

SUMMARY OF CHANGES – Protocol

For Protocol Amendment #7

NCI Protocol #: 9922

Local Protocol #: NCI9922

NCI Version Date: 05/08/2018

Protocol Date: 05/08/2018

#	Section	Page(s)	Comments
1	Header	Global	Changed header date from August 27, 2017 to May 8, 2018
2	Protocol cover page	p. 2	Version Date: May 8, 2018
3	2.2.1.4	11	<p>Revised text to cite most recent version of Ibrutinib Investigator's Brochure</p> <p>2.2.1.4 Summary of Clinical Data</p> <p>For more detailed and comprehensive information, please refer to the most recent Investigator's Brochure for ibrutinib (version 11 10.2 February <u>November</u> 2017). As of the April 6 2015 cutoff date, 26 studies were ongoing and 19 studies had completed their primary analysis.</p>
4	2.2.1.4.3	14	<p>Revised text to cite most recent version of Ibrutinib Investigator's Brochure</p> <p>2.2.1.4.3 <i>Summary of Safety</i></p> <p>Please refer to section 7.1.1.1 of this protocol and the most recent version of the Ibrutinib Investigator's Brochure (<u>November</u> February 2017) for the most up to date safety information on ibrutinib.</p>

#	Section	Page(s)	Comments										
5	3.1.21	29	<p>Cite NCI CTCAE criteria v5.0 instead of 4.0.</p> <p>3.1.21 Subjects who are unable to tolerate BRAF inhibitor and/or MEK inhibitor therapy due to grade ≥ 2 toxicity (CTCAE <u>v5.0</u> 4.0) from these agents, irrespective of antitumor response, are eligible on condition that: (a) toxicities persisted despite change from doublet to singlet therapy (i.e. from concurrent BRAF inhibition plus MEK inhibition to BRAF inhibition alone), (b) toxicities are attributed to a class effect, and therefore switch from one drug to another is expected to induce the same type of toxicity (e.g. ocular toxicities or cardiac dysfunction from MEK inhibitor), (c) drug-specific toxicities that do not resolve with switch from one BRAF inhibitor to another (i.e. dabrafenib to vemurafenib, or vice versa), will be eligible for enrollment in 9922. In other words, patients will be allowed to enroll into the NCI9922 study despite lack of progression to MAPK inhibitor treatments, on condition that grade 2 or higher toxicities attributed to MAPK inhibitors resolve to grade 1, or less, at the time of study enrollment.</p>										
6	4.1	32	<p>Please delete the language in these 2 subsections 4.1.1 and 4.1.2 and replace with the following, in accordance with the new Registration and Credentialing Repository language:</p> <p>4.1 Investigator and Research Associate Registration with CTEP</p> <p>Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP’s web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.</p> <table><tr><th>Documentation Required</th><th>IVR</th><th>NPI VR</th><th>AP</th><th>A</th></tr><tr><td>FDA Form 1572</td><td>✓</td><td>✓</td><td></td><td></td></tr></table>	Documentation Required	IVR	NPI VR	AP	A	FDA Form 1572	✓	✓		
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			<table><tr><td>Financial Disclosure Form</td><td>✓</td><td>✓</td><td>✓</td><td></td></tr><tr><td>NCI Biosketch (education, training, employment, license, and certification)</td><td>✓</td><td>✓</td><td>✓</td><td></td></tr><tr><td>HSP/GCP training</td><td>✓</td><td>✓</td><td>✓</td><td></td></tr><tr><td>Agent Shipment Form (if applicable)</td><td>✓</td><td></td><td></td><td></td></tr><tr><td>CV (optional)</td><td>✓</td><td>✓</td><td>✓</td><td></td></tr></table> <p>An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:</p> <ul style="list-style-type: none">• Added to a site roster• Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN• Act as the site-protocol PI on the IRB approval• Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL). <p>For questions, please contact the RCR Help Desk by email at <RCRHelpDesk@nih.gov>.</p>	Financial Disclosure Form	✓	✓	✓		NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		HSP/GCP training	✓	✓	✓		Agent Shipment Form (if applicable)	✓				CV (optional)	✓	✓	✓	
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7	4.1	32	<p>Please add the following sentence to the end of this section.</p> <p>Additional information can be found on the CTEP website at <https://ctep.cancer.gov/investigatorResources/default.htm>.</p>																									
8	4.2	33	<p>Please revise this section as indicated:</p> <p>This study is supported by the NCI Cancer Trials Support Unit (CTSU).</p> <p>Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to</p>																									

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			<p>enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:</p> <ul style="list-style-type: none"> • An active Federal Wide Assurance (FWA) number • An active roster affiliation with the Lead Network or a participating organization • A valid IRB approval • Compliance with all protocol specific requirements <p>In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:</p> <ul style="list-style-type: none"> • Active registration status • The IRB number of the site IRB of record listed on their Form FDA 1572 • An active status on a participating roster at the registering site <p>Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to: an active Federal Wide Assurance (FWA) number, an active roster affiliation with the Lead Network or a participating organization, a valid IRB approval, and compliance with all protocol specific requirements.</p>
9	4.2.2	34	<p>Please remove the first bullet as the CTSU Transmittal Sheet is no longer in use:</p> <p>• CTSU Transmittal Sheet (optional)</p>
10	4.2.3	34	<p>Please update the CTSU Regulatory Office suite number to 3000.</p> <p>CTSU Regulatory Office 1818 Market Street, Suite 3000 1100 Philadelphia, PA 19103</p>
11	4.2.3	34	<p>Please remove the DTL requirement. This is not designated as a protocol requiring DTL implementation.</p>
12	4.3.2	36	<p>Please revise as indicated:</p>

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			<p>OPEN/IWRS users must meet the following requirements:</p> <ul style="list-style-type: none"> • Have a valid CTEP-IAM account (<i>i.e.</i>, CTEP username and password). • To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type. If a DTL is required for this study, the registrar(s) must also be assigned the OPEN Registrar task on the DTL. • Have regulatory approval for the conduct of the study at their site.
13	7.2	49	<p>Cite NCI CTCAE criteria 5.0 instead of 4.0.</p> <ul style="list-style-type: none"> • CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 <u>5.0 will be utilized for AE reporting beginning April 1, 2018.</u> All appropriate treatment areas should have access to a copy of the CTCAE version <u>5.0.</u> 4.0 A copy of the CTCAE version <u>5.0</u> 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
14	12.3	82	<p>Please revise the following paragraph as specified:</p> <p>Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at < https://ctepcore.nci.nih.gov/iam >) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL. (https://capps-ctep.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read Only, or Site Investigator) on either the Corresponding Organization or Participating Organization roster at the enrolling site</p>

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ClinicalTrials.gov Identifier: NCT02581930

TITLE: A Phase 2 Study of Ibrutinib (PCI-32765) in Refractory Distant Metastatic Cutaneous Melanoma: Correlation of Biomarkers with Response and Resistance

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LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-MN026 / Mayo Clinic Cancer Center LAO
LAO-NJ066 / Rutgers University - Cancer Institute of New Jersey LAO
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NCI-Supplied Agent: Ibrutinib (PCI-32765) (NSC 748645)

Study Exempt from IND Requirements per 21 CFR 312.2(b)

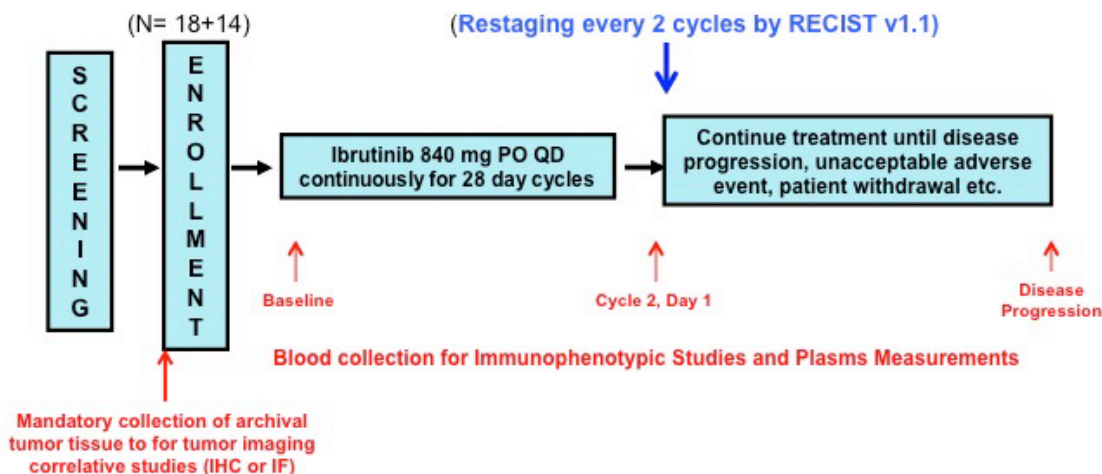
Study Sponsor: DCTD, NCI

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Protocol Type / Version # / Version Date: Version #8 / November 22, 2016
Protocol Type / Version # / Version Date: Version #9 / December 12, 2016
Protocol Type / Version # / Version Date: Version #10 / June 2, 2017
Protocol Type / Version # / Version Date: Version #11 / July 25, 2017
Protocol Type / Version # / Version Date: Version #12 / November 6, 2017
Protocol Type / Version # / Version Date: Version #13 / May 8, 2018

SCHEMA

Design: Open-label, Single-Arm, Phase II Study

Primary objective: Estimate Overall Response Rate to Single Agent Ibrutinib



Key Eligibility Criteria

- Stage IV melanoma; measurable disease per RECIST1.1
- Disease refractory or ineligible to MAPK & Immune checkpoint inhibitors (CTLA4/PD1/PDL-1)
- Life expectancy > 3 months & ECOG PS ≤ 2

This is an open-label, single-arm, phase 2 trial designed to estimate the rate of objective response [OR: complete response (CR) + partial response (PR)] to ibrutinib administered as a single agent in up to 32 patients with immune checkpoint inhibitor-refractory (all patients) or immune checkpoint inhibitor ineligible and mitogen-activated protein kinase (MAPK) inhibitor-refractory (if BRAFV600-mutant) or MAPK inhibitor-intolerant distant metastatic cutaneous melanoma. Patients will receive ibrutinib 840mg orally once daily, which is dose under investigation for refractory distant metastatic cutaneous melanoma, until intolerable toxicity, disease progression, or withdrawal from the trial. Four weeks (28 days) will constitute a treatment cycle. Archived or freshly collected (immediately prior to enrollment to this study) tissue formalin-fixed paraffin embedded (FFPE) tumor blocks/slides will be requested at baseline to investigate tumor tissue-based biomarkers of response. Peripheral blood will be collected at baseline, 4 weeks (day 29, i.e., day 1 of cycle 2), and at disease progression to explore ibrutinib-mediated effects on immune cell subsets associated with immunosuppression. Additional correlative blood samples will be collected at specified time points for protein marker and pharmacogenomics exploratory analysis.

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1. OBJECTIVES

1.1 Primary Objective

- 1.1.1 Estimate rate of objective response [OR: complete response (CR) + partial response (PR)] to ibrutinib administered as single agent in patients with immune checkpoint inhibitor-refractory, or immune checkpoint inhibitor ineligible and MAPK inhibitor-refractory (if BRAFV600-mutant) or MAPK inhibitor-intolerant distant metastatic cutaneous melanoma.

1.2 Secondary Objective

- 1.2.1 Estimate progression-free survival (PFS) rate at 6 months after initiation of ibrutinib in patients with immune checkpoint inhibitor-refractory or immune checkpoint ineligible and MAPK inhibitor-refractory (if BRAFV600-mutant) or MAPK inhibitor-intolerant distant metastatic cutaneous melanoma.
- 1.2.2 Estimate overall survival (OS) after initiation of ibrutinib in patients with immune checkpoint inhibitor-refractory or immune checkpoint ineligible and MAPK inhibitor-refractory (if BRAFV600-mutant) or MAPK inhibitor-intolerant distant metastatic cutaneous melanoma.
- 1.2.3 Explore the association of ITK protein expression with OR and PFS. The ITK protein expression in melanoma cells (pretreatment) is assessed by 2-color immunofluorescence (IF) in representative tissue sections obtained from archival FFPE tumor blocks or in representative tissue sections/slides obtained from freshly collected (immediately prior to enrollment to this study) biopsies from enrolled patients.

1.3 Exploratory Objectives

- 1.3.1 Explore association between other putative targets of ibrutinib (e.g. Tec, ErbB4, Hck, Yes, BTK) in melanoma cells, as assessed by 2-color IF in representative tissue sections obtained from pretreatment archived FFPE tumor blocks or FFPE blocks obtained from fresh tissue biopsy from enrolled patients, with OR and PFS.
- 1.3.2 Explore ibrutinib-mediated effect(s) on immune cell subsets associated with immunomodulation by performing multiparameter flow cytometric analysis in PBMC obtained prior to treatment, on day 29 (i.e., predose day 1 of cycle 2) following initiation of treatment with ibrutinib, and at the time of disease progression (3 time points).
- 1.3.3 Determine pharmacokinetics (PK) of ibrutinib following daily dosing at 840 mg on Day 8 of cycle 1 (Css).

2. BACKGROUND

2.1 Metastatic Cutaneous Melanoma

Metastatic cutaneous melanoma remains an active cancer type for further drug development despite the recent approval of 4 small molecule inhibitors targeting the MAPK pathway (Moschos *et al.*, 2015) and 3 immunotherapies targeted against either the co-inhibitory molecules PD-1 (programmed cell death protein 1) or CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4) (Eggermont *et al.*, 2015). Such treatments have prolonged OS either by stalling melanoma growth or by reversing inhibition of an occasionally present but usually dysfunctional (anergic) immune system. Durable responses lasting at least more than 3 years are possible following both treatment types (Sznol *et al.*, 2015; Flaherty *et al.*, 2016), and have possibly increased the incidence of cure in a small subgroup of patients, but in most cases disease ultimately becomes resistant (Atkins *et al.*, 1999; Lebbe *et al.*, 2014; Daud *et al.*, 2015). Several melanoma subgroups (cutaneous and non-cutaneous) have primary resistance despite these new effective treatments.

The most recent evidence supporting the need for novel therapies comes from two randomized clinical trials in which inhibitory antibodies against PD-1 (nivolumab or pembrolizumab) were administered as a second- (i.e. following the anti CTLA-4 antibody ipilimumab) or third-line therapy (i.e. following ipilimumab and MAPK inhibitor-based therapy, if BRAFV600-mutant) versus investigator's choice chemotherapy (ICC) in metastatic melanoma (Ribas *et al.*, 2015; Weber *et al.*, 2015). As shown in Table 1, OR by RECIST1.1 criteria ranged from ~20-30% for anti-PD1 therapy, as compared to 4% for ICC. The percent of patients alive and free of disease at 6 months for pembrolizumab [data not available for nivolumab at the time of publication (Weber *et al.*, 2015)] versus ICC was 34% and 16%, respectively, highlighting the need for additional approaches to treat metastatic cutaneous melanoma.

Table 1. PD-1 Antibody versus ICC as Second or Third-Line Treatment of Metastatic Melanoma

	Nivolumab, 3mg/kg Q2wks	ICC	Pembrolizumab 2mg/kg Q3wks	ICC
N	272	133	180	179
BRAFⁱ (%)	57	31	47	43
Rate of OR (%)	32	11	21	4
PFS rate at 6 months (%)	Not reported (NR)	NR	34	16

We have recently shown that the non-receptor IL-2 inducible tyrosine kinase (ITK) is highly expressed in metastatic cutaneous melanoma and that its pharmacologic inhibition using the highly specific ITK inhibitor, BI10N, delays tumor growth *in vitro* as well as in various melanoma mouse models (Carson *et al.*, 2015). As shown below, ibrutinib, the recently FDA-approved inhibitor of Bruton's tyrosine kinase (BTK) that also inhibits other non-receptor tyrosine kinases including ITK, delayed cell proliferation in various melanoma cell lines. We would therefore like to conduct a phase II clinical trial of ibrutinib in distant metastatic

cutaneous melanoma.

2.2 CTEP-supplied Agent

2.2.1 Ibrutinib (PCI-32765)

Ibrutinib is a first-in-class, potent, orally administered, covalently binding inhibitor of Bruton's tyrosine kinase (BTK) co-developed by Pharmacyclics LLC and Janssen Research & Development LLC for the treatment of B-cell malignancies.

Ibrutinib has been approved in many regions, including the US and EU, for indications covering the treatment of patients with mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) who have received at least one prior therapy, first-line treatment of patients with CLL with a deletion of the short arm of chromosome 17 (del17p) or a *TP53* mutation, and patients with Waldenström's macroglobulinemia. Ibrutinib is currently under investigation in various indications as a single agent and in combinations.

2.2.1.1 Mechanism of Action

In vitro studies showed that ibrutinib binds covalently to a cysteine-481 residue (Cys-481) near the BTK active site and inhibits the enzymatic activity with subnanomolar half-maximal inhibitory concentration (IC₅₀ 0.5nM)(Pan *et al.*, 2007; Honigberg *et al.*, 2010). Covalent binding to Cys481 results in irreversible inhibition of BTK. Other kinases that are inhibited by ibrutinib have been identified using an Ambit kinome screen, which is the basis for the current clinical proposal. The inhibitory effect of ibrutinib against the activity of these kinases has been characterized in biochemical assays. Only a small subset of kinases from the Tec and Src/abl family of kinase is predicted to contain a modifiable cysteine residue homologous to Cys481 in BTK, and therefore can be irreversibly modified by ibrutinib. The other Cys-containing kinases include EGFR, HER2, HER4, Itk, Bmx, JAK3, Txk, Tec, and Blk. Ibrutinib also has reversible inhibitory activity against other kinases that do not contain Cys481(Honigberg *et al.*, 2010). However, assuming a rapid off-rate, any reversible kinase inhibition *in vivo* is likely to be short-lived, since in humans the effective half-life of ibrutinib following oral dosing is only 1.5 to 3.3 hours (as measured from time of maximum drug concentration [T_{max}] to 6 hours after dose).

A fluorescently-tagged derivative of ibrutinib was shown to bind to BTK in B-cell lysates, suggesting a high degree of specificity within B-cells (Honigberg *et al.*, 2010). Thus, it was expected that ibrutinib would significantly occupy BTK within malignant cells of patients who had maximum plasma concentrations (C_{max}) of ≥100nM.

Therefore, by combining fast irreversible binding to BTK with rapid *in vivo* elimination, ibrutinib provides a unique approach to improve selectivity of BTK *in vivo* relative to reversibly inhibited off-target kinases.

2.2.1.2 Cellular Selectivity of Ibrutinib

BTK expression is limited to cells of hematopoietic origin. The cellular selectivity of ibrutinib was demonstrated by the observation that ibrutinib inhibits antigen-receptor signaling in B-cells, but not in T-cells. In *ex-vivo* stimulation assays, ibrutinib inhibits human BCR activation ($IC_{50} < 10\text{nM}$) in B cells, but does not affect T-cell receptor activation (Honigberg *et al.*, 2010). It has also been determined that ibrutinib inhibits key phosphorylation events downstream of the BCR at similar concentrations. Ibrutinib has been studied in other blood cell types where the function of BTK is understood, including mast cells, basophils, monocytes, macrophages, and platelets. Consistent with the functional role of BTK in mast cells and basophils, it was determined that ibrutinib fully inhibits degranulation following stimulation at the high-affinity Immunoglobulin E (IgE) receptor (Chang *et al.*, 2011; MacGlashan *et al.*, 2011). In monocytes and macrophages, ibrutinib inhibits the secretion of pro-inflammatory cytokines following stimulation at the Fc-gamma receptors (FcγR) by immune complex (Chang *et al.*, 2011). Consistent with the proposed function of BTK in platelets, ibrutinib inhibits shear-force and collagen-induced platelet aggregation *in vitro* (IC_{50} 10-100 nM) (Kamel *et al.*, 2015). The latter may have implications in the treatment of patients who are prone to hemorrhage (i.e. post-surgery, on anticoagulation).

2.2.1.3 Summary of Nonclinical Data

For more detailed and comprehensive information, please refer to the most recent Investigator's Brochure (IB) for ibrutinib.

2.2.1.3.1 Nonclinical Pharmacology Studies

As mentioned above, ibrutinib was designed as a selective and irreversible inhibitor of the BTK protein by inhibiting its autophosphorylation (Pan *et al.*, 2007). The irreversible binding of ibrutinib to Cys481 in the active site of BTK enhanced selectivity over other kinases that do not contain a cysteine at this position. Ibrutinib potently and specifically blocked BCR-mediated lymphocyte activation in primary CD20+ human B cells ($IC_{50} < 10\text{nM}$) whereas the T-cell receptor signaling remained intact at concentrations up to 1,000 nM. Furthermore, ibrutinib inhibited proliferation of cell lines derived from patients with diffuse large B-cell lymphoma (DLBCL), CLL at various concentrations ranging from low nM to sub μM *in vitro* (Zheng *et al.*, 2014) and inhibited tumor growth of OCI-Ly10 xenograft models with a once-daily oral gavage dose of 12mg/kg/day.

Preclinical studies have also revealed a vital and multifaceted role for BTK and the BCR signaling pathway in B-cell leukemogenesis and lymphomagenesis. These data suggest that blockade of BCR signaling pathway by ibrutinib in CLL results in three major effects: 1) direct induction of apoptosis, 2) inhibition of cell homing and migration to chemokines and with subsequent adhesion to cellular substrates, and 3) inhibition of proliferation (Herman *et al.*, 2011; Ponader *et al.*, 2012). Owing to the described mechanism of action of BTK inhibition, administration of ibrutinib to

patients with lymphoproliferative diseases has been associated with mobilization of tumor cells from lymph tissue to the peripheral blood compartment (Advani *et al.*, 2013).

As mentioned above, it is important to emphasize that ibrutinib potently and irreversibly inhibits various other kinases that contain the conserved Cys481 site, in particular those of the Tec and Src/abl family. In particular, the median IC₅₀ values are subnanomolar for ErbB4/HER4 and Blk, in addition to Btk; between 1-5nM for Bmx/Etk, Fgr, Txk, Lck, and Yes/YES1; between 5-10nM for Tec, Csk, and EGFR; between 10-15nM for Brk and Itk; between 15-22 nM for Hck, ErbB2/HER2 and JAK3 (Investigator's Brochure 2015 and (Honigberg *et al.*, 2010)). These "off-target" effects of ibrutinib may account for several immunomodulatory (Stiff *et al.*, 2016) and direct antitumor effects that have been observed with ibrutinib in CLL (Dubovsky *et al.*, 2013), T-cell driven, and alloantibody-driven graft-versus-host-disease (cGVHD) models (Dubovsky *et al.*, 2014), non-small cell lung cancer cell lines (Gao *et al.*, 2014), pancreatic cancer models (Masso-Valles *et al.*, 2015; Gunderson *et al.*, 2016), lymphoma (Sagiv-Barfi *et al.*, 2015) and breast cancer (Wang *et al.*, 2016). The results from these studies suggest that ibrutinib, by being a highly potent and irreversible inhibitor of various kinases, can have antitumor effects beyond the originally observed clinical benefit in BTK-driven malignancies.

2.2.1.3.2 Toxicology Studies

Ibrutinib toxicity following a single po dose as well as repeated po dose of up to 6 months were conducted in rats, and up to 9 months in beagle dogs. The maximum nonlethal total dose of ibrutinib following administration by oral gavage was 2,000mg/kg for mice (human equivalent dose [HED] =160mg/kg), 400 mg/kg for female rats (HED =63mg/kg), and 1,000mg/kg for male rats (HED =160mg/kg), whereas the no-observed-adverse-effect level (NOAEL) was 30mg/kg/day (HED =16.2mg/kg/day). Lymphoid depletion in central and peripheral organs, inflammation of intestines, body weight gain and food intake, as well as squamous epithelial atrophy in some tissues (stomach, pancreas) was observed at high dose levels in the 13-week study. These histopathologic changes were fully reversible within 6 weeks after treatment cessation.

In dogs the no-observed-adverse-effect level was 30mg/kg/day (HED =16.2mg/kg/day) whereas the NOAEL for ibrutinib once daily by oral gavage for up to 9 months was 80mg/kg/day (HED=44.4mg/kg/day). Similar reversible histopathologic changes to rats and mice were also seen in dogs (intestinal inflammation, lymphoid depletion) at the top dose (80mg/kg/day) administered over 9 months or at an even higher dose (220mg/kg/day) administered over up to 13 weeks (Investigator's Brochure, 2015). A cardiac study in telemetry-monitored dogs (single doses of 24 and 150mg/kg) has also been performed (Investigator's Brochure, 2015). This study identified increased PR interval, lower heart rate and QTc with the NOAEL identified as 24mg/kg/day.

2.2.1.3.3 Pharmacokinetics and Metabolism

Orally administered ibrutinib was rapidly absorbed with $\leq 25\%$ oral bioavailability in rats and dogs. Pharmacokinetic studies using orally administered ^{14}C -labeled ibrutinib showed wide distribution in most tissues at 1 hour after dosing, with plasma exhibiting the highest levels followed by the liver whereas central nervous system organs (brain, spinal cord and eye) exhibited the lowest concentrations. Ibrutinib is metabolized in various laboratory animals and humans by CYP3A and primarily via 3 metabolic pathways. (1) hydroxylation of the phenyl moiety, (2) opening of the piperidine with further reduction to a primary alcohol or oxidation to a carboxylic acid, and (3) epoxidation of the acrolein moiety followed by hydrolysis to form dihydrodiol ibrutinib of PCI-45227. This metabolite is a reversible inhibitor of BTK with an IC_{50} of 6.2nM (2.95ng/mL). Because of lower intrinsic potency relative to the parent ibrutinib and a reversible mechanism of action, PCI-45227 most likely does not contribute significantly to the overall pharmacologic activity of orally administered ibrutinib. Ibrutinib is not a substrate for P-glycoprotein (P-gp) or other major transporters substrate, but is a mild inhibitor of P-gp and the breast cancer resistance protein, BCRP.

Ibrutinib is an inhibitor of several CYP450 isoenzymes, with IC_{50} values ranging from 4.23 to 11.0 $\mu\text{g/mL}$ (Investigator's Brochure, 2015). Because of the wide separation between these inhibitory concentrations and the expected maximum plasma levels in patients, inhibition of CYP450 metabolism in humans is not expected. The metabolite PCI-45227 is a weak inhibitor towards CYP450 isoenzymes, with IC_{50} values ranging from 8.1 to 36.1 $\mu\text{g/mL}$. Inhibition of CYP450 metabolism by PCI-45227 is not expected to occur in humans. The metabolite PCI-45227 is a P-gp substrate, but is not an inhibitor of P-gp.

In rats, dogs, and humans, excretion of the ibrutinib-related radioactivity occurred principally via feces. Biliary excretion was confirmed as a major route of ^{14}C -ibrutinib-derived radioactivity in rats. Less than 2-3.3% and between 5.5-9.3% of the administered radioactivity was recovered in urine from rats/dogs and humans, respectively.

2.2.1.4 Summary of Clinical Data

For more detailed and comprehensive information, please refer to the most recent Investigator's Brochure for ibrutinib (version 11 November 2017). As of the April 6 2015 cutoff date, 26 studies were ongoing and 19 studies had completed their primary analysis. More specifically, 143 subjects have been treated in 7 studies (all complete) involving healthy subjects. A total of 1,071 patients have been treated with ibrutinib monotherapy and 423 patients were treated with ibrutinib in combination with chemotherapy or immunotherapy. Of these, 345 patients with B-cell lymphoma in a total of 6 studies (3 ongoing) (Advani *et al.*, 2013). There are 11 ongoing/completed studies in patients with CLL/SLL (6 ongoing, 5 complete)(Byrd *et al.*, 2013; Burger *et al.*, 2014; Byrd *et al.*, 2014; O'Brien *et al.*, 2014; Brown *et al.*, 2015; Byrd *et al.*, 2015; Farooqui *et al.*, 2015;

Jagłowski *et al.*, 2015), 5 studies in patients with MCL (2 complete, 3 ongoing)(Wang *et al.*, 2013; Wang *et al.*, 2015), 4 ongoing/completed studies in patients with DLBCL (one complete, 3 ongoing)(Maddocks *et al.*, 2015; Wilson *et al.*, 2015), 3 ongoing studies in follicular/marginal zone lymphoma (FL/MZL)(Younes *et al.*, 2014), 2 studies in Waldenström's macroglobulinemia (one complete, one ongoing)(Trean *et al.*, 2015), 2 ongoing studies in multiple myeloma (MM), and a single ongoing study in each of the following diseases: MZL, chronic graft-versus-host disease and acute myelogenous leukemia (Appendix A, Investigator's Brochure, 2015).

Of the studies described above, 36 normal subjects (2 studies; CLL1007, CLL1008), approximately 60 patients with CLL/SLL (1102), approximately 35 patients with MM (1111, 1119), have received ibrutinib at a daily dose of 840mg, either alone or in combination with chemotherapy and or immunotherapy. Finally, the following randomized phase III studies are being conducted in: (a) CLL/SLL (total of six studies; for example, ibrutinib alone versus single-agent chlorambucil, single-agent ofatumumab, or single-agent rituximab OR ibrutinib in combination with bendamustine versus bendamustine alone OR ibrutinib in combination with obinutuzumab versus chlorambucil plus obinutuzumab OR ibrutinib based induction therapy versus FCR), (b) MCL [total of two studies; ibrutinib alone versus temsirolimus AND ibrutinib plus bendamustine plus rituximab (BR) versus BR alone], (c) DLBCL (a single study of ibrutinib plus R-CHOP versus R-CHOP alone), (d) FL/MZL (a single study of ibrutinib in combination with BR or R-CHOP versus BR alone or R-CHOP alone), (d) Waldenström's macroglobulinemia (a single study of ibrutinib in combination with rituximab versus ibrutinib alone).

2.2.1.4.1 Summary of Pharmacokinetics

Extensive pharmacokinetic (PK) analysis has been performed in approximately 250 healthy individuals as well as patients with various B-cell malignancies across five studies. Following oral administration of doses ranging from 1.25mg/kg to 12.5mg/kg as well as a fixed-dose level of 560mg daily, area-under-the-curve (AUC) values for ibrutinib increased in an approximate dose-proportional manner with substantial inter-subject variability (Investigator's Brochure, 2012). The coefficient of variation for the AUC ranged from 60% to 107% across all studies. Administration of ibrutinib in a fasted condition resulted in approximately 60% of exposure as compared to administration either in fed condition (30 minutes after a high-fat breakfast) or when drug was taken 30 minutes before or 2 hours after a meal. The plasma protein binding of ibrutinib and PCI-45227 in human plasma is 97.3% and 91%, respectively and the apparent steady-state volume of distribution was approximately 10,000L, suggesting extensive distribution to peripheral tissues and/or binding to macromolecules in the circulation. The mean terminal half-life ($t_{1/2}$) of ibrutinib ranged from 4.3 to 8.9 hours, with median T_{max} of 2 hours. There was no apparent accumulation of ibrutinib exposure after repeated daily oral dosing. Ibrutinib was extensively metabolized and predominantly cleared by 3 primary cytochrome P450 (CYP)3A-mediated metabolic pathways, one of them being epoxidation of the acryloyl moiety, followed by hydrolysis to form dihydrodiol metabolite PCI-45227. The metabolite-to-parent AUC ratio ranged from 1.4 to 3.2. Consistent with the profile observed for ibrutinib,

there was no apparent accumulation of PCI-45227 in the plasma after repeated daily oral dosing of ibrutinib. Metabolite PCI-45227 is a reversible inhibitor of BTK with approximately 1/10th the inhibitory potency of ibrutinib. Excretion is predominantly via the feces with 80% recovered mostly within 2 days, whereas 8% was excreted in urine.

Effects of ibrutinib on the PR and QT interval were studied in 125 subjects with CLL/SLL who underwent central electrocardiogram monitoring. There was a clinically insignificant, dose-independent QTcF interval shortening by an average of 7.5ms and PR interval increase by an average of 9.7ms relative to baseline in CLL/SLL subjects who were dosed with 420mg/d or 840mg/day.

With the exception of the hepatic impairment study, no special population was formally studied. The recommended doses for patients with mild, moderate and severe impairment are 280mg/day and 140mg/day (Child-Pugh Class A and B, respectively). Nevertheless, clinical development of ibrutinib included a large number of patients >65 years old. In the pharmacokinetic studies performed variables, such as sex, race, and renal impairment were not significant.

In accordance with the role of CYP3A pathways in ibrutinib metabolism, co-administration of ketoconazole, a strong CYP3A inhibitor in 18 healthy subjects increased exposure (C_{max} and AUC_{last}) of ibrutinib by 29- and 24-fold, respectively. Grapefruit juice, which inhibits intestinal CYP3A and therefore influences the first-pass effect, induced a 2- and 4-fold increase in ibrutinib's AUC_{last} and C_{max} , respectively. In contrast, 600mg daily of oral rifampin, a strong CYP3A inducer, in combination with 560mg of ibrutinib in 18 healthy fasted males and females led to reduction of AUC_{last} and C_{max} of ibrutinib by 90% and 92%, respectively.

2.2.1.4.2 Summary of Pharmacodynamics

Pharmacodynamics of ibrutinib were determined in 2 studies in subjects with B-cell malignancies by monitoring the BTK active-site occupancy in subjects' peripheral blood mononuclear cells (PBMC) before and after ibrutinib treatment. These studies showed that subjects administered ibrutinib at doses greater or equal to 2.5mg/kg/day achieved more than 90% occupancy at 4 and 24 hours after drug administration. Therefore, to ensure achievement of the full pharmacodynamics effect in the vast majority of patients, fixed dose levels >280mg were likely necessary. In support of the latter, results from a phase 2 study in patients with CLL who received ibrutinib administered at 420mg/day and 840mg/day confirmed that the median BTK occupancy was >90% on all treatment days examined, including the 24-hour post-dose on day 1 and pre-dose on day 8 of the first week of treatment.

2.2.1.4.3 Summary of Safety

Please refer to section 7.1.1.1 of this protocol and the most recent version of the Ibrutinib Investigator's Brochure (November 2017) for the most up to date safety information on ibrutinib.

Overall Adverse Events in Patients Taking Ibrutinib Monotherapy

Pooled safety data for a total of 1071 subjects treated with ibrutinib monotherapy from 9 studies in B-cell malignancies, which includes subjects from two randomized-control studies who crossed over from comparator treatment or placebo to receive ibrutinib monotherapy, are summarized below.

Most frequently reported treatment-emergent adverse events (TEAEs) in subjects receiving ibrutinib as monotherapy (N=1071):

Most frequently reported TEAEs >10%	Most frequently reported Grade 3 or 4 TEAEs >2%	Most frequently reported Serious TEAEs >1%
Diarrhea	Neutropenia	Pneumonia
Fatigue	Pneumonia	Atrial fibrillation
Nausea	Thrombocytopenia	Febrile neutropenia
Cough	Anemia	Pyrexia
Anemia	Hypertension	
Pyrexia	Atrial fibrillation	
Neutropenia		

Overall Adverse Events in Patients Taking Ibrutinib Combination Therapy

Pooled safety data for a total of 423 subjects treated with various therapies in combination with ibrutinib from 4 studies conducted in B-cell malignancies, which included one randomized-control study, are summarized below. Therapies used in combination with ibrutinib in these studies, included BR (bendamustine and rituximab), FCR (fludarabine, cyclophosphamide, and rituximab), ofatumumab, and R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).

Most frequently reported TEAEs in subjects receiving ibrutinib in combination therapy (N=423):

Most frequently reported TEAEs >10%	Most frequently reported Grade 3 or 4 TEAEs >2%	Most frequently reported Serious TEAEs >1%
Neutropenia	Neutropenia	Febrile neutropenia
Diarrhea	Thrombocytopenia	Pneumonia
Nausea	Febrile neutropenia	Atrial fibrillation
Thrombocytopenia	Pneumonia	Pyrexia
Fatigue	Hypertension	

Bleeding-related Events

There have been reports of hemorrhagic events in subjects treated with ibrutinib, both with and without thrombocytopenia. These include minor hemorrhagic events, such as contusion, epistaxis, and petechiae; and major hemorrhagic events, some fatal, including gastrointestinal bleeding, intracranial hemorrhage, and

hematuria. Use of ibrutinib in subjects requiring other anticoagulants or medications that inhibit platelet function may increase the risk of bleeding. Subjects with congenital bleeding diathesis have not been studied.

Infections

Fatal and non-fatal infections have occurred with ibrutinib therapy. At least 25% of subjects with MCL and 35% of subjects with CLL had Grade 3 or greater infections per NCI Common Terminology Criteria for Adverse Events (CTCAE). The most commonly reported infections include pneumonia, cellulitis, urinary tract infection and sepsis. Although causality has not been established, cases of progressive multifocal leukoencephalopathy (PML) have occurred in patients treated with ibrutinib.

Cytopenias

Treatment-emergent Grade 3 or 4 cytopenias (neutropenia, thrombocytopenia, and anemia) were reported in subjects treated with ibrutinib.

Atrial Fibrillation

Atrial fibrillation and atrial flutter have been reported in subjects treated with ibrutinib, particularly in subjects with cardiac risk factors, acute infections, and a previous history of atrial fibrillation. For atrial fibrillation that persists, consider the risks and benefits of ibrutinib treatment.

Second Primary Malignancies

Second primary malignancies, most frequently skin cancers, have occurred in subjects treated with ibrutinib. Second primary malignancies including non-skin carcinomas have occurred in patients treated with ibrutinib. The most frequent second primary malignancy was non-melanoma skin cancer.

Tumor Lysis Syndrome

There have been reports of tumor lysis syndrome (TLS) events in subjects treated with single-agent ibrutinib or in combination with chemotherapy. Subjects at risk of TLS are those with comorbidities and/or risk factors, such as high tumor burden prior to treatment, increased uric acid, elevated lactate dehydrogenase (LDH), bulky disease at baseline, and pre-existing kidney abnormalities.

Diarrhea

Diarrhea is the most frequently reported non-hematologic AE with ibrutinib monotherapy and combination therapy. Other frequently reported gastrointestinal events include nausea, vomiting, and constipation. These events are rarely severe.

Rash

Rash has been commonly reported in subjects treated with either single agent ibrutinib or in combination with chemotherapy. In a randomized Phase 3 study (PCYC-1112-CA), rash occurred at a higher rate in the ibrutinib arm than in the control arm. Most rashes were mild to moderate in severity.

Treatment-Related Lymphocytosis

Similar to other agents targeting B-cell receptor signaling, transient lymphocytosis is a pharmacodynamic effect of ibrutinib, in which the inhibition of BTK-mediated cellular homing and adhesion results in a mobilization of tumor cells to the peripheral blood (Chang *et al.*, 2013).

Upon initiation of treatment, a transient phase of increase in lymphocyte counts (i.e., $\geq 50\%$ increase from baseline and above absolute count 5000/mcL), often associated with reduction of lymphadenopathy, has been observed in most patients (75%) with relapsed/refractory CLL/SLL treated with ibrutinib. This effect has also been observed in some patients (33%) with relapsed/refractory MCL treated with ibrutinib. This observed transient lymphocytosis is usually not associated with an AE and should not be considered progressive disease in the absence of other clinical findings. In both disease types, lymphocytosis typically occurs during the first few weeks of ibrutinib therapy (median time 1.1 weeks) and resolves within a median of 7.1 weeks in the MCL and 18.7 weeks in the CLL patients.

A substantial increase in the number of circulating lymphocytes ($>400,000/\text{mcL}$) has been observed in a subset of patients. There have been isolated cases of leukostasis reported in patients treated with ibrutinib.

A high number of circulating malignant cells ($>400,000/\text{mcL}$) may confer increased risk; these patients should be closely monitored. Administer supportive care, such as hydration and/or leukopheresis, is indicated. Ibrutinib may be temporarily held, and medical monitor should be contacted.

Hemorrhagic Adverse Events

There are reports of hemorrhagic events in patients treated with ibrutinib in both monotherapy and combination clinical studies. The majority of these hemorrhagic adverse events were of grade 1 or 2 in severity, including minor hemorrhagic events, such as contusion, epistaxis and petechiae; and major hemorrhagic events including gastrointestinal bleeding, intracranial hemorrhage and hematuria. Hemorrhagic events of grade 3 or higher, including central nervous system hemorrhage of any grade severity, occurred in 3.4% (17/506) of patients treated in monotherapy studies and in 3.1% (4/130) of patients treated in combination therapy studies. It is not clear whether or not these events are attributable to ibrutinib. However, it is possible that treatment with the study drug could increase the risk of bruising or bleeding.

Patients were excluded from participation in ibrutinib phase 2 and 3 studies if they required warfarin or other vitamin K antagonists. Do not administer ibrutinib concomitantly with warfarin or other vitamin K antagonists. Ibrutinib should be used with caution in patients requiring other anticoagulants or medications that inhibit platelet function. Patients receiving antiplatelet agents in

conjunction with ibrutinib should be observed closely for any signs of bleeding or bruising and ibrutinib should be withheld in the event of any bleeding events. Supplements, such as fish oil and vitamin E preparations, should be avoided. Patients with congenital bleeding diathesis have not been studied.

Patients in the current study will be monitored closely for hemorrhagic AEs. Ibrutinib should be held at least 3 to 7 days pre and post-surgery depending upon the type of surgery and the risk of bleeding. [NOTE: Please refer to prohibitions and restrictions for further details.]

2.2.1.4.4 Summary of Efficacy

Efficacy data are available in 1,494 subjects who received ibrutinib either as monotherapy or combination therapy across 13 studies (10 studies in patients with pure B-cell malignancies; 3 studies in patients with mixed B-cell malignancies; see Table 24 Investigator's Brochure 2015). In summary, in the first-in-human phase 1, dose-escalating study (PCYC-04753), overall response rate in the efficacy evaluable population (n=54) was 57% (18% complete response; 39% partial response). Responses were observed across all B-cell histologies enrolled, with the highest response rates in CLL/SLL and MCL (85% each) and lower response rates in DLBCL and non-Hodgkin's lymphoma (33% each). PCI-32765DBL1102 was a phase 1b, open-label, non-randomized, multicenter, dose-escalation and expansion study to establish the recommended phase 2 dose of ibrutinib (280mg, 420mg, 560mg) in combination with standard R-CHOP in patients with CD20-positive treatment-naïve B-cell NHL. The ibrutinib plus R-CHOP combination demonstrated clinical activity across all dose cohorts in the all-treated population with ORR 85.7%, 100%, and 95%, respectively.

Study 1112, was a randomized phase 3 of ibrutinib versus ofatumumab in patients with CLL/SLL. Ibrutinib was superior to ofatumumab in both ORR (42.6% versus 4.1%) and risk of death (HR=0.215, $p<0.0001$). Long-term follow up in subjects with CLL/SLL who received ibrutinib as front-line versus following previous treatment, as part of the 1102 and 1103 trials, showed that higher clinical benefit of upfront ibrutinib therapy in both OR (90.3% versus 79.2%) and 30-month PFS rate (96.3% and 68.4%).

A number of studies have combined ibrutinib with standard treatments in patients with CLL/SLL. In the study CLL3001, the addition of ibrutinib to BR, as compared with placebo plus BR, significantly improved OR (82.7% vs. 67.8%, $p<0.0001$) and prolonged PFS (HR=0.203, 95% CI: 0.150, 0.276). In study 1109 the addition of ibrutinib in combination with ofatumumab across three dosing sequences resulted in high OR (81.7%, per investigator assessment) and PFS (12-month PFS rate 83.1%).

A number of phase II studies of ibrutinib administered as single agent have shown promising clinical benefit across non-CLL/SLL B-cell malignancies (1104, MCL2001, 1106, 1118E).

2.3 Rationale

Our interest to investigate the antitumor effect of ibrutinib in distant metastatic cutaneous melanoma was developed following studies to characterize the role of ITK, one of ibrutinib's high affinity targets (Honigberg *et al.*, 2010; Dubovsky *et al.*, 2013), in cutaneous melanoma. This section describes our preclinical investigations, which suggest that ITK is a potential therapeutic target in melanoma (Carson *et al.*, 2015). Since ITK is a high affinity target for ibrutinib, we proceeded to characterize the direct antitumor role of ibrutinib in various melanoma cell lines with variable degree of expression of ITK by fluorescent immunocytochemistry (IHC). Our results show that ibrutinib has a direct antitumor effect by at least delaying melanoma cell growth and that this effect is primarily mediated via ITK. Nevertheless, given that ibrutinib has several high affinity targets with a potential role in melanoma (see Table 2), we do not exclude the possibility that ibrutinib's antitumor effect may, in addition, be dependent on the expression and functions of other kinases by melanoma cells.

2.3.1 Role of ITK in Melanoma; *In-vitro*

The promoter for the ITK gene, a member of the TEC family of non-receptor tyrosine kinases that also includes BTK, among others, was found to be significantly hypomethylated in primary cutaneous melanomas compared with nevi (Conway *et al.*, 2011). To investigate this paradoxically aberrant expression of ITK in cutaneous melanomas, a tyrosine kinase that was previously thought to be exclusively expressed in immune cell subset, we evaluated the expression pattern of ITK in cutaneous melanomas, its *in vitro* role in various melanoma cell lines, and the effects of a highly selective small molecule ITK inhibitor, BI 10N, in various mouse models (Riether *et al.*, 2009). In summary, analysis of the proteomic effects of BI 10N in melanoma cells suggest moderate-to-minimal effects on the most frequently involved signaling pathways in melanoma development and tumor progression, namely Ras-Raf-MEK-ERK and PI3K-Akt-mTOR. In contrast, significant suppression of EGFR- and FAK-mediated signaling and increases in AMPK α and p53/MDM2 protein levels were seen. In addition, Ingenuity® pathway analysis, a web-based software application for the analysis, integration, and interpretation of data derived from 'omics experiments (Ganter *et al.*, 2008), has shown effects on TP53 signaling and moderate effects on cytoskeleton and extracellular matrix components that cannot fully account for the effect of ITK inhibition on cell motility.

Details on UNC *In Vitro* Experiments

In our hands, BI 10N exhibited kinase selectivity against ITK (IC₅₀ at 1mM [ATP] 1.43nM), neurotrophic tyrosine kinase receptors type 1, 2 and 3 (NRTK1, 2, and 3; IC₅₀ 0.82nM, 1.28nM, and 1.58nM, respectively), and the non-receptor tyrosine kinase YES (IC₅₀ 5.9nM). The results are summarized as follows (Carson *et al.*, 2015):

- Expression of ITK protein, as determined by 2-color immunofluorescence (IF) is significantly increased along the nevus-to-melanoma progression pathway with the highest expression in metastatic cutaneous melanoma (both regional lymph node positive as well as distant metastatic melanoma). ITK expression is significantly correlated with melanoma

protein expression of PD-1 ligand 1 (PD-L1), but is unrelated to melanoma protein expression of activated AKT (pAKT^{Ser473}), PTEN, activated ERK (pErk1/2^{Thr202/Tyr204}), and BRAFV600E status.

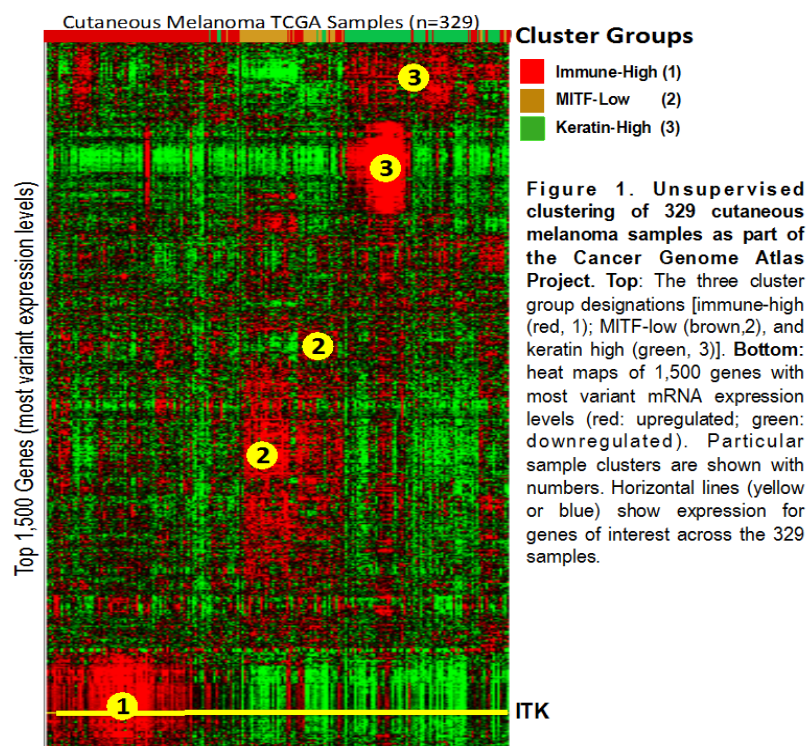
- Suppression of ITK mRNA expression using short hairpin (sh) RNA technology, or pharmacologic inhibition of ITK using BI 10N, inhibits melanoma cell growth and impairs melanoma cell motility without appreciable effects on cell death.
- Reverse phase protein array analysis of approximately 180 proteins in their total or phosphorylated form was performed on whole cell protein extracts obtained from melanoma cell lines that were treated with up to 50nM of BI 10N:
 - With respect to effects on signaling pathways RAF-MEK-ERK and PI3K-Akt-mTOR we **saw minimal or no effect** on components of the PI3K/AKT/mTOR signaling [PI3Kp110/p85, PTEN, pMTOR(S2448)/MTOR, pAkt(S473,T308)/Akt, RSK, TSC1, pP70S6K(T389)/P70S6K, pPRAS40(T246)/PRAS40, pRICTOR(T1135)/RICTOR, S6 [pS6(S235,S236), pS6(S240,S244)], pTuberin(T1462)/Tuberin, Rheb]. Similarly, **minimal or no effects** on the RAS/RAF/MEK1/ERK signaling [NRAS, pBRAF(S445)/BRAF, pCRAF(S338)/CRAF, pMAPK(T202,Y204), pMEK1(S217,S221)/MEK1] were seen.
 - With respect to effects on other signaling pathways, we saw **marked reduction in pEGFR(Y1068)/EGFR, marked increase in pAMPKαT172/AMPKα**, smaller increase in pHER3(Y1289)/HER3 (but not pEGFRY1173/EGFR, or heregulin signaling), reduction in pP38(T180,Y182)/P38, marked increase in pHSP27(S82)/HSP27, reduction in pPEA15(S116)/PEA15, reduction in PLK1, and reduction in Tyro3. No effect was seen on cMET [pCmet(Y1234,Y1235)/cMET], PKCα [pPKCα(S660)/PKCα], Src [pSrc(Y416,Y527)/Src], FoxO3α [pFoxO3α(S318,S321)/FoxO3α], GSK3α [pGSK3α(S21)/GSK3α], IGF1R [pIGF1R(Y1135,Y1136)/IGF1R], NFκBp65 (S536), PKCβ (S660), PKCδ (S664), PLCγ2 (Y759), STAT3 [pSTAT3(Y705)/STAT3], STAT5α, YAP [pYAP(S127)/YAP], YB1 [pYB1(S102)/YB1], Aurora B, PDGFRβ, c-Kit, IRF1, Lck, IRS1, or β-catenin [pβ-catenin(T41,S45)/β-catenin].
 - With respect to effects on cell cycle and growth we saw **marked reduction in p21, an increase in p53 and MDM2 (S166)**, slight reduction in CDK1, cyclin D1, and reduction of Elk1 (S383). No effects were seen for pChk1 (S296, S345)/Chk1, pChk2(T68)/Chk2, pRB(S807,S811)/RB, p16^{INK4A}, pP27^{KIP1}(T157)/P27^{KIP1}, PCNA, cyclin B, cyclin E1, or MIG6. No effects were seen on c-Myc, ETS1, or JNK (T183, Y185).
 - With respect to effects on cell adhesion and motility we saw **marked reduction in pFAK(Y397)/FAK**, reduction in collagen VI, increase in myosin IIa (S1943), and slight increase in P-cadherin and paxillin. No changes were seen in β-actin, tau, α-tubulin, CK19, N-cadherin, E-cadherin, fibronectin, MMP2, or myosin11.

2.3.2 Role of ITK in Melanoma; *In Vivo*

The effect of ITK inhibition on *in vivo* growth was tested in human melanoma xenografts established from melanoma cell lines that were expressing high levels of ITK. BI 10N treatment (15mpk, administered in medicated chow) significantly slowed melanoma growth in both ITK-high expressing human melanoma xenografts without any signs of toxicity (lethargy, weight loss). Treatment of the ITK-high expressing Tyr-CRE-ER^{T2} B-Raf^{CA} Pten^{L/L} genetically engineered murine model (GEMM) with BI 10N significantly reduced melanoma growth and prolonged survival, whereas no such effect was seen with the non-ITK expressing Tyr-HRas^{G12V} Ink4a/Arf^{null}. To investigate the mechanism of action of BI 10N *in vivo*, tumors were stained with cyclin D2, a marker of cell proliferation and LC3, a marker of autophagy by IHC. In BI 10N-treated tumors, cyclin D2, but not LC3, was significantly decreased (Carson *et al.*, 2015).

2.3.3 Immunomodulatory Role of ITK in Melanoma

Several lines of evidence by us and others suggest that ITK may also play an immunomodulatory role in melanoma. UNC-CH served as the hub to perform RNAseq and analysis of melanoma samples as part of The Cancer Genome Atlas Project (TCGA). Unsupervised clustering of 329 untreated melanoma samples using the top 1,500 genes with the maximum absolute deviation identified three RNAseq clusters defined as ‘immune-high’ (n=168, 51%), ‘keratin-high’ (n=102, 31%), and ‘microphthalmia-associated transcription factor (MITF)-low’ (n=59, 18%) based on gene function of discriminatory mRNAs (Cancer Genome Atlas Network (2015)). The ‘immune-high’ cluster is comprised of genes associated with various immune cell subsets (T cells, B cells, NK cells), immune cell signaling (for B-cells, T-cells, or non-immune cell subset-specific), various immune checkpoint proteins, soluble factors and receptors, as well as melanoma antigens. In other words, this sample cluster reflects melanomas with an ‘inflamed’ signature, as previously described (Gajewski *et al.*, 2013). Among the mRNA that comprised the ‘immune-high’ cluster was significantly overexpressed ITK [**Figure 1** and The Cancer Genome Atlas Network (2015)].



Given the role of ITK in distinct immune cell subsets, we have examined ITK expression in Ficoll-separated PBMCs obtained from patients with end-stage metastatic cutaneous melanoma and healthy human subjects. We optimized our anti-ITK antibody (ab32039) for flow cytometry, as there has not been a report to date about assessment of ITK expression in PBMC by flow cytometry.

Figure 2 shows expression of ITK protein in Ficoll-isolated PBMC obtained from a representative normal subject. The mean percentage of ITK⁺ FSC^{low}/SSC^{low} cells among

normal subjects was 51%. More than 50% of CD3⁺CD8⁺ and CD3⁺CD4⁺ were found to be ITK-positive (data not shown). Analysis of PBMCs obtained from patients with metastatic melanoma showed that the expression of ITK was highly variable, but not lower than ITK levels in normal human subjects. Peripheral blood from 3 of 5 subjects with distant metastatic cutaneous melanoma was collected several weeks after the initiation of a BRAF inhibitor-based therapy. In these patients, ITK expression was significantly higher than the expression of ITK in normal subjects. Given the data regarding activation of tumor-infiltrating lymphocytes (TILs) in response to vemurafenib (Koya *et al.*, 2012) and the increased infiltration of tumors by CD8⁺ cells as early as 15 days after BRAF inhibition treatment (Wilmott *et al.*, 2012; Frederick *et al.*, 2013), we postulate that the higher expression of ITK in PBMC from patients who were recently started on BRAF inhibitors may reflect direct or indirect (i.e. compensatory) responses to direct activation of immune cells by BRAF inhibitors. These data as well as other reports describing the role of ITK in type 2 T helper cells (Dubovsky *et al.*, 2013; Gunderson *et al.*, 2016; Stiff *et al.*, 2016), which do not favorably contribute to antitumor response in melanoma (Tatsumi *et al.*, 2002), suggest that ITK inhibition may possibly have an additional immunomodulatory role. In addition, ibrutinib via its effect on BTK may also have an immunomodulatory role towards myeloid-derived suppressor cells (Stiff *et al.*, 2016), B-cells and tumor associated macrophages (Gunderson *et al.*, 2016). In fact, preclinical models have shown synergy in antitumor response when ibrutinib is combined with inhibitors of the PD-1/PD-L1 pathway (Sagiv-Barfi *et al.*, 2015). The clinical significance of these findings is currently being explored in an early phase clinical trial of ibrutinib with corresponding inhibitors of the PD-1/PD-L1 pathway (NCT02403271, NCT02401048).

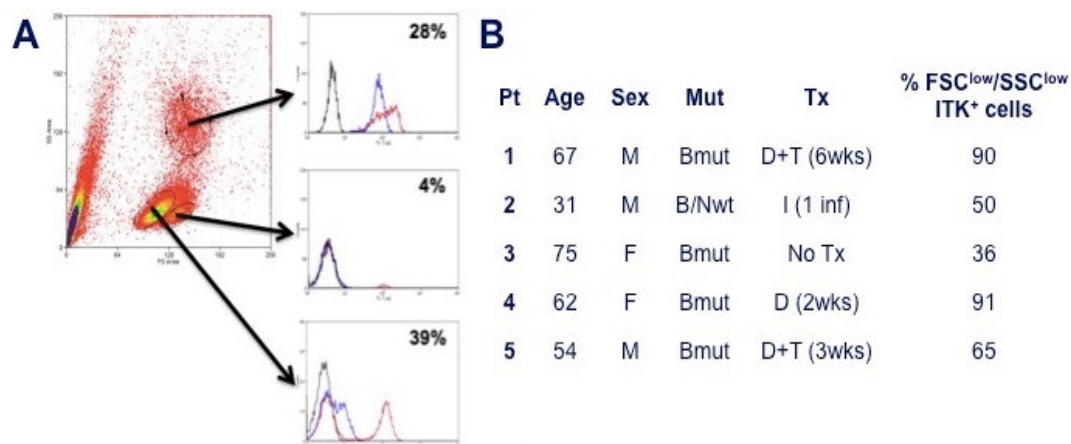


Figure 2.

Flow cytometric analysis of PBMC for total ITK expression. **A.** Gating strategy using forward scatter (FSC) and side scatter (SSC) parameters in a normal subject. Cells with low FSC/SSC features (e.g. lymphocytes) express ITK (red histogram) approximately 1-log difference higher than isotype control (blue histogram) and/or unstained cells (black histogram). **B** Expression of ITK in PBMCs from patients with metastatic melanoma. Abbreviations. B, BRAF; N, NRAS; mut, mutant; Tx, treatment; D, dabrafenib; T, trametinib; I, ipilimumab; inf, infusion; wt, wild type.

2.3.4 Activity Against ITK

In addition to the data on ibrutinib's inhibition of the ITK summarized in Section 2.2.1.1, a number of published reports also document this inhibition (Honigberg *et al.*, 2010; Dubovsky *et al.*, 2013). In fact, ibrutinib is an inhibitor of a large number of Tec and Src/Abl family kinases in addition to ITK. **Table 2** summarizes tyrosine kinases that have the highest affinity ($IC_{50} < 25nM$) with ibrutinib, the number of melanomas that were stained positive for a particular tyrosine kinase over the total number of stained melanomas, as part of the Human Protein Atlas (*, www.proteinatlas.org), as well as the potential biologic role of these kinases in melanoma biology, based on peer-reviewed publications. From this table it is notable that HER4, YES, Csk, EGFR, and HER2 have been previously described to have a role in melanoma. We propose that the expression status of each of these 5 proteins in archived or freshly collected (immediately prior to enrollment to this study) FFPE tumor tissues should be investigated as potential mediators of antitumor response to ibrutinib.

Table 2. High-affinity ibrutinib targets and their relevance to melanoma			
Targets	IC₅₀ (nM)§	Expression in Melanoma	Biological Role in Melanoma
BTK	0.39	11/32*	Unkn
ErBB4/HER4	0.64	18/23*	(Tworkoski <i>et al.</i> , 2011)
Blk	0.94	2/11*	Unkn
Bmx/Etk	1.1	12/21*	Unkn
Fgr	2.86	11/11*	Unkn
Txk	2.87	1/12*	Unkn
Lck	3.49	2/24*	No direct role
Yes	3.92	18/24* (Loganzo <i>et al.</i> , 1993; Lee <i>et al.</i> , 2010; Liu <i>et al.</i> , 2012)	(Marchetti <i>et al.</i> , 1998; Liu <i>et al.</i> , 2012)

Tec	5.49	12/12*	Unkn
Csk	6.17	6/33* (Lee <i>et al.</i> , 2010)	(O'Connor <i>et al.</i> , 1995; Perez <i>et al.</i> , 2001; Niu <i>et al.</i> , 2002; Hwangbo <i>et al.</i> , 2010; Gallagher <i>et al.</i> , 2013)
EGFR	7.8	5/35* (Mattei <i>et al.</i> , 1994; Katunaric <i>et al.</i> , 2014)	(Tworkoski <i>et al.</i> , 2011; Girotti <i>et al.</i> , 2013; Sun <i>et al.</i> , 2014)
Brk	10.1	30/33* (Easty <i>et al.</i> , 1997)	Unkn
ITK	11.7	(91%)	(Carson <i>et al.</i> , 2015)
Hck	16.98	11/12	Unkn
ErbB2/HER2	21.57	10/33* (Kluger <i>et al.</i> , 2004; Mirkina <i>et al.</i> , 2014)	(Zhang <i>et al.</i> , 2013)

To assess the *in vitro* effect of ibrutinib in melanoma cell lines that express ITK at various levels, we treated melanoma cells with increasing concentrations of ibrutinib (up to 50nM). **Figure 3** shows proliferation rates for melanoma cell lines expressing ITK at various levels. Proliferation of two of the three high-ITK expressing, but none of the intermediate or low-ITK expressing melanoma cell lines, was completely suppressed when ibrutinib was administered at concentrations as low as 5nM, which is comparable to the ibrutinib levels that have been previously shown to inhibit various cell lines prepared from patients with various B-cell malignancies (Zheng *et al.*, 2014).

Fluorescent immunocytochemical analysis for protein expression levels of other putative ibrutinib targets (TXK, BMX, TEC, BTK, c-FGR, YES, HCK, ERBB4, EGFR, **Table 3**) in these melanoma cell lines show that the ibrutinib effect is probably not mediated via TXK, BMX, TEC, BTK, and HCK whereas c-FGR, YES, ERBB4, and EGFR may play some role solely on the basis of expression by melanoma cells. We will name the first group of tyrosine kinases as “low priority” ibrutinib targets for melanoma and will name the latter group as “high priority” ibrutinib targets for melanoma. The protein expression status of the “high priority” ibrutinib targets will be systematically investigated in archived or freshly collected (immediately prior to enrollment to this study) FFPE tumor blocks that will be collected at baseline from the subjects who will participate in the study. These results indicate that ibrutinib exerts its anti-proliferative effect via suppression of ITK, although other, yet unidentified targets may also be involved (e.g. ErbB2/Her2, Csk).

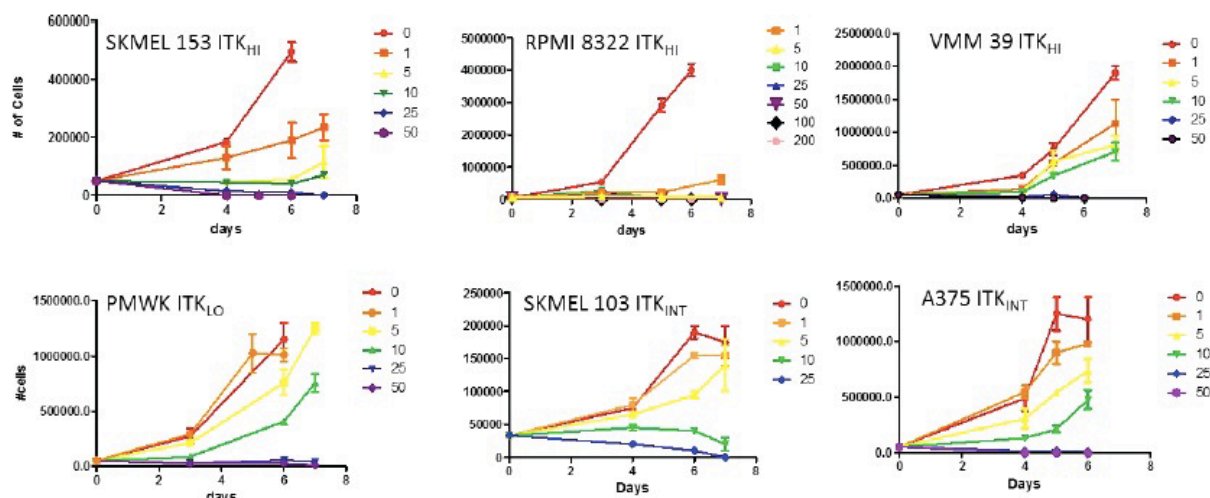


Figure 3. Effect of ibrutinib on proliferation rate of high- (HI), intermediate- (INT), and low (LO)-ITK expressing melanoma cell lines. Cells were added to 10 cm² 6-well dishes at a density of 50,000 cells per well. Ibrutinib was added in DMSO, which was used as a drug vehicle control. Cells were harvested using trypsin (0.025%) in PBS solution (R-001-100, Gibco) containing 0.01% EDTA for approximately 5 min. Cells were counted using the Countless Automated Cell Counter (C10227, Life Technologies).

Table 3. Non-receptor tyrosine kinase expression levels in melanoma cell lines by single color fluorescent immunocytochemistry*										
	Itk**	Txk	Bmx	Tec	Btk	c-Fgr	Yes	Hck	ErbB4	EGFR
SKMel 153	4941	82	39	20	12	136	203	12	106	17
RPMI 8322	193	56	27	18	6	73	120	11	47	1
VMM39	264	50	40	27	25	100	163	23	80	1
PMWK	31	55	37	18	11	68	148	18	56	162
SKMEL103	36	54	36	27	36	78	185	20	74	79
A375	44	63	41	24	17	70	160	11	76	83

*Units for data are arbitrary immunofluorescence (IF) units taking background intensity into account
**Data included in (Carson *et al.*, 2015)
Antibody sources are: ITK (Abcam, ab32039); TXK (Acris Antibodies, AP14483PU-N); BMX (Novus Biologicals, NBP1-19437), TEC (Abcam, ab32368), BTK (Novus Biologicals, NBP1-00728), c-Fgr (Biorbyt, orb160411), Yes (Proteintech, 20243-1-AP), HCK (Proteintech, 11600-1-AP), ErbB4 (Millipore, 05-1133), EGFR (Zymed/Invitrogen, 28-0005)

The rationale for treatment of melanoma with ibrutinib is based on *in vitro* and *in vivo* data highlighting the potential important direct antitumor effect of ITK suppression in melanoma, and perhaps on its possible role as an immunomodulating agent in this disease.

Based on the data just outlined, we hypothesize that ibrutinib will demonstrate clinical activity against melanoma as evidenced by an improved antitumor OR and prolonged PFS compared to historical data in patients with disease refractory to immune checkpoint inhibition and (in those with BRAF600 mutant disease) MAPK inhibition (Ribas *et al.*, 2015; Weber *et al.*, 2015).

2.4 Rationale for 840 mg dose of Ibrutinib

Table 2 shows that the IC₅₀ for ITK (as well as other ibrutinib targets with interest in melanoma and other solid tumors, such as EGFR and HER2) over BTK is 30-fold higher. However, the concentrations required for enzymatic inhibition in cells and inhibition of cellular growth of sensitive cell lines *in vitro*, is similar to or only slightly higher than required for BTK. When ibrutinib was tested at 420mg dose in patients with CLL only up to 70% ITK occupancy was achieved; in contrast, >90% BTK occupancy was observed for that dose. Dedicated pharmacokinetic studies regarding ITK inhibition using ibrutinib do not exist, and are difficult to perform (downstream effectors of ITK signaling are different for different cell lines). However, from the IC₅₀ studies, it can be inferred that a higher dose would be optimal for clinical indications where this target is mechanistically implicated. In addition, the slower-growing property of human tumors and the inherently less efficient penetration of a compound into solid tumor tissues may also contribute to the necessity for higher dose levels. In fact, ibrutinib at doses 840-1,400mg qd was administered for 28 days in normal individuals and did not have any serious side effects; the single dose AUC values corresponding to the 840mg dose was 1,445±869 ng · hr/mL, which is approximately 50% greater than steady-state exposures seen at the highest indicated dose (560 mg; see ibrutinib package insert). At this time there are safety and efficacy data only for the 420mg and 840mg in the dose escalation study of ibrutinib in patients with CLL, which are equivalent for both doses (Byrd *et al.*, 2013). Due to the expected heterogeneity in the sensitivity profile of clinical cancers and patient pharmacokinetics, a starting dose of 840 mg/day has been selected for this study with allowance for dose de-escalation.

2.4.1 Safety, Pharmacokinetics and Efficacy of 840 mg dose

Safety, pharmacokinetics and efficacy data are emerging from ongoing Phase I studies of ibrutinib in patients with lymphoma given 840mg po qd. Briefly, in a study by Dunleavy *et al.* (NCT02203526), 14 patients with central nervous system (CNS) lymphoma received ibrutinib in cohorts (560, 700, and 840 mg po qd) for 14 days prior to cycle 1, followed by dose-adjusted TEDDI-R with ibrutinib every 21 days for 6 cycles. Four patients have been treated with 840mg po qd of ibrutinib; and no DLTs were reported at this dose. Notably, higher ibrutinib doses achieved higher levels in cerebrospinal fluid (CSF) and longer times above the IC₅₀; median time above the IC₅₀ was 4 hours after 560 mg and 8.5 hours after 700 mg doses of ibrutinib were given. CSF penetration corrected for protein binding was 21.4-100% for ibrutinib and 48-120% for its metabolite. Eleven patients were evaluable for response to ibrutinib alone with 10 partial responses and 1 stable disease reported (Dunleavy *et al.*, 2015).

Grommes *et al.* (NCT0232315326) are investigating single-agent ibrutinib in relapsed/refractory primary or secondary CNS lymphoma (PCNSL, N=10) in an ongoing Phase I trial. PK studies have shown ibrutinib penetrates the CNS and has activity in PCNSL. Seven patients have received the 840 mg dose. Higher CSF drug concentrations were achieved and higher drug concentrations were found in CSF after 840 mg ibrutinib. Toxicity data were reported in the first 6 patients enrolled and 2 subjects experienced grade 4 neutropenia and lymphopenia that resolved after ibrutinib was withheld. Thus far, ibrutinib (840 mg) has proven to be tolerable when given as monotherapy or in combination with intensive chemotherapy (Grommes *et al.*, 2015).

2.5 Correlative Studies Background

See Sections 2.3.1 thru 2.3.4 for detailed background information on the biomarkers to be evaluated in this study. In addition to our primary hypothesis related to the clinical activity of ibrutinib, we also hypothesize that increased expression of ITK will be associated with increased antitumor RR and prolonged PFS. However, we cannot exclude that high protein expression of other putative ibrutinib targets in archived or freshly collected (immediately prior to enrollment to this study) FFPE tumor tissues, in particular HER2, HER4, YES, CSK, EGFR, cFGR, may not have a role in predicting response to ibrutinib in metastatic cutaneous melanoma, independent of or in combination with ITK. Finally, we hypothesize that ibrutinib will not show any inhibitory effect on host immune response; rather it may have beneficial effects on host immune response by reversing melanoma-mediated Th2-bias (Tatsumi *et al.*, 2002).

To address our correlative objectives, expression of ITK and 6 other putative ibrutinib targets will be assessed on archived or freshly collected (immediately prior to enrollment to this study) FFPE tumor blocks. Given the higher sensitivity and specificity of IF, the expression of the exact same proteins (ITK and other putative ibrutinib targets) could also be assessed in melanoma cells (S100+) using 2-color IF, if additional tumor sections are available. Analysis of the staining data by IF will be performed by Aperio imaging, as we have previously published (Schlegel *et al.*, 2013; Nikolaishvilli-Feinberg *et al.*, 2014; Carson *et al.*, 2015). Flow cytometry will be performed on PBMC collected at three different time points (prestudy treatment, day 29 (i.e., predose day 1 of cycle 2), and at disease progression) for analysis of immune regulatory cell populations as well as markers for Th1 and Th2 responses [i.e. regulatory T cells, myeloid-derived suppressor T cells, Th1/Th2 ratio, anergic (PD1±, TIM3±, CTLA4±), and CD8+ effector cells].

3. PATIENT SELECTION

3.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to participate in this study:

- 3.1.1 Histologically confirmed melanoma of cutaneous primary; metastatic melanoma from unknown primary are allowed because melanoma of unknown primary is biologically similar to cutaneous melanomas (Egberts *et al.*, 2014).
- 3.1.2 Measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥ 10 mm (≥ 1 cm) with spiral CT scan, MRI, or calipers by clinical exam. See [Section 11](#) for the evaluation of measurable disease.
- 3.1.3 Stage IV disease.

3.1.4 If BRAFV600-mutant, documented refractory disease to at least one BRAF inhibitor (dabrafenib or vemurafenib) and/or a MEK inhibitor (trametinib or cobimetinib), defined as progression of measurable disease as per RECIST 1.1 criteria while on treatment. Subjects with MAPK inhibitor-intolerance are eligible if they meet criteria outlined in inclusion criterion 3.1.21.

3.1.5 Documented disease refractory to at least one PD1/PD-L1 inhibitor, defined as disease progression following at least 2 infusions of the same drug. Radiographic disease progression will be documented by the institutional radiologist based on any radiographic evidence (MRI, CT, PET, or other modalities, etc.) of disease progression on two separate radiographic scans assessment obtained at least 4 weeks apart. This minimum 4-week interval is required to define PD-1 inhibitor resistance based on imaging. Alternatively, Clinical Disease Progression may be documented on examination by the treating investigator.

3.1.6 Prior treatment-related toxicity resolved to \leq Grade 1 or baseline with the exception of alopecia and permanent grade ≤ 2 toxicities related to prior immune checkpoint inhibitor treatment (e.g. PD-1/PD-L1, CTLA-4, CD40, LAG3) treatment with the review and approval by the Lead PI.

3.1.7 Prior radiation allowed (no restriction on amount); measurable lesion(s) may not have been previously irradiated.

3.1.8 Males or female subjects age ≥ 18 years.

Because no dosing or adverse event data are currently available on the use of ibrutinib in patients < 18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.9 ECOG performance status ≤ 2 (Karnofsky $\geq 60\%$, see [Appendix A](#)).

3.1.10 Life expectancy of greater than 3 months.

3.1.11 Patients must have normal organ and marrow function as defined below:

Hemoglobin	$\geq 9.0\text{g/dL}$
Absolute neutrophil count (ANC)	$> 1,500/\mu\text{L}$
Platelets	$> 100,000/\mu\text{L}$
AST (SGOT)/ALT (SGPT)	$\leq 2 \times$ upper limit of normal (ULN); $\leq 5 \times$ ULN, if liver metastasis
Total bilirubin	$\leq 1.5 \times$ ULN (unless Gilbert's syndrome of disease infiltration of the liver is present)
Creatinine clearance	Estimated GFR $\geq 30\text{mL/min/1.73m}^2$ (Cockcroft-Gault)

3.1.12 Patients with brain metastases are allowed provided that:

- No leptomeningeal disease is present,
- Intracranial disease is controlled by prior local therapies (craniotomy, stereotactic radiosurgery, whole brain irradiation), as evidenced by brain MRI 4 weeks post treatment indicating no new intracranial disease,
- Stable or decreasing dose of steroids provided patient on $\leq 20\text{mg}$ of prednisone or its equivalent daily.

3.1.13 Given the effects of ibrutinib on platelet aggregation, ibrutinib should be held at least 3 to 7 days pre- and post-surgery, depending upon the type of surgery and risk of bleeding. Please see Section 5.2.5 for definitions of major and minor surgery.

3.1.14 The effects of ibrutinib on the developing human fetus are unknown. For this reason and because tyrosine kinase inhibitors may be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and for 90 days after completion of ibrutinib administration. 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. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 90 days after completion of ibrutinib administration.

3.1.15 Negative serum pregnancy test within 7 days of treatment initiation with ibrutinib in women of childbearing potential (WOCBP).

3.1.16 Ability to swallow oral medications.

3.1.17 Patients with autoimmune disease requiring systemic corticosteroid treatment (and previously ineligible to receive systemic immunotherapies for melanoma) are allowed on condition that they do not receive more than 20mg of daily dose methylprednisolone, prednisone, or its equivalent. This does not include autoimmune diseases caused by previous immunotherapy treatments for melanoma that require ongoing treatment with corticosteroids (e.g. autoimmune colitis or autoimmune hepatitis receiving corticosteroids).

3.1.18 Willing to consent to allow access to known archival tumor tissue [NOTE: designated pathologist from participating site OR Lead Principal Investigator must sign-off to ensure “sufficient” tumor should be available for support of tumor imaging studies (multi-color immunofluorescence)]; See [Appendix B](#) for a sample of the sign-off letter from pathologist and see Section 9.3.1.1 for process of Lead Principal Investigator to provide sign-off that there is sufficient archival tissue.

- 3.1.19 If archival tumor tissue from a metastatic melanoma lesion is unavailable OR designated pathologist from participating site cannot sign-off to ensure that “sufficient” tumor is available from existing archival tumor block for support of tumor imaging studies (see [Appendix B](#) for a sample of the sign off letter), patients must be willing to consent to undergo a biopsy to collect metastatic tumor tissue. Collection of fresh biopsy tissue does not guarantee enrollment, unless the pathologist from the participating site signs-off that “sufficient” tumor has been collected.
- 3.1.20 Ability to understand and the willingness to sign a written informed consent document.
- 3.1.21 Subjects who are unable to tolerate BRAF inhibitor and/or MEK inhibitor therapy due to grade ≥ 2 toxicity (CTCAE v5.0) from these agents, irrespective of antitumor response, are eligible on condition that: (a) toxicities persisted despite change from doublet to singlet therapy (i.e. from concurrent BRAF inhibition plus MEK inhibition to BRAF inhibition alone), (b) toxicities are attributed to a class effect, and therefore switch from one drug to another is expected to induce the same type of toxicity (e.g. ocular toxicities or cardiac dysfunction from MEK inhibitor), (c) drug-specific toxicities that do not resolve with switch from one BRAF inhibitor to another (i.e. dabrafenib to vemurafenib, or vice versa), will be eligible for enrollment in 9922. In other words, patients will be allowed to enroll into the NCI9922 study despite lack of progression to MAPK inhibitor treatments, on condition that grade 2 or higher toxicities attributed to MAPK inhibitors resolve to grade 1, or less, at the time of study enrollment.

3.2 Exclusion Criteria

Subjects meeting any of the criteria below may not participate in the study:

- 3.2.1 Patients with melanoma of mucosal or ocular primary.
- 3.2.2 Patients who have had chemotherapy or immunotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) or radiotherapy within 2 weeks prior to Cycle 1 Day 1. Patients who have had tyrosine kinase inhibitors (such as Braf or MEK inhibitors) within 15 days of Cycle 1 Day 1.
- 3.2.3 Patients who are receiving any other biologic, cytotoxic or investigational agents.
- 3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ibrutinib (difficulty breathing, lip swelling, itching or rash).
- 3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.2.6 Pregnant and breastfeeding women are excluded from this study.
- Pregnant women are excluded in this study, because ibrutinib is a tyrosine kinase

inhibitor with the potential for teratogenic or abortifacient effects.

- Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ibrutinib, breastfeeding should be discontinued if the mother is treated with ibrutinib.
- 3.2.7 HIV-positive patients on combination antiretroviral therapy are eligible; unless the patient's CD4+ count is below the institutional lower limit of normal.
- 3.2.8 Uncontrolled autoimmune hemolytic anemia or ITP resulting in (or as evidenced by) declining platelet or Hgb levels within the 4 weeks prior to first dose of study drug.
- 3.2.9 Presence of transfusion-dependent thrombocytopenia.
- 3.2.10 Need for daily corticosteroids at high doses (prednisone ≥ 20 mg daily, or an equivalent) is prohibited from 28 days prior to first dose and during treatment with ibrutinib. Brief (up to 7 days) and episodic use of systemic corticosteroids for other general conditions (e.g. pre-medication for radiographic imaging due to IV contrast allergy, COPD exacerbation, poison ivy, etc.) is allowed.
- 3.2.11 Prior exposure to ibrutinib or other ITK inhibitors.
- 3.2.12 History of prior malignancy, with the exception of the following:
- Non-melanoma skin cancers, non-invasive bladder cancer, and carcinoma *in situ* of the cervix,
 - Prostate cancer not under active systemic treatment other than hormonal therapy and with documented undetectable PSA (<0.2 ng/mL),
 - CLL/SLL provided patient has isolated lymphocytosis (Rai stage O), and does not require systemic treatment [for "B" symptoms, Richter's transformation, lymphocyte doubling time (<6 months), lymphadenopathy or hepatosplenomegaly],
 - Lymphoma of any type or hairy-cell leukemia provided patient is not on active systemic treatment and is in complete remission, as evidenced by PET/CT scans and bone marrow biopsies for at least 3 months,
 - History of malignancy provided that patient has completed therapy and is free of disease for ≥ 2 years. If patient had other malignancy within the last 2 years from which he may have been completely cured by surgery alone, he may be considered to be enrolled on condition that the risk of development of distant metastatic disease based on AJCC staging system is less than 30%.
- 3.2.13 Currently active, clinically significant cardiovascular disease, such as uncontrolled arrhythmia, congestive heart failure, any Class 3 or 4 cardiac disease, as defined by the New York Heart Association Functional Classification, or history of myocardial infarction within 6 months prior to first dose with study drug.

- 3.2.14 Unable to swallow capsules, or disease significantly affecting gastrointestinal function and/or inhibiting small intestine absorption, such as malabsorption syndrome, resection of portions of small bowel larger than 3 feet, or poorly controlled inflammatory bowel disease affecting the small intestine.
- 3.2.15 Known serologic status reflecting active hepatitis B or C infection. Patients that are hepatitis B core antibody positive, but antigen negative, will need a negative polymerase chain reaction (PCR) prior to enrollment. [NOTE: Hepatitis B antigen or PCR positive patients will be excluded.]
- 3.2.16 History of stroke or intracranial hemorrhage within 6 months prior to enrollment.
- 3.2.17 Current life-threatening illness, medical condition, or organ system dysfunction, which, in the Investigator's opinion, could compromise the patient's safety, or put the study at risk.
- 3.2.18 Received anticoagulation therapy with warfarin, or equivalent vitamin K antagonists, within the last 28 days prior to day 1 of ibrutinib. Patients with familial coagulopathic diseases (e.g. hemophilia, von Willebrand disease) are also excluded. If applicable, subjects must discontinue fish oil and vitamin E supplements within 7 days prior to initiating ibrutinib therapy.
- 3.2.19 Subjects with known hepatic insufficiency [i.e. Child-Pugh Score A (mild), Child-Pugh Score B (moderate) or Child-Pugh Score C (severe)] according to Child-Pugh Criteria (See [Appendix F](#)).
- 3.2.20 Subjects who received a strong cytochrome P450 (CYP) 3A inhibitor within 7 days prior to the first dose of ibrutinib or subjects who require continuous treatment with a strong CYP 450 3A inhibitor.

3.3 Inclusion of Women and Minorities

NIH policy requires that women, members of minority groups, and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research, unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see <http://grants.nih.gov/grants/funding/phs398/phs398.pdf>. See Section 13.2 for details on projected enrollment.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (<https://ctepcore.nci.nih.gov/iam>). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (<https://ctepcore.nci.nih.gov/rcr>). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✓	✓		
Financial Disclosure Form	✓	✓	✓	
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓	
HSP/GCP training	✓	✓	✓	
Agent Shipment Form (if applicable)	✓			
CV (optional)	✓	✓	✓	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

For questions, please contact the RCR **Help Desk** by email at < RCRHelpDesk@nih.gov >.

Additional information can be found on the CTEP website at <
<https://ctep.cancer.gov/investigatorResources/default.htm>>.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the 9922 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to <https://www.ctsu.org> and log in using your CTEP IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or

- Click on the By Lead Organization to expand, then select LAO-NC010, and protocol # 9922.
- Click on LPO Documents, select the Site Registration documents link, download and complete the forms provided. (NOTE: For sites under the CIRB initiative, IRB data will load to RSS as described above.)

4.2.2 Requirements for 9922 Site Registration

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- Study Chair Approval – Protocol training

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsuo.org (members' area) → Regulatory Tab
→ Regulatory Submission

When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to <https://www.ctsuo.org> and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does

not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

In cooperation with the Corresponding Organization (Duke Cancer Institute LAO), the Lead Protocol Organization or LPO (University of North Carolina at Chapel Hill) is utilizing an online 'study portal' for key study communication from participating sites to the Lead Principal Investigator and Study Coordinator. The study portal operates through REDCap, a secure, web-based application, managed by the Duke Cancer Institute LAO. Participating sites will not require REDCap user accounts or passwords to access the study portal for this study.

The **9922 Study Portal** may be accessed through the following link: <http://j.mp/2eup4vF>. Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

Prior to registering patients into Oncology Patient Enrollment Network (OPEN), participating sites are instructed to access the 9922 Study Portal for the following:

- **Subject Consent** – Within 24 business hours of subject signing consent for the study, the Site Coordinator accesses the study portal to upload consents and notify the Study Coordinator of subject consent. Upon receipt of consent notification, the Study Coordinator will email the Site Coordinator with a subject screening ID number.
- **Pathology Confirmation** – Subject eligibility requires confirmation that the patient has “sufficient” tumor available for support of tumor imaging studies. The Site Coordinator will confirm the subject has sufficient tumor by one of the following processes:
 - a) Pathologist at the participating site reviews available tissue and provides a signed letter (refer to Appendix B for sample letter). The Site Coordinator uploads the letter to the study portal. The Study Coordinator will email the Site Coordinator to confirm receipt of letter.
 - b) Lead Principal Investigator reviews pathology reports, stained slides, and/or digital images provided by the participating site (refer to Section 9.3.1.1 for further instructions). The Site Coordinator ships and/or emails de-identified materials to the Lead Protocol Organization or LPO (University of North Carolina at Chapel Hill). After review and determination of sufficient tumor by the Lead Principal Investigator, the Study Coordinator will email the Site Coordinator regarding the outcome of the pathology review.
- **Eligibility Confirmation** – After completion of all screening evaluations and if possible, within at least 48 business hours of anticipated study treatment start, the Site Coordinator accesses the study portal to upload completed eligibility checklist and de-identified

supporting source documentation including sufficient tumor tissue pathology confirmation documentation. After review of eligibility documents by the Lead Principal Investigator or designee(s), the Study Coordinator will email the Site Coordinator with confirmation or questions/comments regarding subject eligibility. If eligibility is confirmed, the Site Coordinator will be instructed by the Study Coordinator via email to proceed to patient registration in OPEN.

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN/IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (i.e., CTEP username and password).
- To enroll patients: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type. If a DTL is required for this study, the registrar(s) must also be assigned the OPEN Registrar task on the DTL.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated time frames.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.
- Refer to additional instructions in Section 4.3.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at <https://www.ctsu.org> or at <https://open.ctsu.org>. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.4 General Guidelines

Following OPEN registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Lead Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the

study may be cancelled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 Agent Administration

Ibrutinib treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in [Section 7](#). Appropriate dose modifications are described in [Section 6](#). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Treatment will consist of a fixed dose of 840mg of ibrutinib orally once daily on a continuous basis. For purposes of the study, treatment will be divided into 28-day treatment cycles, with treatment starting on Day 1 (D1) of Cycle 1.

5.1.1 Ibrutinib

Premedication for this agent is not required.

Patients will be instructed on the following:

- Take each dose of ibrutinib capsules (six 140 mg capsules = 840mg) once daily by mouth.
- Swallow the capsules whole with water [use a glass (8 ounces, 1cup) of water total for each 840mg dose].
- Take each dose at approximately the same time each day.
- Ibrutinib must not be taken with grapefruit juice. Patients should avoid drinking grapefruit juice while on this study.
- Do not open, break or chew capsules, and do not attempt to dissolve them in water.
- Each dose, along with the date and time, must be recorded on a medication diary provided to you at the beginning of the study. Bring the diary with you to each study visit and return the diary to research staff, as per study instructions.
- If a dose is not taken at the scheduled time, it can be taken as soon as possible on the same day with a return to the normal schedule the following day. Extra capsules should **not** be taken to make up for the missed dose.
- If you vomit immediately after taking your dose, do not replace this dose. Continue dosing the next day, as per usual. Record the vomiting episode on your medication diary.
- Return any remaining ibrutinib capsules to research staff once treatment is complete, and as per study instructions.
- Do not take fish oil and vitamin E supplement while on this study.

5.1.2 Other Agent(s)

N/A

5.1.3 Other Modality(ies) or Procedures

N/A

5.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of ibrutinib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. [Appendix D](#) presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

5.2.1 CYP Inhibiting/Inducing Drugs

Ibrutinib is metabolized primarily by CYP3A4. Avoid co-administration with strong CYP3A4 (e.g., ketoconazole, indinavir, nelfinavir, ritonavir, saquinavir, clarithromycin, telithromycin, itraconazole, and nefazadone) or moderate CYP3A inhibitors and consider alternative agents with less CYP3A inhibition. If a strong CYP3A inhibitor must be used, reduce ibrutinib dose to 140 mg or withhold treatment temporarily. Subjects should be monitored for signs of ibrutinib toxicity. If a moderate CYP3A inhibitor must be used, reduce ibrutinib to 140 mg (for 840 mg/day dose, reduce to 280 mg) for the duration of the inhibitor use. No dose adjustment is required in combination with mild inhibitors. Avoid grapefruit and Seville oranges during ibrutinib/placebo treatment, as these contain moderate inhibitors of CYP3A.

Avoid concomitant use of strong CYP3A inducers (e.g., carbamazepine, rifampin, phenytoin, and St. John's Wort). Consider alternative agents with less CYP3A induction.

For the most comprehensive effect of CYP3A inhibitors or inducers on ibrutinib exposure, please refer to the current version of the IB. [A more frequently updated list of inhibitors and inducers is available at http://medicine.iupui.edu/CLINPHARM/ddis/main-table](http://medicine.iupui.edu/CLINPHARM/ddis/main-table). [Appendix D](#) is a guideline for patients regarding medications/substances that could potentially interact with the ibrutinib study agent(s). Refer to the Ibrutinib IB for a comprehensive list of these agents.

5.2.2 QT Prolonging Agents

Any medications known to cause QT prolongation should be used with caution; periodic monitoring with ECGs and electrolytes should be considered and, if needed, the Medical Monitor may be contacted. See <https://www.crediblemeds.org/> for a list of medications associated with QTc prolongation.

5.2.3 Antiplatelet Agents and Anticoagulants

Warfarin or vitamin K antagonists should not be administered concomitantly with ibrutinib. Supplements such as fish oil and vitamin E preparations should be avoided. Use ibrutinib with caution in subjects requiring other anticoagulants or medications that inhibit platelet function (e.g. aspirin, clopidogrel, non-vitamin K antagonist oral anticoagulants). Subjects with congenital bleeding diathesis have not been studied.

Subjects requiring the initiation of therapeutic anticoagulation therapy (e.g., atrial fibrillation), consider the risks and benefits of continuing ibrutinib treatment. If therapeutic anticoagulation is clinically indicated, treatment with ibrutinib should be held and not be restarted until the subject is clinically stable and has no signs of bleeding. Subjects should be observed closely for signs and symptoms of bleeding. No dose reduction is required when study drug is restarted.

5.2.4 Prohibited Medications

In addition to the above considerations, any non-study chemotherapy, anticancer immunotherapy as well as corticosteroids (at dosages equivalent to prednisone ≤ 20 mg/day), experimental therapy, or radiotherapy are prohibited.

5.2.5 Surgery

The following guidance should be applied during the perioperative period for subjects who require surgical intervention or an invasive procedure while receiving ibrutinib:

- For any surgery or invasive procedure requiring sutures or staples for closure, ibrutinib should be held at least **7 days prior** to the intervention and should be held at least **7 days after** the procedure, and restarted at the discretion of the investigator when the surgical site is reasonably healed without serosanguineous drainage or the need for drainage tubes.
- For minor procedures (such as a central line placement, needle biopsy, thoracentesis, or paracentesis), ibrutinib should be held for at least **3 days prior** to the procedure and should not be restarted for at least **3 days after** the procedure.
- For emergency procedures, ibrutinib should be held after the procedure until the surgical site is reasonably healed, for at least **7 days after** the urgent surgical procedure.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study,

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or
- Death

5.4 Duration of Follow Up

Patients will be followed for up to 2 years post discontinuation of ibrutinib or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Ibrutinib

Dose Escalations of Ibrutinib

No intra-subject dose escalation of ibrutinib is permitted.

Dose Delay

Treatment with ibrutinib should be held for any unmanageable, potentially study drug-related toxicity that is grade 3, or higher, in severity. If anticoagulation therapy is required, an anticoagulant other than warfarin or vitamin K antagonist must be used. Ibrutinib should not be re-started until the patient is clinically stable.

Study drug may be held for a maximum of 14 days for neutropenia, and for 28 consecutive days for any other toxicity. Study treatment should be discontinued if these limits are met and the patient followed up per protocol.

If dose reductions of ibrutinib are required, please follow the guidelines provided in the table below. Ibrutinib should be permanently discontinued in patients who require <420 mg per day, and patients should be followed-up per protocol. The only exception to this rule will be patients on strong or moderate CYP3A inhibitors. If strong CYP3A inhibitor must be used, reduce ibrutinib dose to 140 mg, and if a moderate inhibitor must be used, reduce ibrutinib dose to 280 mg for the duration of the inhibitor use (see section 5.2.1 for additional information). Subjects who develop hepatic insufficiency during the trial must discontinue ibrutinib therapy as described in the section below entitled Discontinuation of Ibrutinib for Patients with Hepatic Impairment.

Missed doses of ibrutinib are not to be made up. In the event that ibrutinib dosing is interrupted, the duration of cycle/treatment will not be extended. Therefore, if treatment is delayed on Day 1 of a cycle due to toxicity, and the patient does not begin therapy until Day 5 (for example), the start date of drug for that cycle remains Day 5, not Day 1.

Dose Level	Ibrutinib Oral Daily Dose	Number of 140mg Capsules
-3	420 mg	3
-2	560 mg	4
-1	700 mg	5
Starting Dose 1	840 mg	6

If a patient experiences serious adverse events and there are conflicting recommendations, the investigator should use the recommended dose adjustment that reduces the dose to the lowest level.

Dose Reduction and Discontinuation for Hematological Toxicities

The actions in the table below should be taken for the following hematological toxicities. Investigator is allowed to take more conservative approaches at his discretion and clinical judgement, after discussing with Principal Investigator:

- ANC <500/ μ L for >7 days (neutrophil growth factors are permitted per ASCO guidelines (Bennett *et al.*, 1999; Smith *et al.*, 2006) and use must be recorded in the electronic case report form (e-CRF).
- A platelet count of <50,000/ μ L in the presence of grade 3 bleeding
- A platelet count of <25,000/ μ L regardless of bleeding

Ibrutinib Dose Delay and Dose Modifications for Hematological Toxicities	
Occurrence	Action
1 st	Hold ^a ibrutinib until recovery to grade ≤ 1 or baseline; may resume at original dose level
2 nd	Hold ^a ibrutinib until recovery to grade ≤ 1 or baseline; resume at one dose level lower ^b
3 rd	Hold ^a ibrutinib until recovery to grade ≤ 1 or baseline; resume at one dose level lower ^b
4 th	Hold ^a ibrutinib until recovery to grade ≤ 1 or baseline; resume at one dose level lower ^b
5 th	Discontinue ibrutinib
^a Ibrutinib should be permanently discontinued and patients followed-up per protocol, if delay >28 days is required	
^b If >3 dose reductions are required, ibrutinib should be permanently discontinued, and patient followed up per protocol.	

This same information is presented by specific hematological toxicity and grade in the tables

below:

Neutropenia^a	Management/Next Dose for Ibrutinib
≤ Grade 1	No change in dose.
Grade 2	No change in dose.
Grade 3	No change in dose.
Grade 4 lasting > 7 days (NOTE: do not reduce for neutropenia lasting < 7 days)	Hold ^b until ≤ Grade 1 or baseline, then: 1 st Occurrence: Resume at same dose level 2 nd Occurrence: Resume reduced by one dose level 3 rd Occurrence: Resume reduced by one dose level (again) 4 th Occurrence: Resume reduced by one dose level ^c 5 th Occurrence: Permanently discontinue ibrutinib
^a Neutrophil growth factors are permitted per ASCO guidelines (Bennett <i>et al.</i> , 1999; Smith <i>et al.</i> , 2006). Use of growth factors should be included in study data. ^b Ibrutinib should be permanently discontinued and patients followed-up per protocol, if delay >14 days is required ^c If >3 dose reductions are required, ibrutinib should be permanently discontinued, and patient followed up per protocol.	

Thrombocytopenia	Management/Next Dose for Ibrutinib
≤ Grade 1	No change in dose.
Grade 2	No change in dose.
Grade 3 with ongoing significant bleeding NOTE: if there is no ongoing significant bleeding, hold but do not dose reduce for Grade 3 thrombocytopenia	Hold ^a until ≤ Grade 1 or baseline, then: 1 st Occurrence: Resume at same dose level 2 nd Occurrence: Resume reduced by one dose level ^b 3 rd Occurrence: Resume reduced by one dose level (again) 4 th Occurrence: Resume reduced by one dose level (again) ^b 5 th Occurrence: Permanently discontinue ibrutinib
Grade 4	Hold ^b until ≤ Grade 1 or baseline, then: 1 st Occurrence: Resume at same dose level 2 nd Occurrence: Resume reduced by one dose level 3 rd Occurrence: Resume reduced by one dose level (again) 4 th Occurrence: Resume reduced by one dose level (again) ^b 5 th Occurrence: Permanently discontinue ibrutinib
^a Ibrutinib should be permanently discontinued and patients followed-up per protocol, if delay >28 days is required ^b If >3 dose reductions are required, ibrutinib should be permanently discontinued, and patient followed up per protocol.	

If therapeutic anticoagulation is clinically indicated, treatment with ibrutinib should be held until the patient is clinically stable and has no signs of bleeding. Patients should be observed closely for signs and symptoms of bleeding. No dose reduction is required when study drug is restarted.

Dose Reduction and Discontinuation for Non-Hematological Toxicities

For the non-hematological events listed, only those which persist despite adequate treatment by the investigator's/institution's standards should be considered before hold/reduction of ibrutinib.

<u>Nausea^a, Vomiting^a Diarrhea^b</u>	Management/Next Dose for Ibrutinib
≤ Grade 1	No change in dose.
Grade 2	No change in dose. ^c
Grade 3	Hold ^d until ≤ Grade 1 or baseline, then: 1 st Occurrence: Resume at same dose level 2 nd Occurrence: Resume reduced by one dose level 3 rd Occurrence: Resume reduced by one dose level (again) 4 th Occurrence: Resume reduced by one dose level (again) ^e 5 th Occurrence: Permanently discontinue ibrutinib
Grade 4	Discontinue ibrutinib permanently ^a

^aRecommended management: anti-emetics
^bRecommended management: Loperamide antidiarrheal therapy; dosage schedule: 4mg at first onset, followed by 2mg with each loose bowel movement until diarrhea-free for 12 hours (maximum dosage: 16mg/24 hours). Adjunct anti-diarrheal therapy is permitted and should be recorded when used.
^cIbrutinib may be withheld at the discretion of the investigator for grade 2 toxicity that is significantly impacting a patient's quality of life.
^dIbrutinib should be permanently discontinued and patients followed-up per protocol, if delay >28 days is required.
^eIf >3 dose reductions are required, ibrutinib should be permanently discontinued, and patient followed up per protocol.

Suspected pulmonary and/or CNS fungal infections

- Investigators should be vigilant about detecting cases of suspected pulmonary and/or CNS fungal infections and, specifically, aspergillosis.
- If a case of aspergillosis is suspected or observed in this trial, ibrutinib should be discontinued.
- All suspected and confirmed cases of fungal infections should be reported to CTEP within 24 hours.

Discontinuation of Ibrutinib for Patients with Hepatic Impairment

Patients who develop mild, moderate, or severe hepatic impairment (ie, Childs-Pugh Class A, B or C, respectively) during the trial should permanently discontinue ibrutinib therapy and be

removed from the study (Please refer to [Appendix F](#) for Childs-Pugh Scoring system of hepatic impairment).

<u>Any other Ibrutinib-related Adverse Event</u>	Management/Next Dose for Ibrutinib
≤ Grade 1	No change in dose.
Grade 2	No change in dose.
Grade 3	Hold ^a until ≤ Grade 1 or baseline, then: 1 st Occurrence: Resume at same dose level 2 nd Occurrence: Resume reduced by one dose level 3 rd Occurrence: Resume reduced by one dose level (again) 4 th Occurrence: Resume reduced by one dose level (again) ^b 5 th Occurrence: Permanently discontinue ibrutinib
Grade 4	Discontinue ibrutinib permanently ^a
^a Ibrutinib should be permanently discontinued and patients followed-up per protocol if delay >28 days is required ^b If >3 dose reductions are required, ibrutinib should be permanently discontinued, and patient followed up per protocol.	

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AE (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. Frequency is provided based on 2082 patients. Below is the CAEPR for ibrutinib (PCI-32765).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPRs for CTEP-supplied Agent

7.1.1.1 CAEPR for Ibrutinib

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Ibrutinib (PCI-32765, NSC 748645)

Frequency is provided based on 2082 patients. Below is the CAEPR for ibrutinib (PCI-32765).

Version 2.5, May 25, 2017

Adverse Events with Possible Relationship to Ibrutinib (PCI-32765) (CTCAE 4.0 Term) [n= 2082]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		<i>Anemia (Gr 2)</i>
		Blood and lymphatic system disorders - Other (leukostasis) ²	
	Febrile neutropenia		
		Leukocytosis ²	
CARDIAC DISORDERS			
	Atrial fibrillation		
		Ventricular arrhythmia	
		Ventricular fibrillation	
		Ventricular tachycardia	
EYE DISORDERS			
	Blurred vision		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Constipation		
Diarrhea			<i>Diarrhea (Gr 2)</i>
	Mucositis oral		
	Nausea		<i>Nausea (Gr 2)</i>
	Vomiting		<i>Vomiting (Gr 2)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
	Fatigue		<i>Fatigue (Gr 2)</i>
	Fever		
		Sudden death NOS	
HEPATOBIILIARY DISORDERS			
		Hepatic failure	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	
INFECTIONS AND INFESTATIONS			
	Infection ³		<i>Infection³ (Gr 2)</i>

Adverse Events with Possible Relationship to Ibrutinib (PCI-32765) (CTCAE 4.0 Term) [n= 2082]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
		Infections and infestations - Other (bronchopulmonary and central nervous system infections) ⁴	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		
INVESTIGATIONS			
	Lymphocyte count increased ²		
Neutrophil count decreased			Neutrophil count decreased (Gr 4)
	Platelet count decreased		Platelet count decreased (Gr 2)
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		
		Hyperuricemia	
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		
	Myalgia		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (benign neoplasm of skin) ⁵		
		Treatment related secondary malignancy ⁵	
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		
		Peripheral sensory neuropathy	
		Cognitive disturbance	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		Cough (Gr 2)
	Dyspnea		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Skin and subcutaneous tissue disorders - Other (angioedema) ⁶	
	Skin and subcutaneous tissue disorders - Other (rash) ⁷		Skin and subcutaneous tissue disorders - Other (rash)⁷ (Gr 2)
		Stevens-Johnson syndrome	
VASCULAR DISORDERS			

Adverse Events with Possible Relationship to Ibrutinib (PCI-32765) (CTCAE 4.0 Term) [n= 2082]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
	Hypertension		
	Vascular disorders - Other (hemorrhage) ⁸	Hypotension	

¹This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

²Leukostasis and/or leukocytosis have been observed especially in patients with chronic lymphocytic leukemia (CLL) and mantle cell leukemia (MCL).

³Infection may include all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

⁴Fungal infections especially respiratory tract infections due to aspergillus and/or pneumocystis and central nervous system (CNS) infections due to aspergillus have been observed in clinical trials of ibrutinib. These reports may include incidents of presumptive fungal infections based on response to anti-fungal agents and/or radiographic evidence.

⁵Other malignant diseases have been observed in patients who have been treated with ibrutinib including solid tumors, skin cancer, and hematological malignancies.

⁶Angioedema may be seen in association with the immune-related adverse event of anaphylaxis.

⁷Rash may include but not limited to the terms dermatitis, erythema, rash generalized, rash maculopapular, rash pustular, rash pruritic, and urticaria.

⁸It is possible that treatment with ibrutinib may increase the risk of hemorrhage which may occur anywhere in the body including CNS hemorrhage (including but not limited to Intracranial hemorrhage, Intraventricular hemorrhage, and Subdural hematoma), Ecchymoses, Purpura (petechia), Gastrointestinal hemorrhage (including but not limited to Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage), Genitourinary tract hemorrhage (including but not limited to Hematuria and Vaginal hemorrhage), Respiratory tract hemorrhage (including but not limited to Epistaxis), and Spontaneous hemorrhage.

Adverse events reported on ibrutinib (PCI-32765) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ibrutinib (PCI-32765) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hemorrhagic diathesis); Blood and lymphatic system disorders - Other (lymphadenitis); Blood and lymphatic system disorders - Other (pancytopenia); Hemolysis

CARDIAC DISORDERS - Atrial flutter; Atrioventricular block complete; Atrioventricular block first degree; Cardiac disorders - Other (bundle branch block left); Cardiac disorders - Other (extrasystoles); Chest pain - cardiac; Heart failure; Myocardial infarction; Palpitations; Pericardial effusion; Pericarditis; Sinus

bradycardia; Supraventricular tachycardia

EAR AND LABYRINTH DISORDERS - Ear pain

EYE DISORDERS - Conjunctivitis; Dry eye; Eye disorders - Other (eye discharge); Eye disorders - Other (macular edema); Eye disorders - Other (ocular hyperemia); Eye disorders - Other (retinal hemorrhage); Eye disorders - Other (visual acuity reduced); Eye pain; Floaters; Glaucoma; Keratitis; Photophobia; Watering eyes

GASTROINTESTINAL DISORDERS - Abdominal distension; Cheilitis; Colitis; Dyspepsia; Enterocolitis; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (gluteal intramuscular bleed); Gastrointestinal disorders - Other (irritable bowel syndrome); Gastrointestinal disorders - Other (tongue discoloration); Oral dysesthesia; Oral pain; Pancreatitis; Periodontal disease; Small intestinal obstruction; Toothache

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (early satiety); General disorders and administration site conditions - Other (multiple organ dysfunction syndrome); General disorders and administration site conditions - Other (sensation of foreign body); General disorders and administration site conditions - Other (temperature intolerance); Infusion related reaction; Injection site reaction; Localized edema; Non-cardiac chest pain; Pain

HEPATOBIILIARY DISORDERS - Cholecystitis

IMMUNE SYSTEM DISORDERS - Immune system disorders - Other (systemic inflammatory response syndrome)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Injury, poisoning and procedural complications - Other (excoriation)

INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Electrocardiogram QT corrected interval prolonged; INR increased; Investigations - Other (cardiac murmur); Investigations - Other (increase CRP); Lymphocyte count decreased; Weight gain; Weight loss; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hyperkalemia; Hypoalbuminemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (cachexia); Metabolism and nutrition disorders - Other (fluid retention); Metabolism and nutrition disorders - Other (hyperphosphatemia); Metabolism and nutrition disorders - Other (hypoproteinemia); Metabolism and nutrition disorders - Other (lactose intolerance)

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Flank pain; Generalized muscle weakness; Joint effusion; Joint range of motion decreased; Musculoskeletal and connective tissue disorder - Other (groin pain); Musculoskeletal and connective tissue disorder - Other (muscle rigidity); Musculoskeletal and connective tissue disorder - Other (pain in jaw); Neck pain; Pain in extremity

NERVOUS SYSTEM DISORDERS - Depressed level of consciousness; Dysgeusia; Encephalopathy; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (mental impairment); Nervous system disorders - Other (parosmia); Nervous system disorders - Other (PML); Paresthesia; Reversible posterior leukoencephalopathy syndrome; Sinus pain; Somnolence; Stroke; Syncope

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Insomnia; Restlessness

RENAL AND URINARY DISORDERS - Cystitis noninfective; Renal and urinary disorders - Other (calculus bladder); Renal and urinary disorders - Other (polyuria); Urinary frequency; Urinary retention; Urine discoloration

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Dyspareunia; Reproductive system and breast disorders - Other (hematospermia); Vaginal dryness

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Hiccups; Laryngeal inflammation; Pleural effusion; Pneumonitis; Productive cough; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (alveolitis allergic); Respiratory, thoracic and mediastinal disorders - Other (nasal ulcer); Sinus disorder; Voice alteration

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Nail discoloration; Nail loss; Periorbital edema; Photosensitivity; Pruritus; Skin atrophy; Skin hyperpigmentation; Skin ulceration; Urticaria

VASCULAR DISORDERS - Flushing; Hot flashes; Thromboembolic event; Vascular disorders - Other (peripheral coldness)

Note: Ibrutinib (PCI-32765) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.2 Serious Adverse Event Reported in 9922

CYTOKINE RELEASE SYNDROME – Dehydration, hypotension potentially requiring pressors, fever without infection

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- **For expedited reporting purposes only:**
 - AEs for the agent that are ***bold and italicized*** in the CAEPR (i.e., those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
 - Other AEs for the protocol that do not require expedited reporting are outlined in Section
- **Attribution of the AE:**
 - Definite – The AE *is clearly related* to the study treatment.
 - Probable – The AE *is likely related* to the study treatment.
 - Possible – The AE *may be related* to the study treatment.
 - Unlikely – The AE *is doubtfully related* to the study treatment.
 - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (<https://eapps-ctep.nci.nih.gov/ctepaers>). The reporting procedures to be followed are presented in the “NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs” which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The Coordinating Center of the Corresponding Organization is responsible for submitting to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 “Disease progression”** in the system organ class (SOC) “General disorders and administration site conditions. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression; clinical deterioration associated with a disease process) should be submitted.

Note: Adverse events that occur from the date of signed informed consent through 30 days after administration of the last dose of study drug, whether or not related to study drug(s), that meets criteria for expedited reporting must be submitted to CTEP-AERS.

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies within 30 Days of the Last Administration of the Investigational Agent/Intervention^{1,2}

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

- 1) Death.
- 2) A life-threatening adverse event.
- 3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- 4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- 5) A congenital anomaly/birth defect.
- 6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.				
Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	
NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR				
Expedited AE reporting timelines are defined as: <ul style="list-style-type: none">“24-Hour; 5 Calendar Days” - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.“10 Calendar Days” - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.				
<p>¹Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:</p> <p>Expedited 24-hour notification followed by complete report within 5 calendar days for:</p> <ul style="list-style-type: none">All Grade 4, and Grade 5 AEs <p>Expedited 10 calendar day reports for:</p> <ul style="list-style-type: none">Grade 2 adverse events resulting in hospitalization or prolongation of hospitalizationGrade 3 adverse events <p>²For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote “1” above applies after this reporting period.</p> <p>Effective Date: May 5, 2011</p>				

7.3.4 Events of Special Interest

Specific adverse events, or groups of adverse events, will be followed as part of standard safety monitoring activities by the Sponsor. These events will be reported to the Sponsor **via CTEP-AERS** within 24 hours of awareness following the procedure described above for SAEs and will require enhanced data collection. **All Events of Special Interest will be submitted within 24 hours of awareness even if they do not meet serious criteria.**

- ***Major Hemorrhage***

Major hemorrhage is defined as any hemorrhagic event that is grade 3 or greater in severity, or that results in one of the following: intraocular bleeding causing loss of vision, the need for a transfusion of two or more units of red cells or an equivalent amount of whole blood, hospitalization, or prolongation of hospitalization. Events meeting the definition of major hemorrhage will be captured as an event of special interest as described above.

- ***Intracranial Hemorrhage***

Any intracranial hemorrhage adverse event, including subdural hematoma/hemorrhage, epidural hematoma/hemorrhage, and intracerebral hemorrhage, of any grade severity, will be captured as an event of special interest, as described above.

- **Pregnancy**

All initial reports of pregnancy must be reported to the Sponsor by the study site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g., spontaneous abortion, stillbirth, and congenital anomaly) are considered SAEs and must be reported in a timely fashion. Any subject who becomes pregnant during the study must discontinue further study treatment.

7.4 Routine Adverse Event Reporting

7.4.1 Definitions

Adverse Event

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

Suspected Adverse Reaction

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event.
- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

7.4.2 Reporting of Adverse Events

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). This includes all new malignant tumors, including solid tumors, skin malignancies and hematologic malignancies. Such malignancies are to be reported for the duration of study treatment and during any protocol-specified follow-up periods, including post-progression follow-up for overall survival. Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 CTEP-supplied Agent

8.1.1 Ibrutinib (NSC #748645)

Chemical Name: 1-[(3*R*)-3-[4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one

Other Names: Imbruvica, PCI-32765

Classification: Selective, irreversible, small molecule inhibitor of Bruton's tyrosine kinase (Btk).

CAS Registry Number: 936563-96-1

M.W.: 440.5 g/mole

Mode of Action: Ibrutinib binds covalently to a cysteine residue in the Btk active site, leading to potent and irreversible inhibition of Btk enzymatic activity of BCR. B-cell maturation is mediated by BCR signal transduction and Btk is an essential part of the signaling pathway.

Description: White to off-white crystalline solid

How Supplied: Ibrutinib is supplied by Pharmacyclics, Inc., and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI. Ibrutinib is supplied as hard gelatin capsules containing micronized ibrutinib and the following excipients: microcrystalline cellulose; croscarmellose sodium; sodium lauryl sulfate; may contain magnesium stearate. Capsules are manufactured as 140mg in a size 0, gray, hard gelatin capsule. Capsules are packaged in high-density polyethylene (HDPE) bottles with an induction seal and a child-resistant screw top cap. Each bottle contains 92 capsules. Ibrutinib capsules are to be dispensed in their original containers.

Storage: Ibrutinib Hard Gelatin Capsules should be stored at 15°C to 25°C (59°F to 77°F) with excursions permitted to 30°C (86°F).

Stability: Shelf life surveillance of the intact bottles is ongoing.

Route of Administration: Orally, with 8 ounces (approximately 240ml) of water. The capsules are to be swallowed intact. Ibrutinib must not be taken with grapefruit juice. Doses are to be taken at about the same time each day. If a dose is missed, it should be taken as soon as possible on the same day with a return to the normal schedule the following day. Patients should not take extra capsules to make up the missed dose.

Potential Interactions: Ibrutinib is primarily metabolized by CYP3A4. Any strong inhibitor or inducer of CYP3A4 (e.g., itraconazole, ketoconazole, clarithromycin, and rifampin) should be administered with caution and only after consultation with the Medical Monitor. Grapefruit and Seville orange juices should be avoided.

Availability

Ibrutinib is an investigational agent supplied by Pharmacyclics and distributed to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Ibrutinib is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (<https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jsp>). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (<https://eapps-ctep.nci.nih.gov/iam/>) and the maintenance of an ‘active’ account status and a ‘current’ password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Oral Drug Accountability Record Form (DARF). (See the NCI Investigator’s Handbook for Procedures for Drug Accountability and Storage.)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Integral Laboratory or Imaging Studies

N/A

9.2 Investigational Device Information

N/A

9.3 Integrated/Exploratory Correlative Studies

9.3.1 Archived/Fresh Tumor Tissue - Expression of the Integrated Biomarker ITK and/or Other Exploratory Putative Targets of Ibrutinib in Melanoma

We hypothesize that the overall response rate (ORR) and/or PFS after ibrutinib initiation will be associated with increased expression of ITK protein by melanoma cells. As shown in Table 2 (see Section 2.3.4), ibrutinib has several high-affinity targets in addition to ITK and BTK; several of these targets have been previously shown to play a role in melanoma biology (e.g. HER2, HER4, Csk, EGFR, YES, cFGR, ErbB4, Tec, Hck, BTK). Therefore, we cannot exclude

that the protein expression of other putative targets by melanoma cells could also be predictive of clinical benefit to ibrutinib, either independent from or in combination with ITK. We will prioritize evaluation of ITK, because it is the only integrated biomarker for this study. The other targets (e.g. HER2, HER4, Csk, EGFR, YES, cFGR, ErbB4, Tec, Hck, BTK) are exploratory markers and will be evaluated if adequate tissue is available for assessment of these proteins.

The protein expression levels of various putative targets of ibrutinib that also have a known biological role in melanoma will be assessed by IF and will be analyzed with Aperio imaging (Schlegel *et al.*, 2013; Nikolaishvili-Feinberg *et al.*, 2014; Carson *et al.*, 2015). More specifically, up to fifteen 5-micron tissue section will be prepared on positively charged slides from corresponding archived or fresh FFPE tumor blocks. Given that abundance of tumor tissue may be variable for such studies the following stains will be performed with a decreasing order of importance:

- standard hematoxylin and eosin stain to assess percent of viable tumor, tumor/stroma ratio, and immunoscore analysis (quality control). Total of one slide.
- 2-color IF using antibodies against ITK and each of the ‘primary’ ibrutinib targets (ITK, EGFR, ERBB4, YES, cFGR). This method will allow differentiation between their expression in melanoma cells (S100+; third stain) versus stromal cells (S100-). Total of five slides with the following decreasing order of importance (ITK>EGFR>ERBB4>YES>cFGR).
- 2-color IF using antibodies against the ‘secondary’ ibrutinib targets (TXK, BMX, TEC, BTK, and HCK). Total of five slides with the following decreasing order of importance (BTK>HCK>TEC>BMX>TXK).

Image analysis involves definition of electronic gates for tumor using an electronic pen tool. Then the S100-positive regions of interest (ROI) will be defined by Definiens Tissue Studio technology (Definiens Architect v.2.5.0 Build 44725 x64, Tissue Studio Library v4.2). Using the "Cell membrane nuclei" composer algorithm of the Definiens Architect XD we will calculate ITK expression in S100+ regions of interest (ROI; melanoma cells). The significance of protein expression measured by H-score (continuous variable) as a predictor of antitumor response and PFS will be explored.

9.3.1.1 Archived Tumor Tissue - Collection of Specimen(s)

Blocks/slides from pre-treatment, FFPE archived tumor tissue from diagnosis of metastatic disease will be collected. Based on our published data, ITK expression is significantly higher in metastatic melanomas, irrespective of stage (regional stage III, or distant metastatic stage IV) compared to primary; therefore, eligible archived tumor blocks can also be from positive sentinel lymph node mapping procedures or regional lymphadenectomies. Consent to allow access to archival tissue is required for enrollment. Once consent is signed, research personnel should begin the process of accessing archival tissue and determining if tissue has sufficient tumor to support tumor imaging studies.

Confirmation of sufficient tumor should be obtained from the participating institution pathologist. However, in situations where obtaining confirmation from the participating institution pathologist may cause significant delay in starting the patient on study treatment, the Lead Principal Investigator may be able to review pathology information to determine eligibility. Below are details for each process.

Pathologist from Participating Site

A pathologist from each participating academic institution (or local pathologist if applicable) may review tumor blocks to ensure sufficient tumor is available for support of correlative studies for this protocol. An institution pathologist is required to sign-off (see [Appendix B](#) for sample letter) regarding the:

- a) availability of metastatic FFPE blocks,
- b) quality of tumor tissue based on review of H&E stained section; tumor tissues with at least 70% viable tumor are suitable for analysis, and
- c) sufficient amount of tumor tissue for tumor imaging analysis (IF).

If metastatic tumor samples are available and of sufficient quantity and quality, metastatic tumors will be used for analysis because we have previously shown that expression of ITK is higher in metastatic melanoma as opposed to primary melanoma (Carson *et al.*, 2015).

The signed pathologist letter should be uploaded with the rest of the eligibility documents to the 9922 Study Portal by accessing: <http://j.mp/2eup4vF>. Additional information about the study portal use is available in LPO Documents for the study on www.ctsu.org.

Lead Principal Investigator

The processes involved for a participating institution pathologist to sign off on archival tumor tissues may be time-consuming and could delay enrollment. Therefore, to minimize delay, in lieu of the institutional pathologist, the Lead Principal Investigator may confirm eligibility of subject's archival tissue based on information provided from participating sites. Confirmation by the Lead Principal Investigator requires the participating site to submit the following materials (**de-identified and labeled with subject screening ID number and initials**) to the Lead Protocol Organization:

- pathology report(s) and stained slides (e.g., hematoxylin-eosin, and/or S100, melan-A/MART-1, tyrosinase, or MITF) shipped directly to address provided below.

-OR-

- pathology report(s) and corresponding digital images emailed directly to Study Coordinators as instructed below.

Study Coordinators will facilitate the pathology review by the Lead Principal Investigator. Requests for the Lead Principal Investigator's review of pathology for determination of

subject eligibility must be emailed with a copy of the subject's pathology report (**de-identified and labeled with subject screening ID number and initials**) attached to the email. The information should be provided to the following:

Kimberly_Keller@med.unc.edu

The stained slides with associated pathology reports should be sent via priority courier delivery (eg. FedEx) to the Lead Principal Investigator's address as follows:

Dr. Stergios Moschos, 9922 Lead PI
University of North Carolina at Chapel Hill
Physician's Office Building, Suite 3116-CB#7305
Chapel Hill, NC 27599
Telephone: 919-843-7713

The Lead Principal Investigator will review subject pathology materials and if confident there is sufficient archival tissue available for study enrollment, confirmation will be provided to the participating site by the Study Coordinator. Refer to [Appendix B](#) for documentation that will be provided by the Lead Principal Investigator in lieu of the site pathologist in this case. If determination of sufficient archival tissue cannot be made by the Lead Principal Investigator based on the information provided, this will be noted. In this case, responsibility for confirming that adequate tissue is available is transferred back to the site pathologist for confirmation of eligibility.

9.3.1.2 Fresh Biopsy of Metastatic Tissue – Collection of Specimens

If patients do not have available archival tumor tissue from metastatic melanoma OR the pathologist or Lead Principal Investigator cannot assure that "sufficient" amount of good quality is available from an existing archived tumor tissue from metastatic melanoma, then patients must consent to undergo a tumor biopsy from a metastatic site. If palpable tumors from a metastatic site are available, then excisional or core biopsies can be performed. If, however, palpable tumors are not available then a core biopsy procedure using an 18-gauge needle will be performed by interventional radiology. Only intra-abdominal lesions will be subjected to core biopsy because intra-thoracic procedures have a significantly higher frequency of complications. In other words, if a patient does not have sufficient metastatic tissue available from archival tumor tissue *and* has lung lesions as the only site of metastatic disease, then the patient is not eligible for this trial. The number of cores obtained will be affected by the patient's clinical condition at the time of biopsy and is determined by the health care professional, who is performing the procedure. Therefore, it is possible that more than one core (up to 5) may be procured for this research project but ultimately, the amount of tissue available for correlative studies can be variable. A rapid on-site evaluation (cytology with quick hematoxylin and eosin stains) should be available in each institution to evaluate the quality of tumor sample (i.e. percent of tumor cells and amount of necrosis).

Fresh biopsy cores will be subject to:

- Immediate and overnight fixation in 10% buffered formalin for paraffin-embedding, usually within 20-24 hours after fixation. For biopsies performed on Friday, fixation time may extend to 48 hours (FFPE samples).
- The FFPE samples must also provide a histological confirmation for the presence and cellularity of melanoma cells as well as amount of necrosis.

Quality Control (QC): All tissue specimens collected will be reviewed by a board-certified pathologist. QC activities for specimens collected include a) histology/cytology examination of the tissues and cells; b) tissue quality assessment of fresh specimens to prepare histology specimens such as whole sections for 2-color IF. All histology stained samples will be scanned and digital images will be available for review.

Histology: H&E-stained sections from core needle biopsies will be used to confirm the presence of tumor cells, as well as their abundance (tumor cellularity), stromal components and lymphocytic infiltrates. H&E-stained sections from all FFPE diagnostic slides will be scanned into Aperio™ digital pathology scanner analysis for pathological evaluation and selection for biomarker analysis.

Distribution: the percent of the section that includes a mononuclear infiltrate. Takes into account whether infiltrate is focal, multifocal or diffuse. 0, 1+, 2+, 3+ if 0, 1-25%, 26-50%, and $\geq 50\%$ of the tumor section, respectively.

Density: the average density of the mononuclear infiltrate in areas where a lymphoid infiltrate is present. 0, 1+, 2+, 3+ if absent, mild, moderate, severe, respectively.

Note: Patients with insufficient (archival or fresh) tissue will NOT be eligible for this study. CTEP will provide remuneration for the fresh biopsy collection.

Tumor Block Preparation:

- Labeled each block with:
 - Study Number NCI9922
 - Subject ID Number
 - Date of Specimen
 - Block Accession Number
- Complete one NCI9922 Archival Tissue Block/Slides Submission Form (see [Appendix E](#) for submission form) per patient.
- Submit the corresponding pathology report(s). Remove name, MRN or any other PHI from the pathology report. Write the study number NCI9922, subject's initials and subject's ID number on the pathology report.

Tumor Slide Preparation:

- Use positively charged microscope slides, such as Superfrost Plus Fisher Catalog #22-034-979 or #12-550-15.
- Cut a minimum of 11 unstained slides to a maximum of 15 unstained slides.
- Section thickness should be 5 microns.

- Stain one additional slide for H&E; the 11-15 slides are unstained.
- Slide drying should be performed at 37°C overnight.
- Storage of cut slides at room temperature should ideally not exceed more than 7 days.
- Longer-term storage should be at 4°C, with slide boxes carefully parafilmed to prevent moisture entering the slide box or alternatively, tissue can be stored in a dessicator or a nitrogen chamber.
- Mark each slide with:
 - Study Number NCI9922
 - Subject ID Number
 - Date of Specimen
 - Block Accession Number
 - Serial Number of Slide (1, 2, 3...)
- Complete one NCI9922 Archival Tissue Block/Slides Submission Form (see [Appendix E](#) for submission form) per patient.
- Submit the corresponding pathology report(s). Remove name, MRN or any other PHI from the pathology report. Write the study number NCI9922, subject's initials and subject's ID number on the pathology report.

9.3.1.3 Archived/Fresh Tumor Tissue - Handling of Specimens(s)

Each participating institution will collect archived or fresh specimens and ship them to the UNC Tissue Procurement Facility within 8 weeks after the start of study treatment for each subject. FFPE blocks (or representative tissue sections from FFPE blocks) will be handled following standard operating procedures for biohazardous materials (i.e., tumor blocks placed on individual biohazardous bags whereas glass slides placed of slide holders).

9.3.1.4 Archived/Fresh Tumor Tissue - Shipping of Specimen(s)

Specimens collected for correlative analysis of FFPE blocks or representative 5-micron tumor tissue sections (i.e., slides) must be shipped within 8 weeks after each patient starts study treatment to the UNC-CH Tissue Procurement Facility (TPF) for analysis in the end of the trial. This means that upon study enrollment of each patient, shipping of tumor tissue is NOT required; only the sign-off letter by the institutional pathologist (or Lead Principal Investigator) is sufficient to enroll the patients.

Archival tissue specimens along with corresponding de-identified pathology reports and completed NCI9922 Archival Tissue Block/Slides Submission Form must be shipped within 8 weeks after each subject starts study treatment by courier (Monday-Thursday) guaranteeing overnight delivery to the following address:

Mei Huang, Lab Manager
c/o Tissue Procurement Facility
108 MacNider Building, CB 7304
University of North Carolina
333 South Columbia St

Chapel Hill, NC 27599
Phone: 919-966-2620
Unc_tpf@med.unc.edu

Note: If shipping in hot weather, include a refrigerated gel pack with the slides. Do NOT ship on dry ice or wrap the slides in gauze.

Please notify the UNC Tissue Procurement Facility (unc_tpf@med.unc.edu) of incoming shipment by providing notification on the day of shipment via the 9922 Study Portal. The shipment notification will include upload of the associated Archival Tissue Block/Slides Submission Form (see [Appendix E](#)). Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

9.3.1.5 Site(s) Performing Correlative Study

Tumor imaging studies (2-color IF) will be performed at the UNC-CH Translational Pathology Laboratory (<https://tpl.med.unc.edu>).

9.4 Exploratory/Ancillary Correlative Studies

See Sections 2.3 and 2.4 for background information on correlative studies.

9.4.1 Peripheral Blood Mononuclear Cell (PBMC) - Effect of Ibrutinib on Immune Regulatory Cell Populations

We hypothesize that ibrutinib does not have any inhibitory effect upon host immune response, but may have beneficial effects by reversing melanoma-mediated Th2-bias, which does not lead to effective antitumor response (Tatsumi *et al.*, 2002).

PBMC from three different time points (pre-study, pre-dose on Day 1 of Cycle 2, and at disease progression) will be stained for various immune regulatory cell populations, as well as markers for Th1 and Th2 responses to investigate the effect of ibrutinib on host immune response, in particular the reversal of Th2-to-Th1 bias, as previously described (Dubovsky *et al.*, 2013). Specifically, the following immune cell subsets will be assayed using multi-parameter flow cytometry:

ITK expressing T cell subsets

T-helper:	CD3 ⁺ CD8 ⁻ CD4 ⁺ ITK [±]
T-cytotoxic:	CD3 ⁺ CD4 ⁻ CD8 ⁺ ITK [±]
Invariant natural killer T cells (iNKT):	Vα24 ⁺ Vβ ⁺ CD3 ⁺ ITK [±]
Gamma delta γδT cells:	CD3 ^{bright} gdTCR ⁺ CD16 ⁺ CD57 ⁺

T-helper cell subtypes

Pro-inflammatory (Th1):	CD3 ⁺ CD4 ⁺ IFNγ ⁺ (or T-bet ⁺)
Anti-inflammatory (Th2):	CD3 ⁺ CD4 ⁺ IFNγ ⁺ (or GATA3 ⁺)
Th9:	CD3 ⁺ CD4 ⁺ IL9 ⁺

Th17:

CD3⁺CD4⁺IL17A⁺

Immunoregulatory populations

Naturally occurring T regulatory cells:	CD4 ⁺ CD25 ^{high} FoxP3 ⁺
Type I regulatory T cells:	CD4 ⁺ CD25 ⁺ (IL10 or TGFβ) ⁺ ,
Myeloid-derived suppressor cells, monocytic type:	HLA-DR ^{low} CD14 ⁺ ;
Myeloid-derived suppressor cells, other,	lin1 [−] HLA-DR [−] CD33 ⁺ CD11b ⁺
Myeloid-derived suppressor cells, lymphoid type:	lin1 [−] /HLA-DR [−] /CD33 ⁺ /CD11b ⁺

Within-patient changes in the expression of particular immune cell subsets from pre-study (i.e., baseline) to pre-dose on Day 1 of Cycle 2 (i.e., Day 29) and at disease progression will be assessed and used to develop a profile of immune response in peripheral blood that distinguishes patients who respond to treatment versus those who do not.

A total of 50 mL of blood will be collected for flow cytometric analysis for each time point. This amount of blood is justified by the fact that: (a) patients with metastatic melanoma are usually lymphopenic, (b) certain immune cell subsets are rare (<1% of immune cell subsets) events [e.g. myeloid-derived suppressor cells, invariant natural killer T cells (iNKT), γδT cells], and therefore sufficient peripheral blood is required to detect those rare populations.

9.4.1.1 PBMC - Collection of Specimen(s)

Collection and Processing:

- Collect peripheral blood in five 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog no. 366480) at the following time points (**on Monday, Tuesday, and Wednesday only**):
 - a) pre-study (i.e., baseline);
 - b) pre-dose on Day 1 of Cycle 2 (i.e., Day 29); and
 - c) disease progression.
- Label each sample with:
 - Study Number NCI9922
 - Sample Type PBMC
 - Subject ID Number
 - Collection Date
 - Collection Time

9.4.1.2 PBMC - Handling of Specimens(s)

Plasma extractions and Ficoll-enriched PBMC isolation will be performed per UNC institutional standard procedures in the UNC Biospecimen Facility (BSP) on the same day samples arrive. Whole blood samples will be separated into plasma and PBMCs as described below. Blood tubes will be spun at 400 x g for 10 minutes, after which plasma will be removed and aliquoted as follows. Two mls of the plasma will be used to prepare twenty 100ul aliquots. The remainder of the plasma will be aliquoted into 1ml aliquots up to a total of 18. Plasma aliquots will be stored at -80°C. After plasma extraction the rest of the whole blood will be diluted with calcium and magnesium free PBS and PBMCs

will be isolated by centrifugation over Ficoll-Paque Plus. After isolation PBMCs will be counted and cryopreserved in as many 5×10^6 aliquots as possible. PBMCs will be stored at -80°C overnight before transfer to long-term storage into vapor-phase LN2 storage.

9.4.1.3 PBMC - Shipping of Specimen(s)

Whole green-top blood tubes will be shipped **on Monday, Tuesday, and Wednesday only** at ambient temperature via priority overnight delivery to the following address:

ATTN: Patricia Basta, PhD
Biospecimen Processing Facility
135 Dauer Dr.
Michael Hooker Research Center Room 3213
University of North Carolina – Chapel Hill, Chapel Hill 27599
Phone: 919-966-7738

Please notify the UNC Biospecimen Facility (patricia_basta@unc.edu) of any incoming shipment by providing notification two days prior to shipment via the 9922 Study Portal. The shipment notification will include upload of the associated PBMC Shipment Log (see [Appendix G](#)). Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

9.4.1.4 PBMC - Site(s) Performing Correlative Studies

Analysis will be performed at the UNC-CH Flow Cytometry Core Laboratory.

9.4.2 Plasma - Protein Markers Associated with Sensitivity or Resistance to Ibrutinib

Multiplex ELISA assays have been developed to analyze over 25 angiogenic, inflammatory and immune-related markers in less than 0.5 ml of plasma. Coefficients of variation for most analytes are $<10\%$. This platform has been successfully applied to several in-house phase I and II studies, as well as several phase III, Alliance-conducted studies with bevacizumab in colorectal and other cancers at Duke University. Markers of inflammation will be analyzed in the Duke Phase I Biomarker Lab, which serves as a core lab for these analyses for the US Cooperative Group, the Alliance.

Analyses will be performed on pre-treatment and on-treatment plasma samples and on samples at disease progression. Analyte levels, and changes in analyte levels, will be correlated with clinical outcome (ORR, PFS, and OS). Plasma will also be evaluated for protein markers that may be associated with sensitivity or resistance to ibrutinib. These may include CRP and other markers of inflammation, including but not limited to $\text{IFN}\gamma$, $\text{IL1}\beta$, IL6, sILR6R, sGP130, IL4, IL7, IL10, IL12, IL17A, IL17E, and IL23. Additional markers of proteins regulated by the PD-1/PD-L1 interaction and the IL6/JAK-STAT axis will also be assessed, including but not limited to VEGF, HER and TGF β family members.

9.4.2.1 Plasma - Collection of Specimen(s)

Collection and Processing:

- Draw one 10ml lavender top (K₂EDTA) tube (BD Vacutainer, Catalog no. 366643) at the following time points:
 - a) pre-study (i.e., baseline);
 - b) pre-dose on Day 1 of Cycle 2 (i.e., Day 29);
 - c) pre-dose Day 1 on each odd numbered cycle (i.e., at each restaging at Cycles 3, 5, etc.)
 - d) disease progression.
- Invert tubes 10 times to mix blood.
- Centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions).
- Remove plasma from the tube and transfer equally into two separate clean 15ml polypropylene tubes (or institutional equivalent).
- Repeat centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions).
- Aliquot approximately 1.0 ml of plasma per cryovial (i.e., 1 ml of plasma per cryovial into four 2.0 ml cryovials).
- Snap freeze (put into liquid nitrogen) or place into -80°C freezer as soon as possible after collection
- Label each cryovial with:
 - Study Number NCI9922
 - Sample Type Plasma
 - Subject ID Number
 - Collection Date
 - Collection Time
 - Frozen Time

9.4.2.2 Plasma - Handling of Specimens(s)

Plasma samples will be stored at -80°C until further use.

9.4.2.3 Plasma - Shipping of Specimen(s)

In the end of trial accrual, all protein marker (plasma) and pharmacogenomics (whole blood) specimens will be batched (approximately 2 batches per institution) and shipped on dry ice by courier guaranteeing overnight delivery (Monday-Thursday) to the following address:

Phase I Biomarker Laboratory
ATTN: Andrew Nixon, PhD
Duke University Medical Center
395 MSRB, Research Drive
Durham, NC 27710

Phone: 919- 681-2239

Please notify the Phase I Biomarker Laboratory (jchris.brady@duke.edu) of any incoming shipment by providing notification on the day of shipment via the 9922 Study Portal. The shipment notification will include upload of the associated Biomarker Shipment Log (see [Appendix H](#)). Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

Directions for how to prepare and ship samples on dry ice are available at:
http://www.iata.org/whatwedo/cargo/dangerous_goods/Documents/DGR52_PI650_EN.pdf

9.4.2.4 Plasma - Site(s) Performing Correlative Studies

Protein marker analysis will be performed at the Duke Phase I Biomarker Laboratory.

9.4.3 **Whole Blood - Pharmacogenomics Assessment**

A one-time, blood sample will be drawn prior to initiation of therapy for assessment of variants in genes anticipated to be involved in the pharmacokinetics or pharmacodynamics of ibrutinib.

9.4.3.1 Whole Blood - Collection of Specimen(s)

Collection and Processing:

- Draw one 6ml pink top (K2EDTA) tube (BD Vacutainer, Catalog no. 367899) or a purple-top (K2EDTA) tube (BD Vacutainer, Catalog no. 367863) at the following time point:
 - a) prestudy (i.e., baseline) only.
- Invert tube 10 times to mix blood.
- Freeze tube in an upright position as soon as possible at -20°C for 24 hours, and then transfer to -80°C for long term storage
- Label the tube with:
 - Study Number NCI9922
 - Sample Type Whole Blood
 - Subject ID Number
 - Collection Date
 - Collection Time
 - Frozen Time

9.4.3.2 Whole Blood - Handling of Specimens(s)

Whole blood samples will be stored at -80°C until further use.

9.4.3.3 Whole Blood - Shipping of Specimen(s)

In the end of trial accrual, all protein marker (plasma) and pharmacogenomics (whole

blood) specimens will be batched (approximately 2 batches per institution) and shipped on dry ice by courier guaranteeing overnight delivery (Monday-Thursday) to the following address:

Phase I Biomarker Laboratory
ATTN: Andrew Nixon, PhD
Duke University Medical Center
395 MSRB, Research Drive
Durham, NC 27710
Phone: 919-681-2239

Please notify the Phase I Biomarker Laboratory (jchris.brady@duke.edu) of any incoming shipment by providing notification on the day of shipment via the 9922 Study Portal. The shipment notification will include upload of the associated Biomarker Shipment Log (see [Appendix H](#)). Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

Directions for how to prepare and ship samples on dry ice are available at:
http://www.iata.org/whatwedo/cargo/dangerous_goods/Documents/DGR52_PI650_EN.pdf

9.4.3.4 Whole Blood - Site(s) Performing Correlative Studies

Pharmacogenomic analysis will be performed at the Duke Phase I Biomarker Laboratory.

9.5 Special Studies

9.5.1 Pharmacokinetic (PK) Study

Plasma concentrations of ibrutinib and metabolite PCI-45227 after repeated dosing will be determined using a validated analytical method. Refer to [Section 10](#) of Study Calendar for PK collection time points (see footnote #17).

9.5.1.1 PK – Collection of Specimen(s)

Collection and Processing:

1. Draw one 2ml **GREEN TOP PLASTIC SODIUM HEPARIN TUBE** (Greiner Bio-One via Fisher Scientific, Catalog no. 454302) for each PK collection at the following time points:
 - pre-dose on Day 1 of Cycle 1
 - pre-dose on Day 8 of Cycle 1
 - post-dose on Day 8 of Cycle 1 at
 - 0.5 hour
 - 1 hour
 - 2 hours
 - 4 hours

- 6 hours
 - 24 hours
2. Allow tube to fill COMPLETELY, as far as the vacuum will allow.
 3. Mix the tube immediately upon completion to avoid clotting by inverting gently 5 times. DO NOT SHAKE.

NOTE: Sample should be processed and stored within 1 hour of collection.

4. Place the tube on melting ice until centrifugation.
5. Place the tube in a refrigerated centrifuge (0-4°C).

NOTE: Use a refrigerated centrifuge bucket in cases where a refrigerated centrifuge is not available. Maintain cold temperature during the plasma preparation process.

6. Centrifuge the tube within 60 minutes of collection at 4°C for 15 minutes at 2500 rpm.
7. Transfer plasma with pipette equally into two 2mL cryovials (approximately 0.5 mL of plasma in each tube).
8. Label each cryovial with:
 - Study Number NCI9922
 - Sample Type PK
 - Subject ID Number
 - Collection Date
 - Collection Time
 - Frozen Time

9.5.1.2 PK – Handling of Specimen(s)

Store plasma samples in a freezer at -70°C or below, within approximately 60 minutes of blood collection.

NOTE: Every effort should be made to collect the full 2mL blood sample at each time point. In the event that less than 1mL of blood is collected, the sample will be processed as described above except that the plasma will not be divided into two cryovials. All deviations will be recorded on the PK Shipment Log provided in [Appendix I](#). This single plasma sample should be frozen, stored and shipped with the primary set of samples.

9.5.1.3 PK – Shipping of Specimen(s)

Each subject's primary samples from Cycle 1 Day 1 and Cycle 1 Day 8 should be shipped together. Make sure to separate primary and back-up samples. Do NOT ship back-up aliquots of plasma in the same shipment as the primary samples from the same subject. **Back-up samples should be held until authorized to ship.**

On a quarterly basis, PK specimens will be batch shipped **on Monday, Tuesday, and Wednesday only FROZEN on dry ice** via priority overnight delivery to the following address:

Mei Huang, Lab Manager
c/o Tissue Procurement Facility
108 MacNider Building, CB 7304
University of North Carolina
333 South Columbia St
Chapel Hill, NC 27599
Phone: 919-966-2620 / Fax: 919-843-9501

Contact delivery (ex. FedEx) customer service to determine the latest pickup time for your site and the scheduling deadline. Allow ample time for delivery. Shipments cannot be received on Friday afternoons, weekends, days preceding holidays, or holidays. Any questions regarding holiday schedules may be addressed to contact person below.

Mei Huang, Lab Manager
Phone: 919-966-2620
Email: mei_huang@med.unc.edu

For each shipment include the completed *NCI 9922 Pharmacokinetics Sample Submission Form* (see [Appendix I](#)) on the outside of the Styrofoam box for each patient's samples contained in the shipment. If shipping samples for different subjects in the same shipping container, make sure they are labeled properly and placed in separate bags or boxes.

Please notify the UNC Tissue Procurement Facility (unc_tpf@med.unc.edu) of any incoming shipment by providing notification on the day of shipment via the 9922 Study Portal. The shipment notification will include the upload of the associated Pharmacokinetics Sample Submission Form (see [Appendix I](#)). Additional information about study portal use is available in LPO Documents for the study on www.ctsu.org.

Directions for how to prepare and ship samples on dry ice are available at: http://www.iata.org/whatwedo/cargo/dangerous_goods/Documents/DGR52_PI650_EN.pdf

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 3 weeks prior to patient OPEN registration. (Patient must start protocol therapy within 7 days after OPEN registration.) Scans must be done ≤ 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study ¹	Cycle 1 ^{1,2} D1	Cycle 1 D8	Cycle 1 D9	Cycle 1 D15	Cycles 2,4,etc. ² D1	Cycle 2 D15	Cycles 3, 5, etc. ^{2,3} D1	Treatment Discontinuation ⁴	Safety Follow-up ⁵	Long-term Follow-up ⁶
Ibrutinib		Begin									
Informed Consent	X										
Demographics	X										
Medical History ⁷	X	X				X		X	X	X	
Physical Exam ⁸	X	X ⁸			X	X	X	X	X	X	
Performance Status (Appendix A)	X	X			X	X	X	X	X	X	
Concomitant Medications	X	Review				Review			Review	Review	
Serum β -HCG ⁹	X ⁹										
CBC w/diff	X	X	X ¹⁹		X	X	X	X	X		
Serum Chemistries ¹⁰	X	X			X	X	X	X	X		
LFTs ¹¹	X	X			X	X	X	X	X		
Radiographic Evaluations with Tumor Measurements	X ¹²							X ¹²	X ¹²		
Patient Diary (Appendix C)	X	Review				Review			Review		
Toxicity Monitoring		X			X	X	X	X	X	X	X ⁶
Request Archive Tissue -OR- Obtain Fresh Tissue (correlative)	X ¹³										
PBMC (correlative)	X ^{14, 17}					X ¹⁴			X ¹⁴		
Plasma (correlative)	X ^{15, 17}					X ¹⁵ Cycle 2		X ¹⁵	X ¹⁵		
Whole Blood (correlative)	X ^{16, 17}										
PK (correlative)		X ¹⁸	X ¹⁸	X ¹⁸							
Survival											X

Key to Footnotes

¹ Pre-study screening evaluations other than imaging must be done within 21 days prior to patient OPEN registration. (Patient must start protocol therapy within 7 days after OPEN registration.) Imaging studies must be conducted within 28 days prior to Day 1 of ibrutinib. If pre-study screening hematology and serum chemistries were performed within 7 days of Day 1 of Cycle 1 of treatment, these do not need to be repeated.

² A window of 2 days will be applied to all study visits including treatment; for every other cycle tumor imaging, imaging may take place within 7 days prior to the study visit.

³ Tumor imaging will continue every other cycle on odd-numbered cycles until progression.

⁴ This visit should occur in patients when treatment stops, for whatever reason (toxicity, progression, or at discretion of the investigator).

⁵ Subjects who discontinue therapy will be followed for safety for 28 days (± 3 days) following the last administration of ibrutinib.

⁶ Patients who have ongoing Grade 4 AE or SAE at the time of discontinuation from treatment, and those who come off treatment prematurely for safety reasons will continue to be followed at least every 30 days until the event is resolved or deemed irreversible by the investigator. After ibrutinib treatment ends, anti-cancer medications taken by the patient should be documented every 3 months (± 1 month) in the eCRF if this information is available for up to 2 years or until death, whichever comes first.

⁷ Complete medical history at baseline including documentation of BRAFV600 mutation status, and documentation of resolution of any toxicities from prior treatment to baseline or \leq Grade 1 (other than alopecia); thereafter focused history on symptoms/toxicity.

⁸ Physical exam to include height (baseline only), vital signs and weight; repeat physical exam on Day 1 of Cycle 1 is at discretion of investigator.

⁹ Serum β -HCG (pregnancy test) will be obtained within 7 days prior to Day 1 of ibrutinib in women of child-bearing potential.

¹⁰ Serum chemistries will be obtained to include Na, K, Cl, Mg, creatinine, BUN, glucose, phosphate, calcium.

¹¹ Liver function tests (LFTs) to include AST, ALT, total bilirubin, alkaline phosphatase.

¹² Tumor response assessment will be performed by the investigator via CT scans of neck (if applicable), chest, abdomen, pelvis; CT scans will be done at treatment discontinuation irrespective of whether clinical progression is suspected by the investigator. If tumor assessments are available for patients who have not yet experienced progressive disease (PD) at the time

treatment is discontinued, the follow-up tumor evaluations will be documented in the electronic case report form (eCRF) until PD or death is confirmed, or until another treatment is initiated.

¹³Request access to archival tissue (FFPE blocks/slides) to support biomarker studies; consent for the use of any residual material from diagnostic biopsy (archival tissue) will be required for enrollment. [NOTE: Investigator must review pathology report to ensure sufficient tumor should be available for support of correlative studies; patients with insufficient tissue will NOT be eligible for this study]. If sufficient archival tissue is unavailable, a fresh biopsy must be obtained and the same rules apply for the fresh biopsy sample i.e., sufficient metastatic tumor must be available to support correlative studies.

¹⁴Blood for isolation of PBMCs (50 mL total; 10 mL each in 5 tubes) will be collected into 5 green-top (sodium heparin) tubes at pre-study (i.e., baseline), pre-dose on Day 1 of Cycle 2, and at disease progression.

¹⁵Blood for plasma (10 mL) will be collected into a lavender-top (K₂EDTA) tube at pre-study (i.e., baseline), pre-dose on Day 1 of Cycle 2 (i.e., Day 29) at each odd numbered cycle (i.e., at each restaging predose on D1 at Cycles 3, 5, etc.), and at disease progression.

¹⁶Whole blood (6 mL) will be collected into a pink-top or purple-top (K₂EDTA) tube at pre-study (i.e., baseline) only.

¹⁷Pre-study (i.e., baseline) blood for PBMC, plasma, and whole blood may be obtained pre-dose on Cycle 1 Day 1.

¹⁸Blood (2 mL per sample time point) for plasma isolation will be collected into 2mL plastic green-top (sodium heparin) tubes for PK studies. A single sample should be collected on Day 1 of Cycle 1 before the ibrutinib dose is given. Serial PK samples will be collected on Day 8 of Cycle 1 at pre-dose and at 0.5, 1, 2, 4, 6 and 24 hours after ibrutinib administration as outlined in table below.

Cycle	Day	Predose ^b (trough value)	Time after dosing ^a					
			0.5 h ± 5 min	1 h ± 15 min	2 h ± 15 min	4 h ± 30 min	6 h ± 1 h	24 h ± 2 h
1	8	x	x	x	x	x	x	x ^b

a. Record actual time of sample collection

b. Sample collected 24 hours (± 2 hours) after last intake and prior to dosing on that PK day (ie, Cycle 1 Day 8 and Day 9).

¹⁹A CBC with differential test should be performed on Day 8 of Cycle 1 to monitor for decreases in platelet count that may occur during ibrutinib therapy. Typically, thrombocytopenia associated with ibrutinib is asymptomatic and resolves without the need for dose adjustment. However, dose reduction should be considered if platelet counts do not recover and symptomology related to thrombocytopenia develops (e.g., petechiae, frequent nose bleeds, etc.). It is prudent to

monitor blood counts more closely during the first cycle because the 840 mg dose of ibrutinib under study is higher than the approved dose in the label. There is limited safety information available in subjects who have received this higher dose of ibrutinib and data that are available reside in patients with hematologic malignancies.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks (2 cycles). In addition to a baseline scan, confirmatory scans should also be obtained 8 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)(Eisenhauer *et al.*, 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with ibrutinib.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (NOTE: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable, but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq 20\text{mm}$ ($\geq 2\text{cm}$) by chest x-ray or as $\geq 10\text{mm}$ ($\geq 1\text{cm}$) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

NOTE: Tumor lesions that are situated in a previously irradiated area must not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be $\geq 15\text{mm}$ ($\geq 1.5\text{cm}$) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5mm [0.5cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter $< 10\text{mm}$ [$< 1\text{cm}$] or pathological lymph nodes with ≥ 10 to $< 15\text{mm}$ [≥ 1 to $< 1.5\text{cm}$] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but, in addition, should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or, in rare cases, unequivocal progression of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should 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 should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable if they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and $\geq 10\text{mm}$ ($\geq 1\text{cm}$) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm (0.5cm) or less. If CT scans have slice thickness greater than 5 mm (0.5cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data that may

bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator-dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot 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. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published (Bubley *et al.*, 1999; Rustin *et al.*, 2004; Scher *et al.*, 2008). In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria, which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer (Vergote *et al.*, 2000).

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing

site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

- c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR, due to limitations of FDG-PET and biopsy resolution/sensitivity.

[NOTE: A 'positive' FDG-PET scan lesion means one which is FDG-avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.]

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm (0.5cm). [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 diameters while on study.

11.1.4.2 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 [<1cm] short axis).

[NOTE: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.]

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): Appearance of one or more new lesions and/or *unequivocal*

progression of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 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.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥ 4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
<p>* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.</p> <p>** Only for non-randomized trials with response as primary endpoint.</p> <p>*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.</p> <p>NOTE: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “<i>symptomatic deterioration</i>.” Every effort should be made to document the objective progression even after discontinuation of treatment.</p>				

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.5 Duration of Response

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 progressive 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, including the baseline measurements.

11.1.6 Progression-Free Survival (PFS)

PFS is defined as the duration of time from Day 1 of treatment to time of progression (based on clinical or radiographic grounds) or death as a result of any cause, whichever occurs first.

11.1.7 Overall Survival (OS)

OS is defined as the duration of time from Day 1 of treatment to death as a result of any cause.

11.1.8 Response Review

N/A

11.2 Other Response Parameters

N/A

12. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in [Section 7](#) (Adverse Events: List and Reporting Requirements).

12.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, routine SAEs; reporting of expedited AEs; and accumulation of reported AEs from other trials testing the same drug(s). The Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

During the Phase 2 portion of the study, the Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance. Decisions to proceed to the second stage of a Phase 2 trial will require sign-off by the Principal Investigator and the Protocol Statistician.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.2 Data and Safety Monitoring Plan

The Lead Principal Investigator and co-investigators will provide continuous monitoring of patient safety in this trial, including regularly scheduled teleconferences with participating ETCTN sites and representatives from CTEP/Pharmacoclinics. Summary information regarding toxicity and accrual patterns, including information from all multicenter sites participating in the trial will be prepared by Lead Principal Investigator or designee. More specifically, the Lead Principal Investigator will review all AE data monthly and all grade 4 to 5 AEs weekly and all SAEs within 72 hours (or earlier, if required). In addition, the Lead Principal Investigator will review all treatment discontinuations and their attribution to the study drug.

Meetings/teleconferences will be held on a bi-weekly basis while the study is enrolling patients. These meetings will include the clinical investigators as well as study coordinators, clinical research associates, regulatory associates, data managers, a GMP facility representative, biostatisticians, and any other relevant personnel the Lead Principal Investigator may deem appropriate (e.g. CTEP personnel), including an external experienced investigator (preferably a lymphoma specialist with experience in ibrutinib administration) who is not involved in the study conduct. The frequency of meetings can change depending on the number of active

patients (i.e. once a month if there are no active patients; every 21 days if only one active patient; or weekly if >5 active patients remain on study). Ad hoc meetings will be convened if an unexpected safety concern is identified during the conduct of the trial. A table of all AEs to be discussed will be shared ahead of the scheduled teleconference. After enrollment is complete, meetings will be held monthly until there are no remaining patients on study. During these meetings, the research team will discuss all issues relevant to study progress, including topics such as enrollment, safety, regulatory, data collection, etc. Decision options will be: continue as is, hold or modify accrual, evaluate AEs in more detail with or without accrual on hold, and/or modify monitoring or management plan (including supportive care), amend, or close. The team will produce summaries or minutes of these meetings, which will be archived. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human patients and the integrity of data for ETCTN trials.

Following accrual of the last patient in stage 1, the study will be suspended for interim analysis of the primary (overall response rate) and two of the secondary endpoints (PFS and toxicity). During interim analysis all grade 4/5 and SAE rates will be reviewed and a decision regarding their attribution to study drug will be resolved by consensus. Suspension can take up to 16 weeks, in particular if the primary endpoint is not met, and therefore estimation of the 6-month PFS endpoint is required for assessment of clinical benefit. See [Section 13.2](#), Sample Size/Accrual Rate for details. A meeting will be subsequently convened to discuss results from the interim analysis to determine if the trial should be terminated due to lack of efficacy. As described above, an agenda and minutes will be generated to document attendees and the outcome of the interim analysis.

12.3 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at < <https://ctepcore.nci.nih.gov/iam> >) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (<https://login.imedidata.com/selectlogin>) using their CTEP-IAM user name and password, and click on the “accept” link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site

registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctscontact@westat.com.

12.3.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: <http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11>. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

12.3.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol-specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent, if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the

recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (<http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models>). Customized eCRFs will be included, when appropriate, to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

Further information on data submission procedures can be found in the ETCTN Program Guidelines
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm

12.4 CTEP Multicenter Guidelines

N/A

12.5 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as “Collaborator(s)”) and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the “Intellectual Property Option to Collaborator” (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient’s family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: <http://ctep.cancer.gov>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):
 - a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

- b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.
 - c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used, and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the Principal Investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This will be an open label, single arm, phase 2 study of patients who have failed, or are not eligible for, all other prior systemic FDA-approved treatments for metastatic melanoma. The primary endpoint will be assessment of antitumor response, defined as the sum of complete response and partial response evaluated by RECIST criteria 1.1 every two cycles (8 weeks) as assessed by the investigator. Secondary clinical endpoints include estimates of PFS at 6 months, OS at 1 year, and toxicity assessment. Another secondary objective is to explore the association of ITK protein expression with OR and PFS. The ITK protein expression in melanoma cells at pretreatment will be assessed by 2-color immunofluorescence (IF) in representative tissue sections obtained from archival formalin-fixed embedded blocks or in representative tissue sections/slides obtained from fresh biopsies from enrolled patients. Exploratory endpoints include protein expression of putative targets of ibrutinib (e.g. Tec, ErbB4, Hck, Yes, BTK) in melanoma cells assessed by 2-color immunofluorescence (IF) in representative tissue sections obtained from pretreatment archived formalin-fixed paraffin-embedded (FFPE) tumor blocks or from FFPE blocks prepared from fresh biopsy tissue from enrolled patients, with OR and without progression, multiparameter flow cytometric endpoints in PBMC obtained prior to treatment, on day 29 following initiation of treatment with ibrutinib, and at the time of disease progression (3 time points).

13.2 Sample Size/Accrual Rate

Our null hypothesis is that the true antitumor response rate is 5%, as per the response rate seen in patients who were treated with investigators' choice chemotherapy as part of the recent study of PD-1 inhibitors for patients with ipilimumab-refractory, BRAF inhibitor-refractory (if BRAFV600-mutant) metastatic cutaneous melanoma (Ribas *et al.*, 2015; Weber *et al.*, 2015), against an one-sided alternative. The assessment of objective response at interim and final analysis will be based upon investigator's assessment of response. We will use Simon's two-stage design. In the first stage, 18 patients will be accrued and study will be suspended for up to 16 weeks for interim analysis. If there are no antitumor responses in these 18 patients, then we will assess the 6-month PFS rate. In the 6-month PFS rate scenario the null hypothesis is that ibrutinib is not effective if the 6-month PFS rate is 18% and the alternative hypothesis is that ibrutinib is promising if the 6-month PFS rate is 35%. The null and alternative hypotheses scenarios for the 6-month PFS rate are based on the KEYNOTE-006 trial in which the 6-month PFS rate for the investigators' choice chemotherapy versus the pembrolizumab arm was 16% versus 34% (Ribas *et al.*, 2015).

If either the antitumor response and/or the 6-month PFS rate endpoint are met then 14 additional patients will be accrued for a total of 32. The null hypothesis will be rejected if 4 or more responses are observed in 32 patients. This design yields a type I error rate of 0.1 and power of 90% when the true response rate is 0.2. If the null hypothesis for the antitumor response endpoint is not met, then we will investigate the 6-month PFS rate endpoint. The Simon's design would reject the null if 9 or more patient out of 32 have PFS better than 6 months.

13.3 Toxicity Assessment

The phase 1 dose escalation study of ibrutinib in patients with CLL showed that both the safety and efficacy are similar for this population irrespective of the dose cohort (420mg versus 840mg)(Byrd *et al.*, 2013). Nevertheless, it is possible that there may be higher rates of AEs from ibrutinib treatment for a solid tumor malignancy, such as melanoma. Continuous toxicity monitoring will be performed based on the data and safety monitoring plan described in section 12.1. More specifically, sequential boundaries will be used to monitor the high grade AE rate, defined as all grade 3/4/5 events attributed to the ibrutinib. The accrual will be halted if excessive numbers of study drug-related high grade AEs or SAEs are seen, that is, if the number of patients with at least one AE grade 3/4/5 is equal to or exceeds b_n out of n patients with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.1 when the AE rate for grade 3/4/5 events is equal to the acceptable rate of 0.38. The 38% acceptable grade 3/4/5 AE rate is based on the recent phase III study of ibrutinib (560mg) versus temsirolimus in patients with relapsed or refractory mantle cell lymphoma(Dreyling *et al.*, 2016).

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	-	4	5	5	6	7	7	8	8	9	9	10	10	11	11	12	12	13
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	13	14	14	14	15	15	16	16	17	17	18	18								

A separate boundary will be used to monitor the rate of thrombocytopenia and hemorrhagic events attributed to the study drug. The accrual will be halted if excessive numbers of these events are seen; that is, if the total number of patients with at least one Grade 3/4/5 thrombocytopenia or hemorrhagic event is equal to or exceeds b_n out of n patients with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.1 when the rate of high grade thrombocytopenia and hemorrhagic events is equal to the acceptable rate of 0.09, as recently reported (Dreyling *et al.*, 2016):

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	2	2	3	3	3	3	3	3	4	4	4	4	4	4	5	5	5	5	5
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	5	5	6	6	6	6	6	6	6	7	7	7								

We anticipate accruing 3 patients/month across the UM1 consortium.

Below are estimates of accrual for this trial. These estimates are based on Cancer Facts & Figures 2015(Siegel *et al.*, 2015) and experience at a tertiary care center. Cutaneous melanoma is most common in non-Hispanic whites, so we expect this to be the largest group of patients in this trial. We anticipate accruing at least a few black and Hispanic patients, however, and every effort will be made to include all minority groups. While incidence of cutaneous melanoma is similar between men and women, mortality is higher for men. Given that this trial enrolls patients with refractory metastatic disease, we anticipate enrolling more men than women into the study.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	1	0	0	2
White	9	16	1	3	29
More Than One Race	0	1	0	0	1
Total	10	18	1	3	32

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015)

OMB No. 0925-0001/0002

13.4 Stratification Factors

N/A

13.5 Analysis of Primary Endpoint

We will compute point estimate and 95% confidence interval for antitumor response.

13.6 Analysis of Secondary Endpoint

PFS and OS will be estimated using the Kaplan Meier method. To explore the association of a baseline ITK protein expression in melanoma cells with OR, we will compute the mean ITK protein expression in patients with CR, PR, SD and PD, and perform the Jonckheere–Terpstra test for trend. To explore the association of a baseline ITK protein expression with PFS, we will compute the mean ITK protein expression in patients with PFS less than 6 months and longer than 6 months. We will also fit a Cox model with ITK protein expression as a continuous covariate.

13.7 Analysis of Exploratory Endpoints

The protein expression levels of ITK and various putative targets of ibrutinib in melanoma cells from pretreatment archived FFPE tumor blocks or from FFPE blocks prepared from fresh biopsy will be assessed 2-color IF, and analyzed by Aperio imaging, as we have previously published (Schlegel *et al.*, 2013; Nikolaishvilli-Feinberg *et al.*, 2014; Carson *et al.*, 2015). Briefly, the Color Deconvolution v9 area quantification algorithm (ImageScope) software will be used to calculate the average H-score for each stain and for the cells of interest (S100+). To investigate the predictive ability of protein expression measured by H-score (continuous variable) as a predictor of antitumor response or PFS to ibrutinib. Exploratory analysis will also be performed to assess the predictive ability of each tissue biomarker by fitting logistic model or Cox model with biomarker as a covariate. For the latter analysis, antitumor response rate or PFS information will be used to investigate possible cut-points for the biomarker.

PBMC from three different time points (baseline, Day 29, and at disease progression) will be stained for various immune regulatory cell populations, as well as markers for Th1 and Th2 responses. Within-patient changes in the expression of particular immune cell subsets in samples taken prior to study treatment, day 29 (i.e., predose D1 of cycle 2), and at disease progression will be assessed by comparisons using ANOVA followed by paired *t*-test or other tests (Wilcoxon rank-sum test), if normality assumption is not satisfied even when data transformation is performed. Within-patient changes in peripheral blood will be characterized for patients who respond to treatment and for those who do not.

Non-compartmental pharmacokinetic (PK) analysis will be performed on ibrutinib (PCI-32765) concentrations in plasma using WinNonlin. The following parameters will be estimated: maximum concentration (C_{max}), time of maximum concentration (T_{max}), area under the concentration versus time curve (AUC), half-life (t_{1/2}), apparent clearance (CL), apparent volume of distribution (V_d). The PK from this study will be compared to the prior study of ibrutinib (PCI-32765) at the lower dose of 560 mg.

13.8 Reporting and Exclusions

13.8.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with ibrutinib.

13.8.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [NOTE: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration will not result in exclusion from the analysis of the response rate. Subanalyses of the patients who have received at least one cycle (4 weeks) of ibrutinib therapy will be also undertaken.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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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 (<i>e.g.</i> , 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 ADEQUACY OF ARCHIVED/FRESH TUMOR TISSUE

Below is a sample letter from the pathologist stating the adequacy of archived or fresh tumor tissue for tumor imaging analysis.

Stergios Moschos
University of North Carolina at Chapel Hill
Department of Medicine, Division of Hematology/Oncology
Physician's Office Building, Suite 3116-CB#7305
170 Manning Drive
Chapel Hill, NC 27599
Phone (O): 919-843-7713
Cell: 412-915-5014
Fax: 919-966-6735
E-mail: Stergios_moschos@med.unc.edu

Dear Dr. Moschos,

I have reviewed the hematoxylin and eosin stained slides corresponding to [SCREENING SUBJECT ID NUMBER and SUBJECT INITIALS]'s archived or fresh tumor block from the [PATIENT'S SURGICAL PROCEDURE TYPE]. I have confirmed that the amount and quality of remaining tumor tissue material contained in this block is sufficient to generate 11 (min) to 15 (max) 5-micron blank sections for histopathologic analysis.

- ☐ I will release 11-15 5-micron tissue sections on charged slides to your institution for research analysis.
- ☐ I will release the entire paraffin block to your institution for research analysis.

Please return the paraffin block (if the paraffin block checkbox is checked off) to the mailing address noted in this letter.

Sincerely,

[INSTITUTION PATHOLOGIST SIGNATURE]
[PRINTED NAME AND MAILING ADDRESS]

Below is a sample documentation from the Lead Principal Investigator stating the adequacy of archived tissue ONLY.

PARTICIPATING INSTITUTION

Screening Subject ID #: _____ **Subject Initials:** _____ , _____
Last, First Middle

Site Name: _____ **Site Contact Person Name:** _____

Site Contact Person Email: _____

Pathology materials provided (check appropriate box):

- ☐ Slides
- ☐ De-identified pathology reports
- ☐ Digital images

LEAD PRINCIPAL INVESTIGATOR

Dr. Stergios Moschos has reviewed the above materials provided and has determined that [SCREENING SUBJECT ID NUMBER and SUBJECT INITIALS] (check appropriate box):

- ☐ Adequate tissue is available to confirm eligibility
- ☐ Insufficient tissue is available to confirm eligibility (provide comments):

Lead Principal Investigator Signature

Date

APPENDIX C PATIENT DIARY FOR IBRUTINIB

PATIENT DIARY FOR IBRUTINIB

Subject ID Number: _____

Cycle # _____

Time Period:

From

--	--

month

--	--

day

--	--

year

to

--	--

month

--	--

day

--	--

year

Patient Instructions: Use this calendar as a reminder to take your medication every day. Be sure to write the number of capsules taken each time. Start recording your first day of taking pills in the first box (DAY 1). Be sure to bring this calendar and the medication bottle with you for all of your return appointments. Thank you.

- Take each dose of ibrutinib capsules (# __ of 140 mg capsules = ____ mg) once daily by mouth.
- Swallow the capsules whole with water (use a glass (8 ounces, 1 cup) of water total for each ____ mg dose).
- Take each dose at approximately the same time each day.
- Fish oil and vitamin E products should not be taken while taking ibrutinib
- Ibrutinib must not be taken with grapefruit juice.
- Do not open, break or chew capsules, and do not attempt to dissolve them in water.
- If a dose is not taken at the scheduled time, it can be taken as soon as possible on the same day with a return to the normal schedule the following day. Extra capsules should **not** be taken to make up for the missed dose.
- If you vomit immediately after taking your dose, do not replace this dose. Continue dosing the next day as per usual. Record the vomiting episode on your medication diary.
- Return any remaining ibrutinib capsules to research staff once treatment is complete, and as per study instructions.

START HERE DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____
# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____
# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____
# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____	# capsules__ Time:_____

Returned Pill Count: _____ Subject Signature: _____ Date: _____

Site Personnel Signature: _____ Date: _____

APPENDIX D INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agent ibrutinib. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

Ibrutinib interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet.** These are the things that you and they need to know:

- Ibrutinib is metabolized by a certain specific enzyme in your liver.
- The enzyme in question is the CYP3A enzyme and ibrutinib is broken down by this enzyme in order to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
 - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors of the CYP3A isoenzyme.
- Your prescribers should look at this web site <http://medicine.iupui.edu/CLINPHARM/ddis/main-table> or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it is usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
 - If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.

- If you take herbal medicine regularly: You should not take St. John's wort while you are taking ibrutinib.
- Other medicines can be a problem with your study drugs.
- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____ and he or she can be contacted at _____.

<p>INFORMATION ON POSSIBLE DRUG INTERACTIONS</p> <p>You are enrolled on a clinical trial using the experimental agent _____. This clinical trial is sponsored by the NCI.</p> <p>_____ interacts with drugs that are processed by your liver. Because of this, it is very important to:</p> <ul style="list-style-type: none"> ➤ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➤ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial. ➤ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement. 	<p>_____ interacts with a specific liver enzyme called CYP_____, and must be used very carefully with other medicines that interact with this enzyme.</p> <ul style="list-style-type: none"> ➤ Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of CYP_____." ➤ Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/ for a list of drugs to avoid, or contact your study doctor. ➤ Your study doctor's name is _____ and can be contacted at _____.
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APPENDIX E ARCHIVAL/FRESH TISSUE SUBMISSION FORM

<u>NCI9922 Archival/Fresh Tissue Block/Slides Submission Form</u>						
Subject ID #: _____ Subject Initials: _____ , _____ <div style="text-align: center; font-size: small;">Last, First Middle</div>						
Site Name: _____ Contact Person: _____						
Phone: _____ Email: _____						
Date Shipped: _____ Courier/Airbill Tracking #: _____						
Date of Biopsy (mm/dd/yyyy)	Type of Submission	Number of Slides/Blocks	Accession Number	Comments	Tissue Type	Metastatic Site (eg, liver, LN etc)
	<input type="checkbox"/> Blocks <input type="checkbox"/> Slides				<input type="checkbox"/> Archive <input type="checkbox"/> Fresh	

Shipping Address:

Mei Huang, Lab Manager
 c/o Tissue Procurement Facility
 108 MacNider Building, CB 7304
 University of North Carolina
 333 South Columbia St
 Chapel Hill, NC 27599
 Phone: 919-966-2620 / Fax: 919-843-9501
 Email: mei_huang@med.unc.edu
 unc_tpf@med.unc.edu

FOR RECEIVING LABS ONLY

	Date Received	Confirmed by	Comments
Archival Tissue			
Fresh Tissue			

APPENDIX F CHILD-PUGH SCORE

Measure	1 point	2 points	3 points
Total bilirubin, $\mu\text{mol/L}$ (mg/dL)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/L (g/dL)	>35 (>3.5)	28-35 (2.8-3.5)	<28 (<2.8)
PT INR	<1.7	1.71-2.30	>2.30
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Points	Class
5-6	A
7-9	B
10-15	C

Source:

Child CG, Turcotte JG. "Surgery and portal hypertension". In Child CG. The liver and portal hypertension. Philadelphia:Saunders. 1964. pp. 50-64.

Pugh RN, Murray-Lyon IM, Dawson L, et al . "Transection of the oesophagus for bleeding oesophageal varices". The British journal of surgery, 1973;60: 646-9.

APPENDIX G PBMC SHIPMENT LOG

Duke-UNC-Washington University (DUNCWU)
An Early Therapeutics Clinical Trials Network (ETCTN) Partnership

NCI9922 PBMC Shipment Log

Site Name: _____

Date of Shipment: _____ FedEx ☐ UPS ☐ Other: _____

Shipment Tracking Number: _____

Insert copy of log with shipment and notify lab of incoming shipment via 9922 Study Portal.

(Ship to UNC: Patricia Basta - address provided in Section 9.4.1.3 of protocol)

Subject ID#	Collection Date (mm/dd/yyyy)	Study Visit (Prestudy, Day 29 i.e., predose D1 of Cycle 2, Disease Progression)	Number(s) of Tubes

APPENDIX H BIOMARKER SHIPMENT LOG

Duke-UNC-Washington University (DUNCWU)
An Early Therapeutics Clinical Trials Network (ETCTN) Partnership

NCI9922 Biomarker (Whole Blood or Plasma) Shipment Log

Site Name: _____

Date of Shipment: _____ FedEx ☐ UPS ☐ Other: _____

Shipment Tracking Number: _____

Insert copy of log with shipment and notify lab of incoming shipment via 9922 Study Portal.

(Ship to Duke: Andy Nixon - address provided in Section 9.4.2.3 of protocol)

Subject ID#	Collection Date (mm/dd/yyyy)	Study Visit [Prestudy, predose on Day 1 Cycle 2 (Day 29), predose at odd number Cycle# Day#, or Disease Progression]	Sample Type (plasma or whole blood)	Number(s) of Tube/Cryovials

Page __ of __

APPENDIX I PK SUBMISSION FORM

NCI 9922 Pharmacokinetics Sample Submission Form

Please include a completed submission form for each sample in with every shipment.

If a sample was not collected, please give reason in comment box below.

Subject ID: _____ Subject's Initials: _____ , _____
Last, First Middle

Site: _____ Contact Person: _____

Phone: _____ Email: _____

FedEx/UPS Tracking #: _____ Date Shipped: _____

Pharmacokinetic Time points Day/Cycle	Projected		Obtained		Problems/ Comments
	Date (m/d/yy)	Time	Date (m/d/yy)	Time	
Pre-dose D1/Cycle 1					
Pre-dose D8/Cycle 1					
0.5 hour \pm 5min Post-dose D8/Cycle 1					
1 hour \pm 15min Post- dose D8/Cycle 1					
2 hours \pm 15min Post- dose D8/Cycle 1					
4 hours \pm 30min Post- dose D8/Cycle 1					
6 hours \pm 1 hr Post- dose D8/Cycle 1					
24 hours \pm 2 hr Post- dose D8/Cycle 1					

Send all specimens on dry ice by a courier guaranteeing overnight delivery to the following address:

Mei Huang, Lab Manager
c/o Tissue Procurement Facility
108 MacNider Building, CB 7304
University of North Carolina
333 South Columbia St
Chapel Hill, NC 27599

Phone: 919-966-2620 / Fax: 919-843-9501

Email: mei_huang@med.unc.edu Lab email: unc_tpf@med.unc.edu

FOR RECEIVING LABS ONLY

	Date Received	Confirmed by	Comments
Pharmacokinetics			