infusion), and c) Week 7 (untimed sample, pre-infusion). No PK collections after Week 7.
5. Research blood collection for correlative studies is to be performed at Screening/Week -2 (pre-treatment, untimed), Week 1 (pre-infusion, untimed), Week 7 (pre-infusion, untimed), and Week 13 (pre-infusion, untimed). Baseline research blood and tumor samples may be performed anytime prior to the start of therapy. Ideally, the research blood samples should be collected 3-14 days prior to the fresh tumor biopsy, but it is also acceptable to collect them on the same day. No research bloods after Week 13.
6. Tumor Tissue Collection. Archival tissue (a block or 6-10 unstained slides) will be obtained for all subjects, when available. Research biopsies are optional but strongly encouraged at Baseline/Week -2, and Week 1, and Week 7. No biopsies after Week 7.
7. Tumor Assessment must be performed at Screening, Weeks 7, 13, 25, 37, 49, 61, 73, 85, 97, and 109.
8. Q4 weeks starting from Week 13: Weeks 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, etc.

Trigriluzole + Pembrolizumab Study Calendar

	Screening/ Baseline[1]	Treatment Week							
		Week -2	Week -1	Week 1	Week 4	Week 7	Q3 weeks (Weeks 10 – 109)	Q6 weeks[8] (Weeks 13 – 109)	Weeks 13, 25, 37, 49
Scheduling Window (Days):		Within 14 d	±3	±1	±7	±7	±7	±7	±7
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Demographics and Medical History, including BRAF status	X								
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X
Assess Baseline Symptoms/Adverse Events and Compliance	X	X	X	X	X	X	X	X	X
Vital Signs and Weight	X	X	X	X	X	X	X	X	X
ECOG Performance Status, Physical Examination	X	X		X	X	X		X	X
Routine Safety Laboratory Studies[2]	X	X[2]		X	X	X		X	X
Serum GGT	X			X		X			X
ECG[3]		X	X	X		X			X[3]
Trigriluzole Administration (PO continuous dosing)		X	X	X	X	X	X	X	X
Pembrolizumab Administration				X	X	X	X	X	X
Research Blood Collection for PK[4]		X		X		X			
Research Blood Collection for correlative science[5]	X			X		X			X[5]
Tumor Tissue Collection[6]	X			X		X			
Tumor Assessment[7]	X					X			X

Footnotes

1. Screening evaluations must be performed no more than 28 days prior to registration. The subject must begin study treatment no more than 14 days after registration.

2. Routine safety labs: CBC with Differential, Comprehensive Metabolic Panel, Amylase, Lipase, TSH, Free T4, LDH. Routine safety labs do not need to be repeated on Week -2 if performed in the past 14 days. More frequent labs may be performed as clinically necessary, as determined by treating investigator. WOCBP must have a negative serum beta-HCG pregnancy test prior to the first dose. During study treatment, pregnancy testing may be performed as indicated at the treating physician's discretion.

3. ECG monitoring is to be performed at the following treatment visits: a) at baseline (prior to dose on start date Week -2), and at expected Cmax 4 hours post trigriluzole (window ± 30 minutes), b) at steady state on Week -1 and c) at steady state at Week 1 (untimed), and d) at steady state at weeks 7, 13, 25, 37, and 49. If trigriluzole has been discontinued, EKG monitoring may be discontinued.

4. Research blood collection for PK analysis is to be performed at the following treatment visits: a) start date (Week -2) at pre-treatment, and at 2 hours and 4 hours post trigriluzole (window ± 30 minutes), b) Week 1 (untimed sample, pre-infusion), and c) Week 7 (untimed sample, pre-infusion). No PK collections after Week 7.

5. Research blood collection for correlative studies is to be performed at Screening/Week -2 (pre-treatment, untimed), Week 1 (pre-infusion, untimed), Week 7 (pre-infusion, untimed), and Week 13 (pre-infusion, untimed). Baseline research blood and tumor samples may be performed anytime prior to the start of therapy. Ideally, the research blood samples should be collected 3-14 days prior to the fresh tumor biopsy, but it is also acceptable to collect them on the same day. No research bloods after Week 13.

6. Tumor Tissue Collection. Archival tissue (a block or 6-10 unstained slides) will be obtained for all subjects, when available. Research biopsies are optional but strongly encouraged at Baseline/Week-2, Week 1, and Week 7. No biopsies after Week 7.

7. Tumor Assessment must be performed at Screening, Weeks 7, 13, 25, 37, 49, 61, 73, 85, 97, and 109.

8. Q6 weeks: Weeks 13, 19, 25, 31, 37, 43, and 49, etc.

Study Calendar: Follow-Up (Nivolumab and Pembrolizumab cohorts)

	Off treatment but on study Q6 weeks[1]	**Off treatment but on study Q12 weeks[1]**	**Safety Follow-up** (12 weeks after last dose of rx)[3]	**Long-Term Follow-up[4]** Q12 weeks
Scheduling Window (Days):	±14	±14	±28	± 28
Prior and Concomitant Medication Review	X	X	X	
Assess Baseline Symptoms/Adverse Events and Compliance	X	X	X	
Vital Signs and Weight, ECOG Performance Status, Physical Examination	X	X	X	
Routine Safety Laboratory Studies	X	X	X	
Tumor Assessment[2]		X	X	
Post-study survival status, and receipt of subsequent anticancer therapy				X

Footnotes

1. If a subject has discontinued active treatment due to reasons other than PD (such as toxicity or patient preference), the subject will continue to have CT scans and follow-up visits to monitor them until Week 49.
2. Tumor Assessment must be performed at the same Weeks as subjects who remain on active treatment (Week 7, Week 13, and then Q12 weeks up until Week 49).
3. Safety follow-up visit will occur at Week 61 for subjects who complete all treatment (Week 49), but it will occur prior to Week 61 if active treatment is discontinued early for PD or other reasons.
4. Long-term Follow-up. Subjects will remain in the follow up phase of the trial for up to 3 years until the start of another therapy, withdrawal of consent or death.

2.0 Project Management

2.1 Research Staff and Qualifications

The Phase I/Developmental research team (Investigators, nurses, data coordinators, regulatory specialists, etc) is part of the RCINJ Office for Human Research Subjects and Rutgers University. Annual compliance and human subjects training is required.

2.2 Resources Available

N/A

2.3 Research Sites

Rutgers Cancer Institute of New Jersey (RCINJ)

3.0 Multi-Site Research Communication & Coordination

N/A

4.0 Research Data Source/s

4.1 Primary Data-Subjects and Specimens

4.1.1 Subject Selection and Enrollment Considerations

Recruitment Details

The subjects will be adults age 18 years and older who have a diagnosis of advanced cancer for whom medical therapy is indicated. Advanced cancer patients will be recruited specifically for this prospective study evaluating the safety of combining trigriluzole with PD-1 blocking antibody. After written informed consent is obtained, each subject will undergo screening for inclusion and exclusion criteria. These criteria, assessed by experienced clinical investigators who are familiar with expected toxicities of the drugs, help ensure the selection of the subjects who stand to benefit the most and the exclusion of medically inappropriate subjects.

Source of Subjects

Subjects will be identified from the medical oncology and surgical oncology clinics at RCINJ and by referring providers.

Method to Identify Potential Subjects

Patients with advanced unresectable or metastatic cancer will be recruited from the surgical oncology and medical oncology clinics at RCINJ. The treating physician will determine if the patient is a potential candidate for the study and discuss the protocol with the patient.

Subject Screening

The treating physician and study nurse will interview the patient and review the chart to determine if the patient is a potential candidate for the study. Patients who have cancer that is curable by surgery are not eligible to participate, as surgery would be a better choice. Patients whom are considered too frail to participate will not meet eligibility criteria for performance status and organ function and will not be eligible to participate. Subjects who do not meet all inclusion criteria are thought to be unable to tolerate study treatment safety and their enrollment will be recorded as screening failures.

Inclusion Criteria

A patient/subject is eligible for enrollment if *all* of the following inclusion criteria are met.

1) Patients must have histologically confirmed solid malignancy or lymphoma that is metastatic or unresectable
2) There is reasonable expectation of response to pembrolizumab or nivolumab, and one of the drugs is available from the commercial supply. This includes (but is not limited to) the following tumor types: melanoma, non-small cell lung cancer, renal cell carcinoma, squamous cell carcinoma of the head and neck, bladder cancer, and classic Hodgkin lymphoma.
3) The patient must have failed at least one line of standard treatment, with the following exceptions in which a PD-1 antibody is FDA approved in the first-line setting:
   a. melanoma patients
   b. non-small cell lung cancer patients without EGFR or ALK genomic tumor aberrations whose tumors have high PD-L1 expression [(Tumor Proportion Score (TPS) ≥50%)] as determined by an FDA-approved test.
4) Age ≥ 18 years old
5) Patients must give informed consent.
6) Prior chemotherapy, immunotherapy, radiotherapy or major surgery (including radiation therapy or surgery for treatment of brain metastases) must be

completed at least 3 weeks before study entry. Prior PD-1 or PD-L1 therapy is acceptable.

7) Patients must have an ECOG performance status ≤2 (Appendix A).
8) Patients must have normal organ and marrow function as defined below:
   a. Hemoglobin >8.0 mg/dL (without transfusion in the preceding 7 days)
   b. Platelets ≥70,000 /µL
   c. Total bilirubin within normal institutional limits (patients with Gilbert's Syndrome must have a total bilirubin < 3.0 mg/dL).
   d. AST(SGOT) <2X institutional ULN
   e. ALT(SGPT) <2X institutional ULN
9) Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm by CT scan, PET/CT scan, MRI or caliper/ruler measurement by clinical exam. Lymph nodes: to be considered pathologically enlarged and measurable, a lymph node must be ≥15mm in short axis when assessed by CT scan. Lesions that have been radiated in the advanced setting cannot be included as sites of measurable disease unless clear tumor progression has been documented in these lesions since the end of radiation therapy.
10) Ability to swallow pills

Exclusion Criteria

A patient /subject will not be eligible for this study if *any* of the following exclusion criteria are met.

1) Systemic immunosuppressive medications such as steroids. The following steroid formulations are permitted: intranasal, intra-articular, and inhaled steroids.
2) History of immune-related adverse event from prior immunotherapy treatment that has not improved to grade 0-1. Subjects with grade 2 hypothyroidism and grade 2 adrenal insufficiency requiring continued medical treatment may enroll provided that they are asymptomatic and stable on their dose of hormone replacement.
3) Serious concomitant systemic disorders (including active infections) that would compromise the safety of the patient or compromise the patient's ability to complete the study, at the discretion of the investigator, including active autoimmune disease requiring treatment within the past 30 days.
4) Any condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other systemic immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses <10 mg daily prednisone equivalents are permitted in

the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if <10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

5) Second primary malignancy, except those second primary malignancies that are not considered to be competing causes of death in the opinion of the treating investigator. Examples include: in situ carcinoma of the cervix, adequately treated non-melanoma carcinoma of the skin, or other malignancy treated at least 5 years previously with no evidence of recurrence.
6) Patients with active, untreated central nervous system (CNS) metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients who have brain metastases that been treated with radiation therapy or surgery will be required to have a washout period of at least 3 weeks prior to study entry, must be neurologically asymptomatic, and must not require systemic steroids.
7) Women of child-bearing potential and men must agree to use adequate contraception prior to the start of treatment, for the duration of treatment, and for 5 months after last dose of study treatment.
8) Patients with immune deficiency have impaired immune responses, therefore, known HIV-positive patients are excluded from the study because of interference with the correlative goals of the study.
9) Patients who require one or more of the following prohibited medications: fluvoxamine, cimetidine, amiodarone, efavirenz, fluoroquinolones, fluvoxamine, furafylline, interferon, methoxsalen, mibefradil, or ticlopidine.

Recruitment Materials

N/A

Lead Site Recruitment Methods

N/A

4.1.2 Subject Randomization

N/A

4.1.3 Secondary Subjects

N/A

4.1.4 Number of Subjects

Total Number of Subjects

A total of 12-27 advanced cancer patients will be recruited specifically for this prospective study.

Total Number of Subjects If Multicenter Study

N/A

Require Number of Subjects to Complete Research

12 - 27 subjects.

Feasibility of Recruiting

Investigators may contact referring doctors to tell them about the study by phone or email. Patients will not be recruited directly, as the information about the study will be shared with them through their treating physician. The study team will contact the patient only after the treating physician has determined that the patient is interested in learning more about the study.

Patients may also learn of the study through websites such as clinicaltrials.gov and cancer patient chatrooms and blogs. No patient recruiting materials will be posted by the study team members without IRB approval.

4.1.5 Consent Procedures

Consent

Documenting Consent

Written informed consent is required. Informed consent must be obtained prior to commencing any research procedures. The informed consent document may not include any exculpatory language through which the subject or representative is made to waive any of the subject's legal rights or releases, or appears to release the investigator, the sponsor or the institution from liability for negligence.

Waiver of Documentation of Consent

N/A

Waiver or Alteration of Consent Process

N/A

Consent Process

Location of Consent Process

The informed consent discussion will take place in the Cancer Institute. Subjects must sign consent in person at the Cancer Institute. If the patient wishes to enroll on the study, he or she will sign the informed consent in the presence of a physician or nurse on the study team.

Ongoing Consent

Subjects will be re-consented if there is new information or protocol amendments and the IRB has required that subjects be verbally informed or reconsent in writing.

Individual Roles for Researchers Involved in Consent

The treating physician will determine if the patient is a potential candidate for the study and discuss the protocol with the patient. The patient will also meet with the research nurse clinician to review the informed consent document.

Consent Discussion Duration

The length of time varies, typically from 30-60 minutes per visit. The patient will be encouraged to take the informed consent document home to read it over in full and if desired, discuss the protocol with their primary care physician or another third party. The process often lasts more than one visit.

Coercion or Undue Influence

The study staff shall seek such consent only under such circumstances that provide the prospective patient the opportunity to consider whether or not to participate, and that minimize the possibility of coercion or undue influence.

Subject Understanding

The patient and his or her family will be given the opportunity to ask questions. The discussion with the patient, or the representative, shall be in a language understandable to the subject or representative.

4.1.6 Special Consent/Populations

Minors-Subjects Who Are Not Yet Adults - N/A

Children under the age of 18 will be excluded from this study. Riluzole and PD-1 inhibition have not been adequately tested in children and so should be studied separately. Because solid tumor cancer is primarily a disease of adults it is anticipated that the exclusion of children will not adversely impact accrual to the trial.

Gender and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. No special recruitment will be performed based on gender or minority status. Based on data examining the ethnic and racial mix of patients presenting with cancer at our institution over the past 10 years, we expect the majority of the population to be white, non-Hispanic subjects, but appropriate patients of all race and ethnic groups will be offered participation.

Non-English Speaking Subjects

Process for Non-English Speaking Subjects

A professional medical translation telephone service called Auracom will be used to facilitate the consent discussion.

Short Form Consent for Non-English Speakers

Yes, a short form will be provided.

Adults Unable to Consent / Cognitively Impaired Adults

N/A

4.1.7 Economic Burden and/or Compensation for Subjects

Expenses

The costs of participating on the trial will be addressed during the informed consent process. General questions will be answered to the best of the study team's ability, but patients will be encouraged to call their insurance company for more specific information. Patients or their insurance companies will be billed for all standard-of-care assessments, as defined on the billing grid. Institutional financial SOPs (e.g. pre-authorizations for medications and scans) will be followed to minimize excess financial impact on the subject. Patients will be educated that additional medical

interventions may be needed in the event of adverse events, which may result in more healthcare-related costs to them.

Compensation/Incentives

No payment will be offered to recruit subjects. Patients may be reimbursed if funds are available for expenses such as parking and hotel rooms.

Economic/Financial Considerations

This is an investigator-initiated trial that is sponsored by Fox Chase Chemical Diversity Center, Inc. and Biohaven Pharmaceuticals. Additional funding is being sought from governmental and foundations to support the correlative science aims.

4.1.8 Risks to Subjects

Physical Risk of Study Treatments

Adverse events are expected to occur on this study. Please see section on expected toxicities. These risks are acceptable because pembrolizumab and nivolumab are both FDA-approved drugs for this indication so the risks are within what is acceptable for standard of care. Trigriluzole is an investigational drug, but the related drug riluzole is also FDA-approved and toxicities are expected to be mild. The risks of the combined study treatment are minimized by careful assessments by trained study staff, as well as frequent laboratory monitoring for safety. Once treatment arm assignment takes place, subjects will be treated and monitored according to the study calendar. Treatment is dosed IV once every 2-3 weeks only after clinician and laboratory assessment. Trigriluzole is self-administered by subjects at home as oral daily to twice daily dosing—subjects will be given clinic phone numbers and after-hours contact information and be instructed to call for guidance if they develop any side effects in between the in-person assessments. Pembrolizumab or nivolumab treatment is dosed IV once every 2-3 weeks only after clinician and laboratory assessment. Trigriluzole is self-administered by subjects at home as oral dosing. To minimize risk, subjects will be given clinic phone numbers and after-hours contact information and be instructed to call for guidance if they develop any side effects in between the in-person assessments.

Potential for Drug-Drug Interactions

Clinical drug interaction studies for Trigriluzole have not been conducted yet. Trigriluzole, itself, is not expected to interfere with drug metabolism and its cleavage via plasma peptidases render it unlikely to be affected significantly by liver cytochrome P450 inhibitors. Trigriluzole is not an inhibitor of CYP3A4, CYP1A2, or CYP2D6. In CYP induction studies, the estimated EC50 and Emax for CYP1A2 mRNA was 1.44 µM and 3.47-fold induction, respectively. The estimated EC50 and Emax for

CYP2B6 mRNA was 12.6 µM and 27.0-fold induction, respectively. Trigriluzole did not increase CYP3A4 mRNA at doses up to 30 µM.

Tumor Biopsy and Venipuncture Risks

There is a small risk of bleeding, pain, or infection with tumor biopsies. These risks will be minimized by use of adequate hemostasis, local anesthetic and aseptic technique. This level of risk is considered to be acceptable as patients routinely have similar biopsies performed when pathologic evaluation is necessary to confirm diagnosis or a site of tumor progression. Tumor biopsies are mandatory, however, may be omitted without deviation due to unacceptable risk (warfarin therapy, tumor near location with high risk for bleeding or other complication, etc.) with prior authorization from the PI (see PI contact information on cover page of protocol). There is a very small risk of bleeding, pain, or infection with venipuncture; however this is minimized as the correlative blood samples are being taken at the same time as the standard of care laboratory bloods.

Procedures for Risks to Embryo, Fetus, and/or Pregnant Subjects

Trigriluzole has not yet been assessed in fertility and fetal development studies. Riluzole has been assessed previously and has been characterized as a Category C drug. As described in the USPI, oral administration of riluzole to pregnant animals during the period of organogenesis caused embryotoxicity in rats and rabbits at doses of 27 mg/kg and 60 mg/kg, respectively, or 2.6 and 11.5 times, respectively, the recommended maximum human daily dose on a mg/m^2 basis. Evidence of maternal toxicity was also observed at these doses. When administered to rats prior to and during mating (males and females) and throughout gestation and lactation (females), riluzole produced adverse effects on pregnancy (decreased implantations, increased intrauterine death) and offspring viability and growth at an oral dose of 15 mg/kg or 1.5 times the maximum daily dose on a mg/m^2 basis. There are no adequate and well-controlled studies in pregnant women. Riluzole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Women of child-bearing potential (WOCBP) are defined as women who have the potential to become pregnant. Peri-menopausal women are considered WOCBP and must use effective contraception. Women who are menopausal (defined as no menses in >1 year) are excluded. WOCBP must have a negative serum pregnancy test prior to the first dose. During study treatment, pregnancy testing may be performed as indicated at the treating physician's discretion. WOCBP may opt out

of pregnancy testing if they provide an acceptable reason with their treating provider (such as 100% abstinence from heterosexual intercourse).

WOCBP and men must agree to use effective contraception during the study participation, and for 5 months after the last dose of the drug. Effective contraception options include: 100% abstinence from heterosexual intercourse, surgical (tubal sterilization or partner's vasectomy), intrauterine device, and combination hormonal contraceptives including birth control pills, skin patches, shots, under-the-skin implants, or vaginal rings. Barrier methods of birth control such as condoms are not adequate as primary method of contraception, but may be used as a secondary method.

Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

Risk-Benefit Ratio

The hypothesis to be addressed in the proposed study is potential therapeutic, diagnostic, and/or prognostic importance for patients with advanced malignancy. GRM1 blockade with riluzole is generally safe and well tolerated and operates by a different mechanism from the anti-PD1 antibodies so the toxicity profiles are non-overlapping. Therefore, we do not anticipate any untoward safety findings with trigriluzole. Nonetheless, this protocol includes measures to increase safety such as DLT observation period and AE reporting procedures. Patients may alternative treatment options including chemotherapy or other therapy. The results of this study may assist in the development of new biomarkers to improve diagnosis/prognosis in this population and could lead to new therapeutic targets for patients with advanced cancer. Therefore, the potential benefits of the proposed study outweigh potential risks.

Potential Benefits

There may be direct benefit to the subject for participation in this proposed study because the subject will receive pembrolizumab or nivolumab, which are FDA-approved and have been shown to prolong survival in patients with various advanced malignancies, although this does not guarantee that each individual patient will benefit. This study may demonstrate enhanced efficacy for pembrolizumab/nivolumab when combined with trigriluzole in this patient

population, so patients may benefit from trigriluzole as well. Subjects may experience prolonged survival or decrease in cancer symptoms. Data gathered from the proposed study may allow us to identify molecules have the potential to be used as predictive biomarkers or therapeutic targets that could result benefit to the patient or society as a whole in the future.

Risks to Non-Subjects

N/A

Assessment of Social Behavior Considerations

N/A

Reasonably Foreseeable Risks

N/A

Risk Of Imposing An Intervention On Subject With Existing Condition

N/A

Other Foreseeable Risks

N/A

Observation And Sensitive Information

N/A

Minimizing Risks

To minimize risk, subjects will be evaluated frequently and given clinic phone numbers and after-hours contact information and be instructed to call for guidance if they develop any side effects in between the in-person assessments. Institutional financial SOPs (e.g. pre-authorizations for medications and scans) will be followed to minimize excess financial impact on the subject.

Other foreseeable risks may include risks associated with a possible loss of confidentiality. Patient confidentiality will be strictly maintained according to NIH guidelines. Computerized clinical data are protected by a series of passwords. Detailed clinical information will be kept in a research patient chart,

which will be kept in a separate private and locked location. All of the data that is to be delivered to the non-clinical researchers is non-PHI, and anonymous. The biospecimens will be processed, analyzed and presented by the issued patient number. The patient will not be identified by name and no PHI will be directly attached to the specimens.

Certificate of Confidentiality

N/A

Potential Benefits to Subjects

There may be direct benefit to the subject for participation in this proposed study because the subject will receive pembrolizumab or nivolumab, which are FDA-approved and have been shown to prolong survival in patients with various advanced malignancies, although this does not guarantee that each individual patient will benefit.

This study may demonstrate enhanced efficacy for pembrolizumab/nivolumab when combined with trigriluzole in this patient population, so patients may benefit from trigriluzole as well. Subjects may experience prolonged survival or decrease in cancer symptoms. Data gathered from the proposed study may allow us to identify molecules have the potential to be used as predictive biomarkers or therapeutic targets that could result benefit to the patient or society as a whole in the future.

Provisions to Protect the Privacy Interests of Subjects

Patient confidentiality will be strictly maintained according to NIH guidelines. Computerized clinical data are protected by a series of passwords. Detailed clinical information will be kept in a research patient chart, which will be kept in a separate private and locked location. All of the data that is to be delivered to the non-clinical researchers is non-PHI, and anonymous. The biospecimens will be processed, analyzed and presented by the issued patient number. The patient will not be identified by name and no PHI will be directly attached to the specimens.

Research Team Access To Subject Data

The research team is permitted to access the medical record and research chart.

Annual training on privacy is required.

4.2 Secondary Data – Records/Chart Reviews/Databases/Tissue Banks/etc.

N/A

5.0 Special Considerations

5.1 Health Insurance Portability and Accountability Act (HIPAA)

It is necessary that PHI be accessed to achieve the goals of this study. All study staff are trained in HIPAA and routine protections of PHI will apply.

5.2 Family Educational Rights and Privacy Act (FERPA)

N/A

5.3 NJ Access to Medical Research Act

N/A

5.4 Code of Federal Regulations Title 45 Part 46 (Vulnerable Populations)

N/A

6.0 Research Data Protection and Reporting

6.1 Data Management /Statistical Considerations

Primary Hypothesis and Endpoint

We hypothesize that combination therapy will be safe in patients with metastatic cancer. Data on the adverse event type, severity and frequency will be recorded.

The primary endpoint will be the MTD/RP2D. The MTD/RP2D of trigriluzole in combination with nivolumab is expected to also be the tolerable in combination with pembrolizumab.

Secondary Hypotheses and Endpoints

We hypothesize that combination therapy will lead to regression of tumors. Therefore, the following secondary endpoints will be recorded:

- objective response rate (ORR) according to modified RECIST v.1.1.
- survival time (OS)
- landmark survival rates at 1 and 2 years
- duration of response for responding patients
- time to progressive disease (PFS)

- time to treatment failure
- time to next therapy or death (TTNTD)
- freedom from new metastases

We hypothesize that features in the tumor microenvironment (TME) and/or tumor genome may be associated with response to therapy. Therefore, we will quantify changes in immune cell phenotypes and gene expression, markers of angiogenesis, metabolic effector molecules, and exosomal formation and contents.

Study Design /Analysis Plan for Primary Endpoint

Overview of sequential testing with nivolumab then pembrolizumab

In the first portion of this study, cohorts of subjects will be treated with increasing doses of trigriluzole in combination with nivolumab. After the MTD of trigriluzole is identified, it will be tested in combination with pembrolizumab (Cohort P1). The MTD of trigriluzole with nivolumab is likely to be safe and tolerable in combination with pembrolizumab.

Escalation Procedure

There 4 dosing cohorts of trigriluzole (Cohorts -1, 1, 2, 3, and 4). Please refer to dosing cohort tables.

Starting with Cohort 1, dose escalation of trigriluzole with nivolumab will proceed up to a maximum of 280 mg PO BID (Cohort 4) to identify the MTD. Subjects will be enrolled on the trial in a starting cohort size of 3 subjects.

After the MTD of trigriluzole with nivolumab is identified, the MTD of trigriluzole will be tested in combination with pembrolizumab in 3-6 patients in Cohort P1. If the MTD is not tolerable in Cohort P1, the dose will be decreased by 1 level.

Escalation Rules

The decision to escalate or deescalate to a new dosing cohort or to expand a dosing cohort will be made using the procedure described in the table below. The dose escalation procedure is a Semi-Bayesian modified toxicity probability interval (mTPI) method.[41] Each cohort size will be 3 patients (i.e., same as 3+3 design), but the

method can handle varying cohort size when real data have different cohort sizes. There will be no within-patient dose escalation.

The following table displays the escalation/de-escalation/stay/ decisions using the set-up: maximum number of patients at a given dose =18, true probability of toxicity pT=0.3, and the higher and lower ends of the equivalence interval =0.05.

Table 6. Number of patients treated at current dose:

Number of dose limiting toxicities (DLT's)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
1	D	S	S	S	S	E	E	E	E	E	E	E	E	E	E	E	E	E
2		D U	D	S	S	S	S	S	S	S	E	E	E	E	E	E	E	E
3			D U	D U	D	S	S	S	S	S	S	S	S	S	S	E	E	E
4				D U	D U	D U	D	D	S	S	S	S	S	S	S	S	S	S
5					D U	D U	D U	D U	D U	D	S	S	S	S	S	S	S	S
6						D U	D U	D U	D U	D U	D U	D	S	S	S	S	S	S
7							D U	D U	D U	D U	D U	D U	D U	D	S	S	S	S
8								D U	D U	D U	D U	D U	D U	D U	D U	D U	D	S
9									D U	D U	D U	D U	D U	D U	D U	D U	D U	D U
10										D U	D U	D U	D U	D U	D U	D U	D U	D U
11											D U	D U	D U	D U	D U	D U	D U	D U
12												D U	D U	D U	D U	D U	D U	D U
13													D U	D U	D U	D U	D U	D U
14														D U	D U	D U	D U	D U
15															D U	D U	D U	D U
16																D U	D U	D U
17																	D U	D U
18																		D U

E = Escalate to the next higher dose
S = Stay at the current dose
D = De-escalate to the next lower dose
U = The current dose is unacceptably toxic
MTD = 30%
Epsilon1 = 0.05
Epsilon2 = 0.05

In this procedure, patients are entered at an initial dose (Cohort 1, trigriluzole 140 mg PO daily) in groups of three, and depending on the outcome, the dose is either escalated, de-escalated, or it remains the same. The procedure continues until all dose levels have been exhausted, or until six patients have been entered at a particular dose. The final dose will be the maximum tolerated dose, or MTD.

The table below summarizes the probabilities of observing the numbers of subjects under different assumptions of true DLT rate θ_0, which can be interpreted as the one-sided p-values for testing the null hypothesis that the true DLT rate is less than θ_0. However, these p-values are considered non-binding guidelines; recommendations would not be based solely on statistical grounds, as many other factors (ie, all aspects of the data from the trial) may be part of the decision process.

Table 7. Probability of observing the number (%) of subjects with DLT out of a cohort of 6 subjects

Observed number (%) of subjects with DLT	Probability (p-value) under different assumptions				
	$\theta_0 = 0.20$	$\theta_0 = 0.25$	$\theta_0 = 0.30$	$\theta_0 = 0.35$	$\theta_0 = 0.40$
0 (0%)	0.74	0.82	0.88	0.92	0.95
1 (17%)	0.34	0.47	0.58	0.68	0.77
2 (34%)	0.099	0.17	0.26	0.35	0.46
3 (50%)	0.017	0.038	0.07	0.12	0.18

Through this procedure, the MTD of trigriluzole in combination with nivolumab will be identified. The MTD will then be tested in combination with pembrolizumab using the same escalate/de-escalate/stay rules.

Dose Limiting Toxicity

Definition of DLT

The occurrence of any Grade 3 or higher toxicity during the DLT evaluation period (i.e., occurred during the first 4 weeks of therapy) will be considered a DLT, if judged by the treating investigator to be related to the administration of either study drug or the combination of study drugs.

EXCEPTION: Grade 3 amylase or lipase elevation without clinical symptomatology of pancreatitis (i.e. no epigastric pain and vomiting) will not be considered a DLT and does not require SAE reporting.

DLT observation period

There will be a 5-week evaluation period for observation of DLT during which time new subjects may be accrued within the same dose cohort, but dose escalation or de-escalation beyond the current dose cohort shall not be allowed. The date of reference for the 5 weeks is the first day of treatment including the lead-in period (Day -14); therefore the end of the DLT observation period occurs at Week 3. The end of the DLT observation period will not coincide with an office visit in the pembrolizumab cohorts; if no DLT has been reported by that time, the evaluation period will be considered to be passed.

Sample Size Justification

Four dosing cohorts are planned in order to test a dose of trigriluzole up to 280 mg PO BID. We do not anticipate testing higher doses. With approximately 3 subjects per dosing cohort, the required sample size is 9 - 21 subjects for testing trigriluzole with nivolumab. The MTD will then be tested with pembrolizumab and an additional 3-6 subjects will be required. The total sample size will be 12 - 27 subjects (9 – 21 in the nivolumab group, plus 3 – 6 in the pembrolizumab group).

Analysis Plan for Secondary Endpoints

Clinical Secondary Endpoints. The frequency of AEs and SAEs will be recorded. Clinical measures of efficacy will be described, including overall survival, progression-free survival, etc. (see Secondary Endpoints above). Descriptive statistical will be used, except where otherwise specified. Continuous variables will be presented by summary statistics (such as mean, median, standard error and 90% CI) and the categorical variables by frequency distributions (i.e., frequency counts, percentages and 90% CI).

Correlatives. We will collect pre- and post-treatment tumor samples from responders and non-responders to test for differences in signal transduction in key pathways (MAPK, Pi3K/AKT), presence of TILs and other immune effector cells, PD-L1 expression, the expression of VEGF, IL-8, CD34, CCL2, M-CSF, and immune-related gene expression profile. We will determine if the presence of GRM1 expression, activating mutations in B-RAF or N-RAS, or PTEN inactivation (or activating B-RAF mutations and PTEN inactivation together) in the pre-treatment tumor samples correlates with response to therapy. We will examine the change in key pathways before and after the trigriluzole monotherapy lead-in period (Baseline vs. Week 1) and compare to the samples taken after exposure to combination therapy (Week 7).

We will also collect pre- and post-treatment peripheral blood samples from responders and non-responders and determine the expression of VEGF, IL-8, CD34, CCL2, and M-CSF, and changes in the quantity and contents of tumor-derived exosomes. We will determine if the expression of these markers in pre-treatment peripheral blood samples correlates with response to therapy. Next, we will compare the pre-treatment and post-treatment blood and tumor samples to see if response to treatment correlates with activation or suppression of signal transduction through the MAPK and Pi3K/AKT pathways, changes in immune effector cells in the tumors and peripheral blood, or changes in the quantity or contents of peripheral blood exosomes.

Treatment effect for each patient will be measured as paired differences between pre- and post- measurements of these parameters at various times. Transformation of the data will be performed if appropriate, e.g. log transformation, and hence treatment effect will be expressed on a log scale.

This analysis of the data obtained in these correlative studies will be descriptive in nature. Sample size may not be adequate to test the correlations; these studies will be used to demonstrate the feasibility of including these studies in larger future trials.

Definitions of Evaluable for Toxicity and Response

Evaluable for toxicity. All subjects who received at least one dose of each study drug will be considered evaluable for toxicity.

Evaluable for objective response. All subjects who undergo at least one on-study tumor assessment will be considered evaluable for response.

Compliance and Missing Data

Compliance/Adherence

Compliance with study procedures is defined as the patient making a good faith effort to complete all protocol-related assessments. If a subject is not compliant, the treating investigator should assess barriers to compliance. Patients whose actions cause 2 or more deviations despite efforts to improve non-compliance may be removed from the study.

Compliance with oral medication is defined as the patient taking ≥75% of the prescribed doses/days within a treatment cycle. The desired level of compliance is > 75%. Patients who cannot maintain this level of compliance may be removed from the study. Patients

who consistently cause deviations despite efforts to improve non-compliance may be removed from the study. See monitoring compliance with oral medication.

Subjects who leave the study for non-compliance will not be followed-up.

Missing data

Subjects who do not start study treatment or are not evaluable for toxicity for the entire DLT observation period will be replaced (up to 5 replacements).

Interim Analysis

An interim analysis of safety data in the first 9 - 12 subjects may be reported at academic conference(s).

6.2 Data Collections and Records to be Kept

Case Report Forms

Completion of the electronic CRFs (eCRFs) will be done in accordance with the instructions in a study specific data capture plan. The electronic CRF stored in Oncore® will be the primary data collection document for the study. All eCRFs will be completed by clinical research coordinators of the Office of Human Research Services (OHRS) at the Cancer Institute of New Jersey. The eCRFs will be maintained in a confidential format in a secure database. The CRFs will be updated in a timely manner following acquisition of new source data. Only the key personnel delegated on the delegation of authority log are permitted to make entries, changes, or corrections in the CRF. All users of this system will complete user training, as required or appropriate per regulations. An audit trail will be maintained automatically by the electronic CRF management system.

Data Submission Timeline and Forms

Completion of eCRFs will occur in accordance with NCI guidelines. Baseline (pre-study) eCRFs (e.g., enrollment, medical history, concomitant medications, disease assessment, etc.) will be completed no later than 14 days after the start of treatment. Treatment eCRFs (e.g., drug administration, adverse events, chemistries, etc.) will be completed no later than 14 days following each cycle of treatment. Off-treatment information (e.g., follow-up, best response, etc.) will be completed no later than 14 days after the end of protocol treatment.

Research Charts

A research chart (i.e., shadow chart) is maintained at OHRS for each patient enrolled. Copies of significant study source documents will be maintained in the research chart. Examples of source document copies that will be maintained in the research chart include: signed informed consent form, documents that verify eligibility and treatment and documents that verify Grade 3-4 adverse events and response. This information will be updated on a prospective basis and will be confidentially maintained at the Cancer Institute of New Jersey, OHRS.

Reports

The RCINJ PI is the sponsor-investigator of the IND (IND#133666) and RCINJ will be responsible for episodic and annual reporting to the FDA. Publications for submission and annual reports for the IRB will be written by the RCINJ PI and OHRS using the data captured on the e-CRFs.

6.3 Data and Safety Monitoring Plan

Monitoring of this study will occur in accordance with the NCI approved Data and Safety Monitoring Plan (DSMP) of RCINJ. The study team reviews all toxicity during the dose-escalation phase of studies and presents this data to the weekly Phase I meetings. The RCINJ Human Research Oversight Committee (HROC) is responsible for annual data and safety monitoring of phase I and phase II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the HROC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The HROC in concert with the Quality Assurance Monitoring Team oversees the conduct of RCINJ cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements. All study staff must complete training and maintaining active certification in the use of human subjects in research.

Periodic Data Evaluation

HROC meets approximately twice a month and each disease group is reviewed at least every 3 months. HROC will review all Dose Administration Memos (DAM) and dose-limiting toxicities (DLTs) for Phase I dose-escalation trials. Protocol suspensions and re-opening of accrual to the next cohort based on DLT evaluation, fall under the purview of the HROC.

An "initiation audit" will be conducted at the RCINJ in accordance with the DSMP following enrollment of the first two (2) or three (3) patients. Subsequent audits will occur on an annual basis prior to annual IRB continuing review, if the findings from the initiation audit were satisfactory. More frequent audits of patient data and study conduct will occur if necessary. Prior audit findings and/or situations that may arise during the course of the study will determine the need for more frequent auditing. All audit findings will be discussed with the principal investigator and reported to the RCINJ HROC and the Rutgers University IRB.

Type of Data Evaluated

HROC monitors accrual, deviations, and SAEs.

Collection of Safety Information

Safety information is collected and tallied in reports using OnCore.

Frequency Of Data Collection

Data is collected at each study visit and follow-up contact. Safety data collection (including SAEs) starts after the first study treatment.

Reviewer of Data

The members of OHRS management staff review the data for completeness. HROC monitors accrual, deviations, and SAEs. The PI will review data as needed for accuracy and consistency.

Schedule Of Review Of Cumulative Data

There will be one planned interim analysis after 9-12 subjects are enrolled.

Tests for Safety Data

N/A

Suspension of Research

If the combination is found to not be tolerable (MTD reached at lowest dosing cohort), accrual will halt until further review/amendment.

6.4 Reporting Results

Sharing of Results with Subjects

Individual subject results of routine diagnostic clinical tests and incidental findings will be shared with subjects at their office visits or by telephone by appropriate study staff (clinicians).

Individual Results

Individual subject results of routine diagnostic clinical tests and incidental findings will be shared with subjects at their office visits or by telephone by appropriate study staff (clinicians).

Aggregate Results

N/A

Professional Reporting

The policies and procedures of Rutgers University's legal department (see: Investigator's Brochure) will govern publication of the trial. It is expected that the results of this trial will be submitted for publication in a timely manner following the conclusion. The RCINJ PI, and all co-authors prior to submission or use, must review any abstract or manuscript.

ClinicalTrials.Gov Registration And Data Reporting

This study is registered at clinicaltrials.gov.

7.0 Data and/or Specimen Banking

N/A

8.0 Other Approvals/Authorizations

N/A

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Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Trigriluzole (BHV-4157) + Anti-PD-1 Lab Appendix

I. Overview of samples to be collected

Tumor biopsies with be performed and blood samples will be collected for correlative analyses. We will measure changes in the tumor microenvironment and peripheral blood in pre- and post-treatment samples in the following categories:

- Immune cell phenotypes and gene expression
- Angiogenesis markers
- Metabolic effector molecules
- Exosome biogenesis
- Pharmacokinetic (PK)

The expected sample size is 12 to 27 subjects. Detailed Study Calendars are located in the Protocol. The following table lists the samples that are expected to be collected from each subject.

Time	Samples needed
Screening or Baseline	• Archival tissue (block or 6-10 unstained slides) when available • Tumor biopsy (optional but strongly encouraged) • Peripheral blood collection for biologic correlative studies (1 green top tube and 1 red top tube)
Week -2	• PK samples x 3 (lavender top tubes) 1. Pre-treatment 2. 2 hours post-trigriluzole (window +/- 30 minutes) 3. 4 hours post trigriluzole (window +/- 30 minutes)
Week 1	• Tumor biopsy (optional but strongly encouraged) • Peripheral blood collection for biologic correlative studies (1 green top and 1 red top tube) • PK sample (lavender top)
Week 7	• Tumor biopsy (optional but strongly encouraged) • Peripheral blood collection for biologic correlative studies • PK sample (lavender top)
Week 13	• Peripheral blood collection for biologic correlative studies (1 green top and 1 red top tube)

II. Handling of archival tumor tissue

Archival tumor tissue (for example, in paraffin blocks or pathology slides) should be logged and labeled in BRS and then delivered to the laboratory and kept at room temperature until analysis.

III. Collection of fresh tumor tissue

a. Method of biopsy: Excisional biopsy or punch biopsy is preferred over core needle biopsy for purposes of tissue sampling for correlative studies. An optimal size is 15 x 15 mm, but 5 mm x 5 mm is also acceptable. There is no minimum size that is required. If a core needle biopsy is performed, 1-3 cm of core is recommended. If the lesion is < 2 cm in longest diameter and it is a RECIST target lesion, care should be taken to ensure that the overall size of the target lesion is not affected by the tissue sampling. In this case, a small excisional biopsy or core biopsy should be performed.
b. The RCINJ Biospecimen Repository Service (BRS) will process, label and store the tissues samples.
c. Fresh tumor tissue for Western blotting will be collected by biopsy and immersed (completely) in sterile cold (2-7^{o}C) RPMI in a sterile specimen cup (for example, VWR catalog# 15704-088) on ice.
d. Fresh tumor tissue for murine experiments will be collected in sterile cold (2-7^{o}C) RPMI in a sterile 50 ml centrifuge tube.
e. The specimen cup or centrifuge tube will be labeled with the patient ID, date, and time of tumor tissue collection.
f. The sample will be immediately transported on ice from the collection site to the BRS laboratory.
g. Tissue processing should be performed as soon as possible after tissue collection/receipt (maximum time from tissue acquisition and processing should be no more than 3 hours).

IV. Processing of tumor tissue

The RCINJ Biospecimen Repository Service (BRS) will process, label and store the tissues samples until delivery to the analysis lab.

As clinical samples are often small, the allocation of small tumor samples for different correlative studies will be determined per the following table:

Prioritization Table for Fresh Tumor Biopsies

Priority Level	Type of Preserved Tissue	Biomarker Analysis Method
1st	Flash frozen in liquid nitrogen	- Western blotting ○ Expression of MAPK and PI3K/AKT signaling pathway components ○ M-CSF and CCL2 expression ○ HIF-1a, IL-8, and VEGF expression Gene expression profiling
2nd	Formalin fixed paraffin embedded (FFPE)	- IHC ○ GRM1 expression ○ pERK and pAKT - H&E - Lymphocytic Infiltration
		○

Fat will be trimmed from the tumor. Tumor tissue specimens will be measured in the 2 longest dimensions. Tumor tissue will be sectioned into specimens by the following prioritization rules:

Prioritization Rules for Allocation of Tumor Tissue at Baseline

Tumor Size in 2 dimensions	Allocation of Tissue
<5 x <5 mm	Cut into 2 equal sections. 2. Allocate one of the sections to liquid nitrogen 3. Allocate one of the sections to formalin
≥5 x ≥5 mm	Split tumor into 3 equal sections: 1. Allocate one third to RPMI 2. Allocate one third to liquid nitrogen 3. Allocate one third to formalin

Prioritization Rules for Allocation of Tumor Tissue at Weeks 1 and 6

Tumor Size in 2 dimensions	Allocation of Tissue
<5 x <5 mm	1. Allocate entire specimen to liquid nitrogen
≥5 x ≥5 mm	Split tumor into 2 equal sections. 1. Allocate one of the sections to liquid nitrogen 2. Allocate one of the sections to formalin

1) RPMI preservation
   A Sterile forceps will be used to gently place the specimen in a sterile labeled cryovial or specimen container (whichever is large enough to hold the tissue full submerged and surround by cold [2-8°C] RPMI. Additional RPMI will be added (under sterile conditions) with the volume required to fully submerge the tissue in the solution and fill the vial/container completely without air pockets.
   B Care should be taken to avoid crushing artifacts and a separate forceps must be used for each specimen to avoid cross contamination.
   C The cryovial/specimen container lid will be secured.
   D The cryovial/specimen container must be kept at 2-8°C and transported immediately to the Immune Monitoring Core laboratory for processing.

2) Liquid nitrogen storage
   A The sample will be placed in a 30 ml cryovial and immediately placed in liquid nitrogen.
   B The Dewar will be transported within 60 minutes to the cryo-storage facility
   C The cryovial will be transferred to a liquid nitrogen freezer or a mechanical -80°C freezer for long-term storage until processed for Western Blotting.

3) Formalin fixation
   A Specimens intended for formalin fixation should be processed after the completion of other fresh tissue procedures, such as flash freezing and submersion in RNA stabilizing reagent.
   B The tissue sample should be trimmed such that it is maximally 0.5 cm in thickness. Specimens that exceed this dimension may not allow for adequate perfusion of the formalin.
   C After trimming, the specimen will be transferred to a container with formalin using a forceps. To avoid cross-contamination, a separate forceps should be used for each tissue sample. The volume of formalin should be a minimum of 15-20 times the volume of the

tissue sample - e.g., 20 ml of formalin per 1 cm^3 of tissue. A 15 ml sterile centrifuge or conical tube or alternatively, a specimen cup can be used.

D The specimen must be completely submerged in the formalin fixative and the container lid securely tightened to avoid any spillage.

E The tissue will be fixed at room temperature for a minimum of 24 hours and maximum of 48 hours.

F Formalin should not be discarded down the drain. The chemical disposal plan at the institution will be followed for an appropriate method of disposal.

V. Blood sampling for biologic correlative studies

1) Collection of peripheral blood

A Research blood collection for correlative studies is to be performed at Baseline/Week -2, Week 1, Week 7, and Week 13.

a) Baseline/Week 2: Approximately 20 mL of blood will be collected in 1 green top tubes and 1 red top tube.

b) Week 1: Approximately 20 mL of blood will be collected in 1 green top tube and 1 red top tube.

c) Week 7: Approximately 20 mL of blood will be collected in 1 green top tube and 1 red top tube.

d) Week 13: Approximately 20 mL of blood will be collected in 1 green top tube and 1 red top tube.

B The RCINJ Biospecimen Repository Service (BRS) will process, label and store the blood samples.

C Peripheral blood will be collected by venipuncture into venous blood collection tubes (Red Top tubes with no additive; for example, BD vacutainers catalog# 366430 and Green Top tubes with sodium heparin; for example BD vacutainers catalog# 366480).

D The samples will be transported at room temperature (18°C to 25°C) in a double container from the collection site to the sample processing laboratory.

E Red Top tubes for serum processing should be kept sitting upright at room temperature for at least 30 minutes and at most 60 minutes prior to processing to allow clotting. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 h.

F Green Top tubes for peripheral blood mononuclear cell (PBMC) processing should be kept on a rocker at room temperature until processed (to avoid clotting) and processed as soon as possible (within 4 h maximum) after blood collection.

G The collection tubes will be labeled with the patient ID, date, and time of venous blood draw.

2) Processing of peripheral blood

A The RCINJ Biospecimen Repository Service (BRS) will process, label and store the blood samples until delivery to the analysis lab.

B Peripheral blood from Red Top tubes will be used for serum acquisition and peripheral blood from green top tubes will be used for PBMC acquisition..

C Red Top tubes will be processed as follows:

a) Red Top tubes will be centrifuged for 20 minutes at 1400 rpm at room temperature.
b) Using a pipette, serum from both Red Top tubes will be transferred into a 50 ml conical tube and mixed.
c) The serum will then be pipetted into the labeled cryovials at an aliquot volume of 250-500 µl per tube. The caps on the vials will be closed tightly.
d) This process should be completed within 1 hour of centrifugation.
e) Care must be taken to not pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.
f) All aliquots will be stored upright at -80ºC or colder freezer in a specimen box.

D Green Top tubes will be processed as follows:

a) Green Top tubes will be mixed by inverting the tube gently 6 to 8 times.
b) Up to 10 ml of peripheral blood will be added to 10 ml of PBS in a 50 ml Falcon tube and mixed by inverting the tube gently 6 to 8 times.
c) The 20 ml peripheral blood/PBS mixture will be overlayed by slow careful pipetting onto a 20 ml layer of Ficoll in a 50 ml tube.
d) The peripheral blood/PBS/Ficoll tube will be centrifugated for 20 min at 1750 rpm at room temperature without the break.
e) Using a transfer pipette the clear top plasma layer will be removed and discarded as biological waste.
f) Using a new transfer pipette, the cloudy PBMC layer will be transferred to a 50 ml conical tube.
g) Care must be taken not to disrupt the erythrocyte layer during the transfer by using gentle pipetting above the Ficoll layer and keeping the tube stationary.
h) The cells will be counted (using a standard hemocytometer) and the total cell count recorded.
i) PBS will be added to the PBMC tube up to the 50 ml mark and the tube will be centrifugated for 5 min at 1750 rpm at room temperature with the break.
j) The supernatant will be discarded and the pellet dissolved in Cryopreserve solution (1 ml Cryopreserve solution per 1 ml of blood) and transferred to cryovials (1 ml per cryovial). The cell concentration and solution volume will be recorded.
k) All aliquots will be stored upright at -80ºC or colder freezer in a specimen box.

VI. Delivery to Analysis Laboratory and Storage

1) Upon receipt of the samples an entry will be made and sample number issued for the expected sample in the Biospecimen Repository Database.

2) Samples stored in liquid nitrogen and formalin will be delivered to the laboratory as needed for batch processing. BRS will deliver samples to:

Daniel J. Medina, PhD
Immune Monitoring Core
195 Little Albany Street, Room 4531
New Brunswick, NJ 08901
Phone: (732) 235-5641

Once specimens arrive at analysis lab, they will be immediately placed at 4ºC (for formalin-fixed tumor tissue) or -80ºC (or lower for all other tissues and PBMCs) for pending correlative science work or for storage.

VII. Correlative Analyses

A. Immunophenotyping

a) Immune cells in the blood and the tumor will be profiled using cells obtained from RPMI-preserved tumor tissue samples and PBMCs (controls) obtained from peripheral blood using flow cytometry to look for TILs, Tregs, MDSC, TAM and other immune cells.
b) Tumor biopsy tissue will be preserved in RPMI solution and PBMCs frozen at -80ºC or colder and delivered to the Rutgers CINJ Immune Monitoring Core.
c) Tumor samples will be dissociated into a single-cell suspension by the Rutgers CINJ Immune Monitoring Core, as described below.
d) Cell in single suspension of tumor or PBMCs (controls) will be analyzed by the Rutgers CINJ Immune Monitoring Core utilizing 14-parameter flow cytometry with an LSR-II flow cytometer, whereby cells will be stained with fluorescent antibodies to the validated cell markers (purchased primarily from eBioscience, Biolegend, and BD Biosciences).
e) Cell population ratios will include identifying cells as cytotoxic CD8+ T cells (Tcs), CD4+ helper T cells (Ths) regulatory CD4+ T cells (Tregs), MDSCs, B cells, NK cells, and DCs.
f) Markers evaluated will include CD3, CD4, CD8, CD11b, CD11c, CD14, CD16, CD19, CD25, CD33, CD45RA, CD56, CD80, CD86, CCR7, FoxP3, Granzyme B, HLA-DR, NKp46, PD-1, and PD-L1.
g) Specifically:
    (i) The tumor tissue will be mechanically dissociated upon receipt using a GentleMACs OctoDissociator (Miltenyi) using the B_01 program.

(ii) The resulting tumor-derived cells will be passed through a 70 µm screen (BD Biosciences) to ensure a single cell suspension, and washed in PBS, and frozen down, as described previously at $2x10^6$ in cryopreservation solution at -80°C or colder.

h) Frozen cells from tumor tissue will be thawed quickly by immersion of the bottom 90% of the cryovial in a 37°C water bath and washed once in PBS (with centrifugation and reconstitution of the cell pellet in 50 µl PBS per sample in a 96 well plate) prior to flow cytometric antibody staining.

i) For extracellular markers:

j) Plates with cells to be stained will be centrifuged at standard conditions (4°C, 1500 rpm, 5 min) and decanted by inversion.

k) Extracellular marker antibodies will be added at titrated concentrations (for example, 0.5 µl antibody in 50 µl of PBS for antibodies used at 1:100).

l) The plate will be incubated in the dark for 30 minutes at room temperature and then centrifuged at standard conditions and washed once with PBS.

m) Cells not requiring intracellular staining (ICS) will be analyzed immediately by flow cytometry or fixed in 50/50 v/v 10% formalin in PBS, kept in the dark at 4°C, and analyzed within 24 hours. Prior to analysis, 5 µl of Countbright beads will be added to each well (and the bead concentration recorded from the Countbright bottle).

n) For intracellular markers:

o) Cells requiring ICS, will be centrifugated at standard conditions and reconstituted with 100 µl of BD Perm/Fix per well, incubated at 4°C for 15 min, receive 100 µl of 10% PermWash to each well, and centrifuged immediately at standard conditions.

p) The plate will be then decanted and a 100 µl ICS stain mixture (with antibodies) added to each well in a 10% PermWash mixture.

q) The plate will be incubated at 4°C for 30 minutes, centrifuged at standard conditions, decanted, washed 2x with 200 µl of 10% PermWash per well, centrifuged at standard conditions, decanted, and analyzed by flow cytometry or fixed in 10% formalin for up to 24 hours.

r) Prior to analysis, 5 µl of Countbright beads will be added to each well (and the bead concentration recorded from the Countbright bottle).

s) Flow cytometry panels will be based on Optimized Multicolor Immunofluorescence Panel (OMIPs) published in the journal *Cytometry Part A.* http://qap2.onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4930/homepage/information_on_omips.htm

1) PD-L1 Expression

a) Determination of PD-L1 expression in the tumor will be conducted using tumor

tissue samples obtained from biopsy and formalin fixed/paraffin embedded.

b) Tumor biopsy tissue will be formalin fixed at each site.
c) Formalin-fixed tumor tissue samples will be paraffin embedded by the Immune Monitoring Core at Rutgers CINJ.
d) The samples will be batched by the Immune Monitoring Core at Rutgers CINJ.
e) PD-L1 will be determined by immunohistochemistry staining.
f) This assay will be performed by QualTek Molecular Laboratories biomarker and tissue-based assay services using the BMS DAKO antibody (Dako, Carpinteria, CA).
g) Details regarding accuracy, precision, controls, reference standards, etc. of this assay can be obtained from the conducting laboratory.

2) Cytokine levels
   a) Analysis of cytokines within the tumor microenvironment will be performed using tumor tissue obtained from RPMI-preserved samples and frozen serum samples.
   b) Tumor biopsy tissue will be preserved in RPMI solution at each site and serum frozen obtained from peripheral blood and delivered to the Rutgers CINJ Immune Monitoring Core.
   c) Tumor tissue samples will be mechanically homogenized upon receipt using the GentleMACs OctoDissociator (Miltenyi), as per the manufacturer instructions.
   d) Samples will be frozen at -80^{o}C or colder until analysis of cytokines.
   e) Cytokine standards will be prepared as per manufacturer instructions from the manufacturer's master standard stock, for the cytokines of interest.
   f) Biolegend flow cytometry-based LEGENDplex technology for cytokine multiplexing will be utilized. The LEGENDplex Human Anti-Virus Response Panel (Cat. No. 740390) is a bead-based multiplex assay, utilizing fluorescence-encoded beads suitable for use on flow cytometers. This panel allows simultaneous quantification of 13 human proteins, including interferons (α, β, γ, and λ), interleukins (1, 6, 8, 10, 12), TNF-α, IP-10 and GM-CSF.
   g) The panel has been validated by the manufacturer and is a standard assay of the Immune Monitory Core at Rutgers CINJ for determination of cytokine levels using flow cytometry (LSR2; BD).
   h) Details regarding accuracy, precision, and published references for this system are available from the manufacturer.
   i) The plate will be analyzed on an LSR-II Flow Cytometer, as per the manufacturer instructions within 1 hour of last incubation.

B. Immune gene and protein expression profiling

a) Immune gene and protein expression profiling will be conducted from RNA derived from RNA preservation media and protein extracted from cell obtained from RPMI-preserved tumor tissue samples.
b) Tumor biopsy tissue will be delivered to the Rutgers CINJ Immune Monitoring Core.
c) RNA will be extracted from frozen tissue upon receipt using RNeasy mini spin columns (Qiagen) according to the manufacturer's protocol, and protein will be extracted from RPMI-preserved tissue cells (derived as previously described).
d) The concentration and quality of the total RNA preparation will be determined by measuring absorbance at 260 and 280 nm using the Nanodrop system (Thermo Scientific).
e) Each hybridization reaction will require 100-150 ng of total RNA. The integrity of the total RNA preparation will be verified on a Bioanalyzer (model 2100, Agilent Technologies) before proceeding with the hybridization reaction. RNA should be aliquoted (to avoid freeze/thaw cycles) and stored for up to several years at −80◦C.
f) Hybridization of the target mRNA to the gene-specific probe pairs will be carried out in triplicate with each sample containing 5 µl RNA (150 ng), 10 µl reporter probe (final 40 pM), 5 µl capture probe (final 200 pM), and 10 µl hybridization buffer (5× SPPE, pH 7.5, with 0.1% Tween-20).
g) Using a thermocycler, the hybridization reaction will be conducted will be at 65°C for optimally 16 h (at least 12 h but not more than 30 h).
h) After the hybridization reactions are completed, post-hybridization processing will immediately continue using the nCounter Prep Station, as per the manufacturer instructions on using the instrument.
i) After post-hybridization processing, the nCounter Digital Analyzer will be used to acquire images of the immobilized fluorescently labeled target mRNA and protein molecules in the sample cartridge using a CCD camera and a microscope objective lens and the expression level of a gene will be measured and tabulated in a CSV format by the system.
j) Using Excel, the individual data files will be imported into a collector file template provided by the analyzer manufacturer. The abundance of target mRNA and protein will be compared across multiple different samples and thus normalized for all target genes in all samples based on the positive spike-in controls to account for differences in hybridization efficiency and post-hybridization processing, including purification and immobilization of complexes.
k) To determine if the normalized, background-subtracted counts are statistically above background, a Student's t test will be performed against eight human negative controls. A gene will be considered to be above background if the average count for

the target gene is greater than the average counts for the eight negative control genes and if the P value of the t test is less than 0.05.

l) The relative changes in the abundance of target mRNA molecules and protein will be calculated using the normalized, background-subtracted counts for one or more reference genes/proteins included in the manufacturer set.

C. Angiogenesis Metabolic Pathway Analysis

a) Markers of angiogenesis will be analyzed, including IL-8 and VEGF using Western blotting.

b) Signal transduction in key metabolic pathways will be analyzed using Western blotting and will include total and pERK, total and pAKT, WNT pathway components, beta-catenin, M-CSF, and CCL4.

D. Exosome Analysis

a) Exosomes will be isolated from peripheral blood using a standard isolation kit.

b) Pre- and post-treatment peripheral blood exosomes will be analyzed for exosome quantity (using the NanoSight instrument) and contents (using Mass Spectrometry and microRNA array analysis).

VIII. Blood Samples for Pharmacokinetic (PK) Analysis

Blood samples for PK analysis will be processed, stored, and shipped as per the graphic below.

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PROCESSING INSTRUCTIONS – PHARMACOKINETIC (PK)

Testing	Collection Materials	Mix by Inversion	Processing	Transport tube	Storage temp.	Shipping temp.
Plasma PK Each timepoint	1 x 2mL Lavender top EDTA tube	15 - 20 times Keep on ice until processed	Centrifuge immediately (or at least within 120 minutes) after collection at 2000G ± 20G for 10 minutes at 4°C. (Refrigerated Centrifuge) Do not use centrifuge brake. Transfer plasma	Aliquot at least 0.5 ml of plasma into each cryovial Label tubes 2 x Purple cap Cryovials Freeze Immediately	Frozen at ≤-20°C	Frozen as directed

PHARMACOKINETIC (PK)

Approximately 2 mL of blood will be collected in a 2 mL EDTA tube

*Pharmacokinetic at V3 and V4

A. Samples should be collected at timepoints as directed in the Protocol.
B. After collection, gently invert the sample a minimum of 15-20 times. Place tube into an ice bath until processed.
C. Centrifuge specimen for 10 minutes at 2000G at 4ºC (Refrigerated centrifuge)
D. Make certain there are no cells in the plasma. Re-centrifuge if necessary.
E. Transfer a minimum of 0.5 mL plasma to each of 2 Purple capped Cryovials. Label tubes.
F. Make sure transport tubes are sealed tightly.
G. Store FROZEN at ≤ -20ºC until shipped on Dry Ice as directed with the completed request form.

Labeled samples will be shipped on dry ice to inVentiv with the completed request form. Samples may be stored at < -20 degrees C for up to 30 days prior to shipping and shipped in batches. Samples will be analyzed at inVentiv.

inVentiv Health
2500 rue Einstein
Québec, QC, G1P 0A2
Canada

IX. Other Analyses

If samples are available, some or all of the following analyses may also be performed, including but not limited to: additional gene expression analysis, additional flow cytometry, additional IHC, proteomic analysis, TCR repertoire analysis, and tumor mutational load. No germline genetic sequencing will be performed. The experimental protocol will follow laboratory SOPs and published procedures.

X. Safety Precautions

Universal precautions (*i.e.*, a method of infection control in which all human blood and body fluids are treated as if they are infectious for Hepatitis Viruses, Human Immunodeficiency virus, and other known and unknown infectious agents) will be utilized when handling all unfixed cells and tissues.

1) Hepatitis B and Hepatitis C viruses may be transmitted through blood and other body fluids, and are associated with acute hepatitis, chronic liver disease, and hepatocellular carcinoma in humans. The probability of seroconversion after needlestick exposure is estimated at 7%. Untreated virus can persist for up to one week at room temperature. All staff who work with human tissue must provide evidence of Hepatitis B vaccination.
2) Human Immunodeficiency virus (HIV) is a retrovirus that causes severe immunodeficiency. Infection increases the risk of developing malignancies, infection by opportunistic organisms, and death. The probability of seroconversion after needlestick exposure is estimated at 0.5%. Infectivity of untreated virus persists for up to one week at room temperature.
3) Other potentially infectious agents, both known and unknown, pose hazards to those working with human tissue. Included are tuberculosis, HTLV1, Coccidiomycosis, Creutzfeldt-Jacob disease, amongst others.
4) Individual institutional and OSHA guidelines must be followed when handling human cells and tissues, and referred to for additional information on bloodborne pathogens, laboratory safety, chemical safety, and biohazardous waste disposal. Briefly:
5) Personal protective equipment (PPE) must be used at all times while working with human tissue. These include disposable latex or nitrile gloves, face shield, protective splash-resistant laboratory coat (disposable preferred), and covered protective shoes.
6) Gloves should be immediately removed and replaced in the event that they become torn or perforated. Gloves must be removed prior to leaving the work area, and disposed of in an appropriate waste disposal container. Hands must be washed in a "clean" sink after removal of gloves.
7) Face shields, goggles and masks should be worn whenever a potential for exposure to splashes, spray, splatter, droplets, aerosols of blood or tissue fluid, or other potentially infectious materials may be generated, and if there is a potential for eye, nose or mouth contamination. They should be worn at all times while handling tissue for processing.
8) Protective lab coats, preferably disposable types, must be donned while working with tissue. Contaminated clothing must be removed prior to leaving the work area, and appropriately laundered or discarded, as per individual institutional guidelines.

9) All waste must be disposed of prior to leaving the work area. Biohazardous sharps must be properly disposed of in an approved "sharps" container. All other non-sharp waste must be disposed of in an approved orange or red biohazardous waste disposal bag.

10) After completion of work with human tissue, all work surfaces must be disinfected with a product that has been demonstrated to be effective against bacteria, viruses, pseudomonas, tuberculosis and fungi. Product literature should be referred to for appropriate use.

11) Any injuries or exposure to human tissue or potentially infectious biologic agents must be reported promptly as specified in individual institutional safety guidelines.