Multicenter Phase 1b/2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma

Roswell Park Cancer Institute

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RPCI Study Contacts

FOR NETWORK SITES: Please contact the RPCI Network Monitor for any study-related questions regarding sample handling, processing or shipping.

Study personnel outside of RPCI-CRS who will be housing essential study documents or receiving, analyzing or storing biological samples. This contact list is to include all internal and external laboratories and their primary laboratory contacts. *NOTE: Personnel from any potential network site are NOT to be included on this contact page.*

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SYNOPSIS

Title / Phase	**Multicenter Phase 1b/2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma**
Roswell Park Cancer Institute Study Number	**I 229112**
Roswell Park Cancer Institute Investigator	Renuka Iyer, MD
Sponsor	Roswell Park Cancer Institute
Study Drug	Tivozanib
Objectives	**Primary Objective:** • The primary endpoint of the study is PFS at 24 weeks in patients with advanced HCC. Patients who remain alive without evidence of disease progression (per RECIST) for at least 24 weeks after enrollment will be considered PFS responders. **Secondary Objectives:** • To determine the safety of tivozanib in HCC. • To determine the OS and clinical benefit rate (CR, PR and SD) by RECIST. • To determine the steady state PK and soluble VEGFR-2 baseline/ change with tivozanib and use modeling to correlate exposure with biomarker change and the primary outcome measure of PFS. • To determine the change in viral load (HBV and HCV) during therapy in patients with HBV or HCV associated HCC. • To determine the change in tumor marker (alfa fetoprotein) with tivozanib therapy is in the effect of tivozanib on several tumor-associated immune response markers.
Study Design	Multicenter Phase 1b/2 study of tivozanib in patients with advanced inoperable hepatocellular carcinoma. The Phase 2 portion includes early stopping rules for futility.
Target Accrual and Study Duration	Between 6 and 18 patients will be enrolled in Phase 1b portion of this study. Six Phase 1b patients treated at the recommended Phase 2 dose will be included in the Phase 2 portion of this study. An additional 31 patients will be enrolled for the Phase 2 portion, for a total Phase 2 sample size of 37. This study is expected to accrue 9 patients to the Phase 1b, for a combined sample of size of 40 (9+37-6). If 18 patients are enrolled in the Phase 1b, the maximum combined sample size will be 49 (18+37-6). The number of subjects required is a function of the unknown PFS response rate. Accrual is expected to take up to 4 years. The duration the patients will be on study will be approximately 2 years.
Study Procedures	**Physical Examination** **Hematology** **Chemistry** **PT/INR** **UPCR**

	Alpha-Fetoprotein (AFP) **HBsAg**; **HCV Ab; (if unknown)** **Viral Load** (if either or both HBsAg or HCV Ab are positive) **PK/PD sVEGF-2 Sampling** **Immune Biomarker Sampling** **ECOG Performance Status** **Electrocardiogram** **CT Scan (within 4 weeks)** **Adverse Events**
Statistical Analysis	**Sample Size Determination:** Sample size for the Phase 1 portion depends on the unknown dose-toxicity profile. For the Phase 2 portion, with $\alpha = 0.05$, assessment of 37 patients gives 80% power to detect minimum 20 percentage point improvement in the 24 week PFS response rate, assuming a 50% rate under the standard of care. **Randomization:** No randomization is required. **Efficacy Analysis:** Primary interest is in the PFS response rate 24 weeks after enrollment. The response rate will be described by binomial proportion estimates and 95% confidence intervals. All enrolled patients will be included in this primary analysis. **Safety Analysis:** Adverse events will be tabulated. AE rates will be described using binomial proportions and 95% confidence intervals.

Subject Name: (Network Sites use Initials) _____

Medical Record No.: (Network Sites use Subject ID#)_____

Title: Multicenter Phase 1b/ 2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES" or "N/A" for subject enrollment.**	**DATE**
☐	☐	☐	**1.** Advanced staged HCC (unresectable and not amenable to local or regional therapy; or metastatic HCC). The diagnosis of HCC should be based on at least one of the following: a) MRI or CT consistent with liver cirrhosis AND at least one solid liver lesion measuring ≥ 2 cm, with characteristics arterial enhancement and venous washout regardless of AFP levels. b) AFP ≥ 400 ng/mL AND evidence of at least one solid liver lesion ≥ 2 cm regardless of specific imaging characteristics on CT or MRI. c) Histological/cytology biopsy confirming HCC.	
☐	☐	☐	**2.** Patients must have measurable disease per RECIST 1.1 criteria, defined as at least 1 lesion that can be accurately measured in at least one dimension, and that has not been the target of local or regional therapy including transarterial chemoembolization, intra-arterial chemotherapy, ethanol, or radiofrequency ablation.	
☐	☐	☐	**3.** Age ≥ 18 years.	
☐	☐	☐	**4.** Life expectancy of greater than 3 months.	
☐	☐	☐	**5.** Patients must have organ and marrow function as defined below: • Child-Pugh liver function Class A. • AST ≤ 5 x Institutional upper limits of normal (ULN) • Total bilirubin ≤ 3 mg/dL • INR ≤ 2.0 (unless due to therapeutic warfarin use) • Serum albumin > 2.8 g/dL • Creatinine ≤ 1.5 x Institutional ULN • ANC ≥ 1200/mm^3 • Platelets ≥ 60,000/mm^3 • Hgb ≥ 8.5 g/dL NOTE: The lower absolute neutrophil count (ANC) and platelet cut-offs are designed to accommodate the large number of patients with HCC who have portal hypertension and splenic sequestration. Given the adverse profile of tivozanib these reduced cut-offs are thought to be safe.	

Subject Name: (Network Sites use Initials) _____

Medical Record No.: (Network Sites use Subject ID#)_____

Title: Multicenter Phase 1b/ 2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES" or "N/A" for subject enrollment.**	**DATE**
☐	☐	☐	**6.** Patients must not have any evidence of bleeding diathesis or active gastrointestinal bleeding.	
☐	☐	☐	**7.** Patients must not be known to be HIV positive; drug-drug interaction with study medication and HIV medications is not well-characterized and could lead to unwanted side effects.	
☐	☐	☐	**8.** Patients must not have other uncontrolled intercurrent illnesses (excluding HBV or HCV). This includes (but is 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.	
☐	☐	☐	**9.** Sexually active fertile patients (male and female), and their partners, must agree to use medically accepted methods of contraception during the course of the study and for 3 months after the last dose of the study drug.	
☐	☐	☐	**10.** Female patients of childbearing potential must have a negative pregnancy test at screening.	
☐	☐	☐	**11.** Have an ECOG Performance Status of ≤ 2. Refer to **Appendix B**.	
☐	☐	☐	**12.** Subject or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____ **Date:** _____

Subject Name: (Network Sites use Subject Initials) _____

Medical Record No.: (Network Sites use Subject ID#) _____

Title: Multicenter Phase 1b/2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "NO" or "N/A" for subject enrollment**	**DATE**
☐	☐	☐	**1.** Patients who have had prior anti-angiogenic therapy, including but not limited to sorafenib, brivanib, bevacizumab, or sunitinib.	
☐	☐	☐	**2.** Patients who have had any prior line of systemic therapy including cytotoxic agents or molecularly targeted agents for advanced/unresectable disease. Any number of prior regional therapies with transarterial chemoembolization (TACE), brachytherapy with Yttrium-90 microsphere, intra-arterial chemotherapy, surgery, or ablative therapy is allowed.	
☐	☐	☐	**3.** Prior liver transplantation and on immunosuppression; drug-drug interaction with study medication and immunosuppression is not well-characterized and could lead to unwanted side effects.	
☐	☐	☐	**4.** Known symptomatic or uncontrolled brain metastases or epidural disease.	
☐	☐	☐	**5.** Patient has a corrected QT interval (QTcF) > 500 ms at screening.	
☐	☐	☐	**6.** The patient is unable to swallow pills or diagnosed with a gastrointestinal disorder that are likely to interfere with the absorption of the study drug or with the patient's ability to take regular oral medication.	
☐	☐	☐	**7.** The patient is pregnant or breastfeeding.	
☐	☐	☐	**8.** Patients with second primary cancer (except adequately treated nonmelanoma skin cancer, curatively treated in-situ carcinoma of the cervix or superficial bladder cancer, or other solid tumors including lymphoma without bone marrow involvement curatively treated with no evidence of disease for ≥ 5 years).	
☐	☐	☐	**9.** The patient has a previously-identified allergy or hypersensitivity to components of the study treatment formulation.	
☐	☐	☐	**10.** Patients receiving any medications or substances that are strong inducers of CYP3A4 are ineligible. Moderate and mild inducers of CYP3A4 should be used with caution as they reduce the efficacy of Tivozanib.	
☐	☐	☐	**11.** Urine protein: creatinine ratio > 1 (see Appendix- H)	

Study participant meets all entry criteria: ☐ **Yes** ☐ **No**

Investigator Signature: _____ **Date:** _____

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IN-TEXT TABLES

APPENDICES

1 BACKGROUND

Primary HCC is the sixth most common malignancy diagnosed worldwide. HCC results in between 250,000 and one million deaths globally per year and its incidence continues to increase in the developed world. HCC is an aggressive tumor that often occurs in the setting of chronic liver disease and cirrhosis and is often diagnosed late in its course, as there are no biomarkers to detect it when it is incipient and potentially curable. Median survival following diagnosis is less than 6 months. Treatment options are divided into surgical therapies (i.e., resection, cryoablation, and orthotopic liver transplantation [OLT]), and nonsurgical therapies (i.e., percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), transarterial chemoembolization (TACE), systemic chemotherapy, and radiation). Curative therapies such as resection, transplantation, or percutaneous therapies benefit only 25% of patients. Majority of patients are not eligible for such therapies because of tumor extent or underlying liver dysfunction. Improving treatment outcomes in patients with advanced stage hepatocellular carcinoma (HCC) requires the development of agents with tolerable safety profiles and the identification of biomarkers capable of predicting tumor response or resistance to treatment. The underlying etiology for HCC development is often chronic viral infection and inflammation due to hepatitis and cirrhosis affects both tumor progression and immune cell networks.[1]

1.1 Study Disease – Hepatocellular Carcinoma

Liver cancer is a common and rapidly fatal malignancy, with the third highest cancer mortality in 2008. Hepatocellular carcinoma (HCC) is the most common type of liver cancer, accounting for about 80% of all cases of cancers originating in the liver. HCC is a hypervascular malignancy and VEGF is frequently expressed and associated with poor prognosis.[2] VEGF induces endothelial cell proliferation and vascular permeability and its effects are mediated through multiple receptors, including VEGFR-2.[3] VEGFR-2 is the predominant mediator of VEGF-stimulated endothelial cell migration, proliferation, survival, and enhanced vascular permeability. High VEGF HCC tumor expression has been correlated with vascular invasion and poor patient survival. VEGFR-2 has been shown to be more strongly expressed in HCC compared to dysplastic nodules and background cirrhotic liver, and VEGFR-2 up-regulation is a feature of poor differentiation and tumor progression. Therefore, VEGFR-2 is an important regulator of VEGF-induced tumor development and angiogenesis. Systemic therapy using targeted therapy for advanced-stage HCC appeared promising in Phase 2 studies delaying progression free survival despite no radiographic shrinkage in the tumor and led to confirmatory Phase 3 studies that led to FDA approval of the multikinase inhibitor, sorafenib. Sorafenib targets Raf kinase and receptor tyrosine kinase activity of platelet-derived growth factor receptor β (PDGFR-β) and vascular endothelial growth factor receptors (VEGFRs) 1, 2 and 3.31, 32 Since its approval in 2008, no other targeted therapies have shown superior activity or safety profiles in the treatment of advanced HCC.[4]

Although sorafenib has been approved for use, in clinical practice the drug has many toxicities and overall survival even in western populations with good supportive care remains only 10.7 months, 2 months greater than placebo. In addition, the primary endpoint of improved symptom control was not achieved and 66% of patients either need dose reductions or delays and

discontinue drug for reasons other than disease progression that speak also to the limited tolerability of this drug and also the challenges with developing safe and efficacious therapies for this disease population. Despite these limitations with the approval of sorafenib targeting angiogenesis is and will remain the backbone for HCC therapy for the foreseeable future.[5] Novel agents that target this pathway and have sound preclinical rationale need to be evaluated to improve outcomes for patients with this disease. In addition, there is a need to incorporate and prospectively validate novel biomarkers that will predict response to such therapies.

1.2 Study Drug - Tivozanib Hydrochloride

Tivozanib hydrochloride (also known as AV-951; previously known as KRN951) has the chemical name (*N*- {2-Chloro-4-[(6, 7-dimethoxy-4-quinolyl) oxy] phenyl}-N'-(5-methyl-3-isoxazolyl) urea hydrochloride monohydrate). Tivozanib hydrochloride is a novel and potent pan-vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor with potent activity against all 3 VEGF receptors (VEGFR-1, VEGFR-2, and VEGFR-3).[6] Tivozanib hydrochloride inhibits phosphorylation of VEGFR-1, VEGFR-2 and VEGFR-3 at picomolar concentrations (IC_{50} of 0.21, 0.16 and 0.24 nM, respectively), and inhibits c-Kit and PDGFR at 10-times higher concentrations (IC_{50} of 1.63 and 1.72 nM, respectively). In nonclinical models and studies performed in humans, tivozanib hydrochloride has shown strong anti-angiogenesis and antitumor activity. VEGF is a potent induction factor, playing a central role in angiogenesis and vascular permeability of tumor tissues. By inhibiting VEGF-induced VEGFR activation, tivozanib hydrochloride inhibits angiogenesis and vascular permeability in tumor tissues, leading indirectly to inhibition of tumor growth.[7]

Tivozanib hydrochloride has been studied in several clinical trials in multiple tumor types. A summary of tivozanib's hydrochloride pertinent efficacy results and safety data follow. Please refer to the tivozanib hydrochloride Investigator Brochure (IB) for descriptions of all available data.

1.3 Preclinical Studies with Tivozanib

A detailed discussion of the preclinical pharmacology, pharmacokinetics, and toxicology of tivozanib can be found in the Investigator's Brochure.

1.4 Clinical Studies with Tivozanib

Clinical activity data are available for 3 monotherapy studies and for 2 combination therapy studies. Clinical response was assessed using RECIST (Response Evaluation Criteria in Solid Tumors, Version 1.1).

Forty-one patients with advanced solid tumors received tivozanib hydrochloride monotherapy given orally for 28-consecutive days followed by a 14-day rest period in a Phase 1 study (KRN951/03-B01). The maximum tolerated dose (MTD) of tivozanib hydrochloride was determined to be 1.5 mg/day. Overall, 46.3% of subjects experienced some level of disease control. Most subjects had a best overall response of stable disease [SD] (43.9%) or progressive disease [PD] (43.9%). The median time to disease progression across all treatment groups was 3.9 months (95% CI: 2.7, 6).

In a Phase 2 study (AV-951-07-201) in 272 patients with metastatic renal cell carcinoma (RCC), there were 2 primary efficacy analyses: best tumor response rates throughout the 16-week open-label period and progression-free survival (PFS) rates at 12 weeks post-randomization. Throughout the 16-week, open-label period, the overall response rate (ORR) for all treated subjects was 24.6% by investigator assessment and 18% by independent radiology review (IRR) assessment. By both investigator assessment and IRR assessment, the disease control rate (DCR) for all treated subjects was 84.2% (229/272 subjects). At 12 weeks post-randomization, PFS rates were 57.4% for tivozanib hydrochloride subjects and 28.1% for placebo subjects ($p = 0.001$). By IRR assessment, PFS rates were 49.2% for tivozanib hydrochloride subjects and 21.1% for placebo subjects ($p = 0.001$).

Sixteen subjects with non-small cell lung cancer (NSCLC) were treated with tivozanib hydrochloride in a Phase 1 continuous-dosing monotherapy study. No subject had a confirmed best overall response of complete response (CR) or partial response (PR). Four subjects had SD. Duration of SD, for the 4 subjects with confirmed SD, ranged from 7 weeks to 32 weeks. Disease control was confirmed in 25% of subjects. Time to progression (TTP) was less than 10 weeks for most subjects.

Tivozanib hydrochloride was combined with temsirolimus in a Phase 1b study in patients with metastatic RCC. Of the 22 subjects evaluable for a secondary endpoint of efficacy, no subject had confirmed CR, 5 subjects had confirmed PR, and 15 subjects had confirmed SD. Reported ORR and DCR were 22.7% and 90.9%, respectively.

In a Phase 1b study of tivozanib hydrochloride combined with paclitaxel in a cohort of invasive breast cancer patients, ORR, DCR, and TTP were among the secondary efficacy variables. Thirteen subjects were included in the efficacy evaluable population. No subject had a confirmed CR, 4 subjects had confirmed PR, 6 subjects had SD, and 1 subject had PD. For responses confirmed by RECIST, ORR was 30.8% and DCR was 76.9%. In the 4 subjects demonstrating a PR, the duration ranged from 5.6 to 9.4+ months and TTP ranged from 9.7 to 13.1+ months.

1.5 Tivozanib Hydrochloride Safety Experience

Clinical data are available for 14 completed or ongoing clinical studies of tivozanib hydrochloride. Adverse events were generally manageable using standard medical therapy and/or discontinuation or reduction of study drug.

Related treatment emergent adverse events (TEAEs) that resulted in a fatal outcome occurred in 4 subjects and included post-procedural hemorrhage, cerebrovascular accident, pulmonary embolism, and hypertension.

Related TEAEs for combination therapy studies can be summarized as follows:

- **Combination Therapy Studies:** Related TEAEs were reported in 65/81 subjects (80.2%). The most frequently related TEAEs (occurring in ≥ 10% of treated subjects) were fatigue (50.6%), nausea (40.7%), diarrhea (35.8%), stomatitis (33.3%), vomiting (24.7%), decreased appetite (23.5%), hypertension (22.2%), peripheral sensor neuropathy (22.2%), epistaxis (19.8%), dysphonia (16.0%), thrombocytopenia (16.0%), alopecia (14.8%), headache (12.3%), neutropenia (12.3%), and blood triglycerides increased (11.1%).

- **Combination Therapy of Tivozanib Hydrochloride and mFOLFOX6:** Related TEAEs were reported in 17/30 subjects (56.7%). The most frequently reported related TEAEs (occurring in ≥10% of treated subjects) were nausea (50.0%), vomiting (36.7%), fatigue (40.0%), peripheral sensor neuropathy (33.3%), decreased appetite (30.0%), diarrhea (23.3%), stomatitis (23.3%), dysphonia (23.3%), epistaxis (16.7%), neutropenia (13.3%), anemia (13.3%), hypertension (13.3%), constipation 10.0%), thrombocytopenia (10.0%), headache (10.0%), thrombophlebitis (10.0%), and alopecia (10.0%).

Tivozanib hydrochloride has been generally well tolerated. **Table 1** lists adverse drug reactions that, based upon preliminary safety data from 4 core RCC monotherapy studies, could be reasonably assumed to be associated with tivozanib hydrochloride. The incidence of these adverse drug reactions to date in 4 core RCC monotherapy studies is also presented. For the purposes of regulatory reporting, the preferred terms listed in **Table 1** are considered expected with tivozanib hydrochloride monotherapy.

Table 1. Adverse Events Expected to Occur with Tivozanib Hydrochloride Monotherapy

Preferred Term	Safety Population N = 712 Subjects N (%)
Hypertension (including: arterial, worsened, increased, elevated) ≤ CTCAE Grade 3[1]	291 (40.9 %)
Dysphonia/Hoarseness	144 (20.2 %)
Fatigue	132 (18.5 %)
Diarrhea	130 (18.2 %)
Asthenia	118 (16.6 %)
Dyspnea	94 (13.2 %)
Palmar-Plantar Erythrodysesthesia Syndrome ≤ CTCAE Grade 3[1]	67 (9.4 %)
Nausea	63 (8.8 %)
Proteinuria ≤ CTCAE Grade 3[1]	55 (7.7 %)
Stomatitis	55 (7.7 %)
Headache	53 (7.4 %)
Decreased appetite	46 (6.5 %)
Vomiting	38 (5.3 %)
Arthralgia	35 (4.9 %)
Hypothyroidism	23 (3.2 %)
Arterial thromboembolic events (ischemic stroke, myocardial infarction, transient ischemic attack, acute myocardial infarction, arterial thrombosis, retinal artery thrombosis, and pulmonary artery thrombosis)[2]	20 (2.8 %)
Venous thromboembolic events (pulmonary embolism, vena cava thrombosis, deep vein thrombosis, pelvic venous thrombosis, subclavian vein thrombosis, and thrombophlebitis)[2]	12 (1.7 %)

Pooled data from 4 core RCC monotherapy studies, AV-951-07-201, AV-951-10-202, AV-951-09-902, and AV-951-09-301.

1 The incidence of hypertension, palmar-plantar erythrodysesthesia syndrome, and proteinuria presented in this table include events of all grades. For the purposes of regulatory reporting, these events are considered expected at ≤ CTCAE Grade 3. Higher grades are considered unexpected.

2 Terms from Standardized MedDRA Query.

When tivozanib hydrochloride is administered in combination with 1 or more approved antineoplastic agents, the expectedness determination should take into account the labeling of each specific marketed drug taken in combination based upon reference documents that will be

included or referenced in the clinical study protocol. The labeled events should, in general, be considered expected for at least one of the drugs in the combination. The contribution of tivozanib hydrochloride to the severity or frequency of the events is currently unknown.

1.6 Correlative Studies

1.6.1 Pharmacokinetic/ Pharmacokinetic and VEGFR-2

Given the toxicity and cost of newer targeted therapies, an important goal of early phase clinical studies is to identify biomarkers that will identify subsets of patients most likely to benefit from novel agents as well as to further our understanding of the mechanisms that are not yet not fully known. Research in this area has focused on soluble factors such as changes in soluble VEGF receptors, dynamic vascular changes and permeability changes using newer functional imaging such as DCE-MRI, but no standards have emerged. In a recent study of sunitinib and chemoembolization in HCC, we used PK modeling and developed a model using soluble VEGFR -2 and AUC of sunitinib and its active metabolite to predict PFS.[8] Model predicted PFS of 7 months and actual PFS in the study of 7.8 months strongly correlated. Given this finding and the very similar mechanism of action between sunitinib and tivozanib, we propose to do a limited PK sampling at rationally selected time points to assess both AUC and soluble VEGFR-2 in tivozanib treated patients and correlate the PFS at 24 weeks with model predicted outcomes using the previously described model. Stats methods are described in appropriate sections. Despite this being a good liver function population by inclusion criteria, still represents a population with limited liver reserve, hence we are starting at one dose level below recommended Phase 2 dose, PK studies in the patients included in this initial Phase 1 portion will add to the existing knowledge of tivozanib pharmacokinetic literature and is included for patient safety.

1.6.2 Immune Biomarker

Like many other cancers, HCC cells develop multiple strategies of escaping tumor-specific immunity such as the accumulation of myeloid-derived suppressor cells (MDSC),[9] suppression of tumor-associated antigen-reactive lymphocytes by T regulatory cells (Treg)[10] and dysfunctional dendritic cells.[11] MDSCs will be quantified by flow using a panel of markers that we have already standardized HLA DR-, CD 14-, CD11b +,CD33+. These mechanisms that drive HCC progression makes the development of efficacious immunotherapies for HCC very challenging.

The hypothesis of this study is based on the following rationale: c-Kit is one of the targets of tivozanib and functions as a receptor for stem cell factor (SCF),[12] a well-studied tumor-derived factor expressed by various human and murine tumor cell lines.[13] In mouse tumor models, abrogation of tumor-expressed SCF by RNA interference has been shown to significantly reduce MDSC expansion and restore the effector function of tumor-infiltrating T cells.[14] Furthermore, blockade of c-Kit by immunotherapeutic treatment with an anti-c-Kit monoclonal antibody prevented the development of Tregs, tumor-specific T cell anergy and tumor angiogenesis.[14] In view of this role of c-Kit, we hypothesize that modulation of c-Kit signaling via tivozanib may have a novel role in reversing tumor-induced immune suppression. In RPCI studies, levels of

ERK2 phosphorylation will be compared between c-Kit+ and c-Kit− immune cells to allow us to address the role of c-Kit expression on immune cells as the mechanism by which tivozanib modulates the immunosuppressive network in advanced stage HCC patients. Results will be correlated to outcome measurements on patients (progressed, median survival time.

Targeting of c-Kit to prevent MDSC and Treg accumulation in murine tumor models has been successfully demonstrated with sunitinib.[15,16] Importantly, in our studies with HCC patients we have shown that multifocal immunosuppressive networks are present at high levels in patients compared to healthy controls and that they are reduced following sorafenib treatment (provided in Preliminary Data). Specifically, the frequency of MDSCs and Tregs decreased in advanced stage HCC patients following their treatment with sorafenib.

The downstream signaling effect of receptor tyrosine kinase (RTK) inhibitors, such as tivozanib, is modulation of mitogen-activated protein (MAP) kinase phosphorylation. Although RTKs converge on several MAP kinases, this study will examine tivozanib-mediated modulation of ERK2 phosphorylation by high-throughput multi-parameter flow cytometry. In addition, this study will measure the extent of intracellular ERK2 phosphorylation as this adaptor signaling molecule plays a critical role in the function of immunosuppressive and effector immune cells. The expression level of c-Kit and level of ERK2 phosphorylation will be examined in circulating MDSC and Tregs analyzed from patients with advanced, inoperable hepatocellular carcinoma receiving tivozanib as part of a Phase 2 study. One prediction is that ERK2 phosphorylation will decrease in c-Kit+ MDSC and Tregs upon treatment with tivozanib. Diminished c-Kit signaling will therefore coincide with a reduction in the frequency of MDSC and Tregs relative to effector anti-tumor T cells.

1.7 Risks and/or Benefits

The known adverse events from tivozanib include diarrhea, fatigue, asthenia, hypothyroidism, dysphonia, hypertension, palmo-plantar erythrodysesthesia, and alopecia. Refer to **Table 1** and the current Tivozanib Investigator Brochure.

2 RATIONALE

HCC is a common malignancy worldwide and rising in incidence in the United States. Most patients have incurable/advanced disease at presentation. The subset of Child Pugh Class A and early Class B liver dysfunction patients have been shown to benefit from sorafenib which is considered the ‘standard of care’; as it improved overall survival and PFS when compared with placebo in well conducted large randomized Phase 3 trials despite a lack of radiographic response. The time to symptom improvement endpoint, however, was not met and median survival still remains less than 11 months. Targeting angiogenesis is now the backbone of HCC therapy, but better agents and biomarkers that define subsets of patients who are likely to derive the most benefit from such therapies are still needed. Tivozanib is an oral potent inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3 and represents a tolerable and effective treatment for VEGF driven malignancies based on data from 12 completed trials in cancer patients. It showed safety and efficacy in renal cell cancer and is being evaluated in combination with sorafenib in Phase 3 studies in RCC. Given that HCC is a highly vascular VEGF driven malignancy and like

RCC, is a malignancy where sorafenib has proven efficacy, we hypothesize tivozanib may have meaningful clinical benefit. Although HCC pathogenesis is driven by many factors, of the identified angiogenic factors, VEGF is the most potent and specific and has been identified as a crucial regulator of both normal and pathologic angiogenesis. *In vivo* data suggest that VEGF inhibition with tivozanib has therapeutic potential in HCC. Elevated VEGF levels correlate with advanced stage and poor prognosis in HCC. The preclinical efficacy of tivozanib in HCC models, the tolerability in early clinical trials and success of a targeting angiogenesis in HCC form the rationale for our hypothesis that tivozanib may be tolerable and improve PFS in HCC patients beyond 24 weeks, the expected median PFS for patients receiving sorafenib. In vitro, sorafenib has been reported to have anti-HCV effects and in recently completed human studies we have seen significant differences in survival using baseline immune cell numbers. It is expected that some effects of novel multi-targeted TKIs maybe immune mediated and hence propose to test this hypothesis prospectively using validated assays already in place.

3 OBJECTIVES

3.1 Primary Objective

The primary endpoint of the study is PFS at 24 weeks in patients with advanced HCC. Patients who remain alive without evidence of disease progression (per RECIST) for at least 24 weeks after enrollment will be considered PFS responders.

3.2 Secondary Objectives

- To determine the safety of tivozanib in HCC.
- To determine the OS and clinical benefit rate (CR, PR and SD) by RECIST.
- To determine the steady state PK and soluble VEGFR-2 baseline/ change with tivozanib and use modeling to correlate exposure with biomarker change and the primary outcome measure of PFS.
- To determine the change in viral load (HBV and HCV) during therapy in patients with HBV or HCV associated HCC.
- To determine the change in tumor marker (alfa fetoprotein) with tivozanib therapy is in the effect of tivozanib on several tumor-associated immune response markers.

3.3 Study Design

This is a multicenter Phase 1b/2 study of tivozanib in patients with advanced inoperable hepatocellular carcinoma.

3.3.1 Phase 1b

Based on monotherapy tolerability data of tivozanib from 14 studies an oral dose of 1.5 mg daily has been recommended for Phase 2 testing. Since tivozanib has not been tested in HCC patients, and HCC patients who are candidates for systemic therapies tend to have Child Pugh Class A or early B cirrhosis, this study will start with a Phase 1 dose escalation study.

The Phase 1b portion of the study follows a modified 3 + 3 design. Typical 3 + 3 studies start at the lowest dose. This modified design starts at patients at the middle dose. The first 3 patients will be treated with a 1 mg oral once daily dose. Depending on observed toxicity rates, the dose may be deescalated to 0.5 mg, or escalated to 1.5 mg. The standard 3 + 3 dose (de)escalation rules will be followed, as specified in the table below. Dose level will be considered, tolerable following completion of 1st cycle of treatment. The dose of 1 mg daily, was found to be the tolerated dose in the phase 1b portion and will be the phase 2 dose.

Number of Patients with DLT at a Given Dose Level	Escalation Decision rule
0 out of 3	Enter 3 patients at the next higher dose level.
≥ 2 out of 3	Stop dose escalation and declare this dose level as the maximally administered dose (highest dose administered). Enroll 3 additional patients at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter 3 more patients at this dose level. If 0 of these 3 patients experience DLT, proceed to the next dose level. If ≥ 1 of this group suffer DLT, then stop dose escalation, and declare this dose as the maximally administered dose. Enroll 3 additional patients at the next lowest dose level if only 3 patients were treated previously at that dose.
≤ 1 out of 6 at the highest dose level below the maximally administered dose	This is generally the recommended Phase 2 dose. At least 6 patients must be entered at the recommended Phase 2 dose

3.3.2 Phase 2

The Phase 2 portion is a two-stage, single arm, unblinded trial allowing for early termination in case of futility. The 6 Phase 1b patients treated at the recommended Phase 2 dose will be included in the Phase 2 trial. Details are provided in **Section 11**.

3.3.3 Treatment Cycle

A cycle will be defined as 28 days. At the full dose, this will be 21 days on treatment with 7 days off. However, in the case of a dose reduction patients will be treated starting on day 1 of the cycle, every other day for 10 doses. Treatment will continue until progression or toxicity. Response/ progression will be assessed Q 8 weeks or q 2 months by CT scan. (MRI when patients have contraindication to CT). Steady state PK will be assessed at Day 15, as this is the first study in patients with liver dysfunction.

3.4 Target Accrual and Study Duration

Between 6 and 18 patients will be enrolled in the Phase 1b portion of this study. Six Phase 1b patients treated at the recommended Phase 2 dose will be included in the Phase 2 portion of this study. An additional 31 patients will be enrolled for the Phase 2 portion, for a total Phase 2 sample size of 37. This study is expected to accrue 9 patients to the Phase 1b, for a combined

sample of size of 40 (9+37-6). If 18 patients are enrolled in the Phase 1b, the maximum combined sample size will be 49 (18+37-6).

Accrual is expected to take up to 4 years. The duration the patient will be on study will be approximately 2 years.

4 SUBJECT SELECTION

4.1 Inclusion Criteria

To be included in this study, subjects must meet the following criteria:

1. Advanced staged HCC (unresectable and not amenable to local or regional therapy; or metastatic HCC). The diagnosis of HCC should be based on at least one of the following:
   - MRI or CT consistent with liver cirrhosis AND at least one solid liver lesion measuring ≥ 2 cm, with characteristics arterial enhancement and venous washout regardless of AFP levels.
   - AFP ≥ 400 ng/mL AND evidence of at least one solid liver lesion ≥ 2 cm regardless of specific imaging characteristics on CT or MRI.
   - Histological/cytology biopsy confirming HCC.
2. Patients must have measurable disease per RECIST 1.1 criteria defined as at least one lesion that can be accurately measured in at least one dimension, and that has not been the target of local or regional therapy including transarterial chemoembolization, intra-arterial chemotherapy, ethanol or radiofrequency ablation.
3. Age ≥ 18 years.
4. Life expectancy of greater than 3 months.
5. Patients must have organ and marrow function as defined below:
   - Child-Pugh liver function Class A. (Appendix G)
   - AST ≤ 5 x Institutional upper limits of normal (ULN)
   - Total bilirubin ≤ 3 mg/dL
   - INR ≤ 2.0 (unless due to therapeutic warfarin use)
   - Serum albumin > 2.8 g/dL
   - Creatinine ≤ 1.5 x Institutional ULN
   - ANC ≥ 1200/mm^3
   - Platelets ≥ 60,000/mm^3
   - Hgb ≥ 8.5 g/dL

NOTE: The lower absolute neutrophil count (ANC) and platelet cut-offs are designed to accommodate the large number of patients with HCC who have portal hypertension and splenic

sequestration. Given the adverse profile of tivozanib these reduced cut-offs are thought to be safe.

6. Patients must not have any evidence of bleeding diathesis or active gastrointestinal bleeding.
7. Patients must not be known to be HIV positive; drug-drug interaction with study medication and HIV medications is not well characterized and could lead to unwanted side effects.
8. Patients must not have other uncontrolled intercurrent illnesses (excluding HBV or HCV). This includes (but is 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.
9. Sexually active fertile patients (male and female), and their partners, must agree to use medically accepted methods of contraception during the course of the study and for 3 months after the last dose of the study drug.
10. Female patients of childbearing potential must have a negative pregnancy test at screening.
11. Have an ECOG Performance Status of ≤ 2. Refer to **Appendix B**.
12. Subject or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

4.2 Exclusion Criteria

Subjects will be excluded from this study for the following:

1. Patients who have had prior anti-angiogenic therapy, including but not limited to sorafenib, brivanib, bevacizumab, or sunitinib.
2. Patients who have had any prior line of systemic therapy including cytotoxic agents or molecularly targeted agents for advanced/unresectable disease. Any number of prior regional therapies with transarterial chemoembolization (TACE), brachytherapy with Yttrium-90 microsphere, intra-arterial chemotherapy, surgery, or ablative therapy is allowed.
3. Prior liver transplantation and on immunosuppression; drug-drug interaction with study medication and immunosuppression is not well characterized and could lead to unwanted side effects.
4. Known symptomatic or uncontrolled brain metastases or epidural disease.
5. Patient has a corrected QT interval (QTcF) > 500 ms at screening.
6. The patient is unable to swallow pills or diagnosed with a gastrointestinal disorder that are likely to interfere with the absorption of the study drug or with the patient's ability to take regular oral medication.
7. The patient is pregnant or breastfeeding.
8. Patients with second primary cancer (except adequately treated nonmelanoma skin cancer, curatively treated in-situ carcinoma of the cervix or superficial bladder cancer, or other solid

tumors including lymphoma without bone marrow involvement curatively treated with no evidence of disease for ≥ 5 years).

9. The patient has a previously identified allergy or hypersensitivity to components of the study treatment formulation.
10. Patients receiving any medications or substances that are strong inducers of CYP3A4 are ineligible. Moderate and mild inducers of CYP3A4 should be used with caution, as they may reduce the efficacy of Tivozanib.
11. Urine protein: creatinine ratio > 1 see (Appendix- H)

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study.

5 INVESTIGATIONAL PRODUCT

5.1 Active Substance and Source

Tivozanib is formulated for oral administration as a white opaque number 4 gelatin capsule. Tivozanib 1 mg, capsules will be used in this study.

5.2 Drug Shipment

Tivozanib, manufactured by AVEO/Astellas Pharmaceuticals, will be shipped to the participating site from the drug depot.

Drug shipment records will be retained by the investigational pharmacist or designee.

5.3 Storage and Stability

The Investigator or designate is responsible to store and dispense the investigational product and will be responsible for ensuring that study drug is securely maintained in a locked, limited-access facility, in accordance with the applicable regulatory requirements.

Tivozanib is to be stored at room temperature (15˚C - 25˚C), in a dry place, and in a secure location.

Tivozanib bottle labels will bear the appropriate label text for investigational agents, as required by governing regulatory agencies.

Complete study drug information (including packaging, labeling, storage and disposition) is provided in the Tivozanib Investigator's Brochure (IB).

5.4 Handling and Disposal

The Investigator or designee will be responsible for dispensing and accounting for all investigational drug, exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken per the institution's

environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

All products dispensed will be recorded on a product accountability record. Records of product lot numbers and dates received will be entered on a product accountability form. This record will be reviewed by the Sponsor's staff or representative during periodic monitoring visits. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

6 TREATMENT PLAN

6.1 Preparation and Administration

Treatment will be administered in an outpatient basis. Reported adverse events (AEs) and potential risks are described in **Section 9**. Appropriate dose modifications are described in **Section 0**. No other investigational or commercial drugs or therapies other than that described below may be administered with the intent to treat the subject.

The amount of tivozanib dispensed to the subject at the beginning of each dosing cycle will be sufficient to allow for 3 weeks (21 days) of consecutive once-daily dosing of tivozanib. Only 21 capsules will be dispensed per cycle. In the case of a dose reduction patient will be given 10 capsules for every other day dosing for 10 doses.

Tivozanib capsules will be dispensed for oral use only and taken daily for 21 days and off for 7 days. The intended dose is 1 mg daily on Day 1 through Day 21 and no drug on Day 22 through Day 28, in a 28-day cycle. Patients start Cycle 2 after Day 28 and repeat dosing from Day 1 to Day 21. In the case of dose reduction patients will be intended to take 1 mg every other day starting on Day 1. After 10 doses (every other day) patients will have no drug through the end of the 28-day cycle. Patients will start the next cycle after Day 28 of the previous cycle.

The prescribed daily dose of tivozanib hydrochloride is to be taken, once per day (or every other day in the case of a dose reduction), preferably in the morning, with water, and with or without food. Only one capsule of tivozanib hydrochloride should be taken each dose. If a dose is vomited or otherwise missed that day for any reason, the dose for that day should not be made up. The next dose should be taken as prescribed at the next scheduled time (i.e., one capsule is to be taken even if the previous dose was vomited or otherwise missed; additional dose(s) should not be taken at any time to make up for any missed dose(s)). Grapefruit and grapefruit juice should be avoided during the study.

In the event that tivozanib hydrochloride dosing is interrupted, the duration of cycle/treatment will not be extended; doses missed during the interruption will be captured as omitted rather than delayed.

Figure 1. Dosing Schema

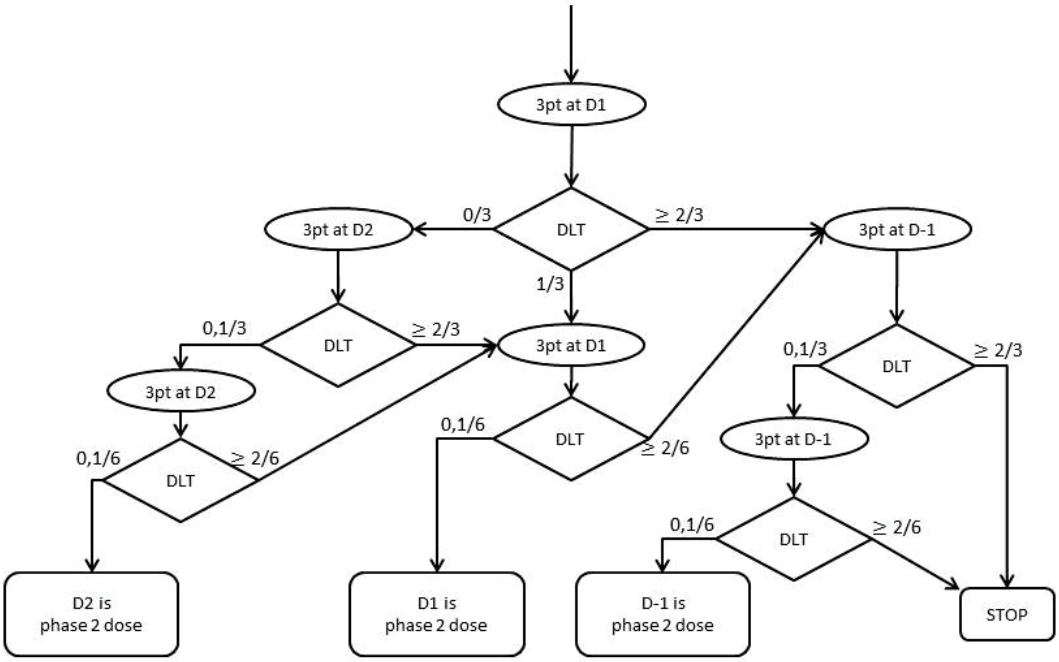


Table 2. Dose Schedule

Level	Dose
-1*	0.5 mg PO daily for 21 days, then 7 days off (q 28 days cycles)
1 (starting dose)	1 mg PO daily for 21 days, then 7 days off (q 28 days cycles)
2	1.5 mg PO daily for 21 days, then 7 days off (q 28 days cycles)

***0.5 mg not manufactured (Per Amendment #11), please see section 6.2.4**

6.2 Dose Modifications

6.2.1 Definition of Dose-Limiting Toxicity

A DLT is any Grade 3 or higher toxicity that is possible, probable, or definitely related to study drug.

Management and dose modifications associated with the above AEs are outlined in the below sections. Dose level will be, considered tolerable following completion of 1st cycle of treatment.

Patients who experience a DLT will be dose reduced to the next lower dose cohort for continuation on the study.

6.2.2 Treatment Delay

Clinical judgment will be used to determine appropriate management of the subject experiencing any adverse event (AE). The suggested criteria for dose modification for tivozanib hydrochloride drug-related AEs are summarized in the table below.

Table 3. Tivozanib Hydrochloride Dose Modification Guidelines for Drug-Related Adverse Events

Drug-Related Adverse Events (excluding Hypertension[1])	Action Taken	Subsequent Dosing Modification
Grade 1	No dose interruption, or reduction required; adverse event management is at the discretion of the Investigator	None required; Dosing may continue at the same dose
Grade 2	No dose interruption, or reduction required; adverse event management is at the discretion of the Investigator	None required; Dosing may continue at the same dose
Grade 3	Interrupt dosing until toxicity resolves to grade ≤ 1	Dosing may resume at reduced dose (see below for dose reduction guidelines)
Grade 4	Interrupt dosing until toxicity resolves to grade ≤ 1.	Dosing may resume at reduced dose (see below for dose reduction guidelines).

1 Hypertension must be treated as described in Section 6.2.5 prior to any dose modification

6.2.3 Tivozanib Hydrochloride Dose Interruption

Subjects with drug related Grade 3/4 AEs should have their dose interrupted to allow for resolution of toxicities to grade $\leq$ 1. The exception is HTN, which must first, be treated as described in **Section 6.2.5** prior to any dose modification. Dosing interruptions of ≤ 2 weeks will be allowed. Upon resolution of toxicities to $\leq$ grade 1, dosing may resume at one dose level below the current dose, based on the Investigator's discretion for an individual subject's safety and tolerability. Doses missed during a drug interruption are not to be made up. If dosing is interrupted for > 2 weeks, dosing should be discontinued, unless there is clear benefit from treatment.

6.2.4 Tivozanib Hydrochloride Dose Reduction

Dose reductions of tivozanib by 0.5 mg/day will be required for patients with $\geq$ Grade 3 drug-related adverse events. This excludes grade $\geq$ 3 hypertension, nausea, vomiting, and diarrhea, without adequate supportive care. Hypertension, nausea, vomiting, and diarrhea may resume at the same dose level after adequate supportive care, in the investigators opinion it is safe to proceed at the same dose level. If hypertension, diarrhea, nausea, and vomiting recurs despite adequate supportive care tivozanib must be dose reduced, up to 2 dose reductions are allowed. Once a patient's dose of tivozanib is reduced, it may not be re-escalated through the

remainder of the patient's participation in the study. 1 mg capsules every other day for 10 doses will be used, as 0.5 mg capsules are no longer manufactured. According to the drug manufacturer AVEO Pharmaceuticals, this dosing regimen will achieve comparable exposure to 0.5 mg QD, based on the half-life of Tivozanib.

6.2.5 Management of Hypertension

Hypertension (HTN) that occurs during study treatment must be treated with anti-hypertensive drugs prior to any dose reduction. Recommended management of HTN for subjects receiving study drug is presented in **Appendix C** and **Appendix D**.

Persistent HTN is defined as 2 consecutive elevated blood pressure (BP) measurements preferably taken in the clinic and obtained at least 1 hour apart. It is recommended that 24-48 hours should elapse between the decision steps. For a listing of recommended DHP (dihydropyridine) calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin receptor blockers, see **Appendix D**, Recommended Anti-hypertensive Medications. **Note:** If a subject has controlled HTN on an anti-hypertensive regimen at baseline and then develops a worsening of HTN requiring more intensive therapy than the previous regimen, this would be considered Grade 3 HTN.

6.3 General Concomitant Medication and Supportive Care

Agents known to be Cytochrome P450 (CYP3A4) strong inhibitors and inducers must be avoided for the duration of tivozanib treatment. Appropriate replacement drugs will be prescribed with the help of clinical Pharmacist. An individual patient's risk/benefit should be taken into account when administration of a CYP3A4 inhibitor or inducer is required as these agents may decrease serum concentrations of tivozanib, potentially impairing efficacy. For a complete list and additional information, refer to the following website: http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

Since there is a potential for interaction of tivozanib with other concomitantly administered drugs through the CYP3A4 system, the electronic case report form (eCRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 inhibitors and inducers.

Patients may be pretreated for nausea and vomiting with appropriate anti-emetics.

6.4 Treatment Discontinuation

Patients with non-hematologic Grade 4 drug related adverse events will permanently discontinue study drug unless the patient has experienced objective evidence of benefit and the investigator has determined that it is reasonably safe to continue treatment despite the occurrence of a life threatening adverse event. If a patient has dose interruption ≤ 2 weeks from the next scheduled dose, missed doses will not be made up (i.e., cycle duration will remain unchanged). If any drug-related toxicity results in interruption of dosing of > 2 weeks from the next scheduled dose, the subject should be discontinued from tivozanib, unless there is clear benefit from treatment.

Upon treatment discontinuation, all end of study evaluations and tests will be conducted. All subjects who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the subject's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Treatment-related toxicity
- Toxicity unrelated to treatment
- Investigator judgment
    - The Investigator may withdraw a subject if, in his/her judgment, it is in the subject's best interest to do so.
- Noncompliance
- Subject voluntary withdrawal
    - A subject may withdraw from the study at any time, for any reason. If a subject discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Early withdrawal of subject(s)
- Sponsor decision.

6.5 Compliance

Patients will be instructed to record date for each day and the total number of pills taken each day (**Appendix E**). Dosing compliance will be monitored at each clinic visit. Phase 1b patients who do not complete at least 80% of the prescribed dose in Cycle 1 for reasons other than DLT will be replaced. Otherwise, if a patient has 2 consecutive cycles of less than 50% compliance, the patient will be deemed non-compliant and come off study. At the discretion of the investigator, if the patient is benefiting from the treatment the patient may continue on study. Patients who come off study, due to non-compliance, will not be replaced.

7 STUDY PROCEDURES

7.1 Baseline Evaluations

The following will be performed within 2 weeks prior to treatment start and prior to receiving any study-related procedures:

- Informed consent: Must be completed prior to receiving any study-related procedures.
- Medical history

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, body weight, and height)
- Hematology (i.e., complete blood count (CBC) with differential, absolute neutrophil count (ANC), and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- Alpha-Fetoprotein (AFP)
- PT/INR
- Urine pregnancy test in females of childbearing potential (performed within 1 week prior to treatment start).
- ECOG Performance Status
- Electrocardiogram
- CT Scan- (within 4 weeks)
- Concomitant Medications (Any ongoing medications with an onset within 1 week of first dose of study drug.)
- Hep B and C testing (if unknown). Blood collection of about 4 teaspoons will be drawn from your arm for HBsAg, (the surface antigen of the hepatitis B virus (HBV). And Hep C Ab with verification.
- Urine will be collected to calculate urine protein and creatinine ratio.

7.2 Evaluations Performed at Cycle 1, Day 1

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, and body weight)
- Hematology (i.e., CBC with differential, ANC, and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- PT/INR
- Pharmacokinetic/Pharmacodynamic sVEGF-2 Sampling
- Immune Biomarker Sampling
- ECOG Performance Status
- Tivozanib (Patients will be administered tivozanib in clinic following their pre-dose pharmacokinetic/pharmacodynamic sample).
- Concomitant Medications
- If either (or both) HBsAg or Hep C Ab with verification are positive, blood will be collected for viral load anytime pre-treatment. (Viral load test: Hep B (or C) virus PCR, Quant).

7.3 Evaluations Performed at Cycle 1, Day 15 (± 3 Days)

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, and body weight)
- Hematology (i.e., CBC with differential, ANC, and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- PT/INR
- Pharmacokinetic/Pharmacodynamic sVEGF-2 Sampling
- ECOG Performance Status
- Tivozanib (Patients will be administered tivozanib in clinic following their pre-dose pharmacokinetic/pharmacodynamic sample).
- Concomitant Medications
- Adverse Events

7.4 Evaluations Performed at Day 1 of Each Cycle After Cycle 1 (± 3 Days)

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, and body weight)
- Hematology (i.e., complete blood count (CBC) with differential, absolute neutrophil count (ANC), and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- PT/INR
- ECOG Performance Status
- Tivozanib (Patients will take at home).
- Concomitant Medications
- Adverse Events
- Alpha Fetoprotein (AFP): Biomarker to detect hepatocellular cancer every 2 cycles, starts with cycle 3 (Only if elevated at baseline).
- A computed tomography scan (CT scan) every 2 cycles, not to exceed 10 weeks apart.
- Urine will be collected to look at protein and creatinine levels every 2 cycles starting with cycle 3
- If either (or both) HBsAg or Hep C Ab with verification are positive, blood will be collected for viral load, (Hep B (or C) virus PCR, Quant) every 2 cycles starting with cycle 3

7.5 Evaluations Performed at Cycle 3, Day 1 (± 3 Days)

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, and body weight)
- Hematology (i.e., CBC with differential, ANC, and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- ECOG Performance Status
- AFP (Only test if AFP was elevated at baseline).
- PT/INR
- Immune Biomarker Sampling
- CT Scan
- Concomitant Medications
- Adverse Events
- Urine will be collected to look at protein and creatinine levels every 2 cycles starting with cycle 3
- If either (or both) HBsAg or Hep C Ab with verification are positive, blood will be collected for viral load. (Viral load test: Hep B (or C) virus PCR, Quant)

7.6 Evaluations Performed at 1 Year (± 7 Days)

- Immune Biomarker Sampling (When the patient progresses/comes off study drug or at 1 year whichever occurs first.)

7.7 Evaluations Performed at End of Treatment

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, and body weight)
- Hematology (i.e., CBC with differential, ANC, and platelets)
- Chemistry (comprehensive metabolic panel (i.e., AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin.
- CT Scan (Only if last CT scan was more than 28 days prior to end of treatment.)
- PT/INR
- Immune Biomarker Sampling (When the patient progresses/comes off study drug or at 1 year whichever occurs first.)
- ECOG Performance Status
- Concomitant Medications

- Adverse Events

7.8 Follow-Up: Medical Record review every 6 months for overall survival.

7.9 Schedule of Procedures and Observations

The schedule of procedures and observations for this study is summarized in **Table 4** below.

Table 4. Schedule of Procedures and Observations

Evaluation	Baseline	Cycle 1 Day 1	Cycle 1 Day 15 (± 3 days)	Day 1 of Each Cycle After Cycle 1 (± 3 days)	Cycle 3 Day 1 (± 3 days)	1 Year (± 7 days)	End of Treatment[15]
Informed Consent	X						
Medical History	X[1]						
Physical Examination , including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure, body weight, and height)[2]	X[1]	X	X	X	X		X
Hematology[3]	X[1]	X	X	X	X		X
Chemistry[4]	X[1]	X	X	X	X		X
HBsAg[17]	X						
Hep C Ab With Verification[17]	X						
Viral Load (Hep B(or C) virus PCR, Quant)		X[13]		X[13, 14]	X [13, 14]		
Alpha-Fetoprotein (AFP)	X[1]			X[5,14]	X[5, 14]		
PT/INR	X[1]	X	X	X	X		X
Pharmacokinetic/Pharmacodynamic and sVEGF-2 Sample Collection		X[6]	X[6]				
Immune Biomarker Sample Collection		X[7]			X[7]	X[7]	X[7]
Pregnancy Test (Urine)	X[8,1]						
Urine Protein/Creatinine ratio	X[1]			X[14]	X[14]		
ECOG Performance Status	X[1]	X	X	X	X		X
Electrocardiogram	X[1]						
CT Scan	X[16]			X[12]	X[12]		X[9]
Tivozanib		X[10]	X[10]	X			

Evaluation	Baseline	Cycle 1 Day 1	Cycle 1 Day 15 (± 3 days)	Day 1 of Each Cycle After Cycle 1 (± 3 days)	Cycle 3 Day 1 (± 3 days)	1 Year (± 7 days)	End of Treatment[15]
Concomitant Medications	X[11,1]	X	X	X	X		X
Adverse Events			X	X	X		X

1 Performed within 2 weeks prior to treatment start and prior to receiving any study-related procedures.
2 Height collected at baseline only.
3 Hematology (i.e., complete blood count (CBC) with differential, absolute neutrophil count (ANC), and platelets).
4 Chemistry (comprehensive metabolic panel (i.e.; AST, ALT, total bilirubin, alkaline phosphatase, creatinine, potassium, glucose, and calcium) and serum albumin).
5 Only test if AFP was elevated at baseline.
6 Refer to Section 7.10.
7 Pre-dosed cycle 1 day 1, cycle 3 day 1,when the patient progresses/comes off study drug or at 1 year whichever occurs first. Refer to Section 7.11.
8 Performed within 1 week prior to treatment start.
9 Only if last CT scan was more than 28 days prior to end of treatment.
10 Patients will be administered tivozanib in clinic following their pre-dose pharmacokinetic/pharmacodynamic sample.
11 Medications ongoing within 1 week prior to first dose of study drug.
12 CT every 2 cycles starting with cycle 3, with maximum of 10 weeks apart.
13 Collect viral load, pre-treatment, if either (or both) HBsAg or Hep C Ab with verification are positive & every 2 cycles starting with cycle 3.
14 Every 2 cycles starting with cycle 3.
15 Patients will be followed for overall survival, every 6 months by medical record review
16 CT scan within 4 weeks prior to treatment start.
17 Only if unknown

7.10 Pharmacokinetic/Pharmacodynamic Blood Sample Collection and Processing

Plasma blood samples for pharmacokinetic/pharmacodynamic analysis of tivozanib levels will be collected via venipuncture using (2) 4 mL purple-top EDTA collection tubes per time point for PK and blood samples for sVEGF-2.

Pharmacokinetic/pharmacodynamic and sVEGF-2 sample collection will be obtained on:

- Cycle 1, Day 1 at pre-dose, 2 hours, and 4 hours post-dose.
- Cycle 1, Day 15 at pre-dose, 2 hours, and 4 hours post-dose.

Plasma will be separated from the total blood within 30 minutes following the extraction. The screw cap polypropylene cryogenic tube will be labeled with the subject identifier, subject's initials, subject's study number, clinical study number, protocol time point, dose, and protocol day. The label for each subject's sample will be supplied by RPCI. The samples will be placed in ice immediately and then frozen at -70ºC or below until analyzed. After hours, samples will be processed in Lab Medicine.

NETWORK SITES: Follow directions above for sample collection and processing. The cryogenic tubes should be labeled with the Subject ID # (unique to Network patients), initials, the participant's study number, clinical study number, protocol time point, dose, and protocol day. The samples will immediately be frozen at -70ºC or below (samples are to be stored until requested for batch mailing). Samples are to be batch shipped frozen, on dry ice.

Frozen samples will be shipped via Fed Express Overnight on dry ice with delivery on Mon-Fri. NO SATURDAY DELIVERY. Do not ship on a Friday or the day before a holiday.

Address shipments and any questions regarding specimen processing to:

Roswell Park Cancer Institute
Bioanalytics, Metabolomics & Pharmacokinetics Core Facility
Center for Genetics and Pharmacology, Room L1-140
Refer to Study Number– I 229112
Elm & Carlton Streets
Buffalo, New York 14263

PKPDCore@RoswellPark.org

For additional information regarding the handling of pharmacokinetic samples please contact RPCI's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility laboratory at 716-845-3303 (Tel) or 716-845-1579 (Fax).

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens.

This is required for both observational and interventional clinical studies collecting clinical samples.

7.11 Immune Biomarker Blood Sample Collection and Processing

Blood samples for measuring immune correlatives by flow cytometry will be collected via venipuncture using (4) 10 mL green-top heparinized collection tubes. Tubes must be completely filled, and kept at room temperature until processing. Tube will be labeled with the subject identifier, subject's initials, subject's study number, clinical study number, protocol time point, and protocol day. The label for each subject's sample will be supplied by RPCI. Peripheral blood mononuclear cells (PBMC) will be isolated immediately by Ficoll-Paque™ PLUS density gradient centrifugation of blood samples. Refer to **Appendix F2.** Patient Pill Diary(Dose Reduced)

Study No.: _____ **Patient's Name: _____**

Drug Name: _____ **Cycle:_____**

Medical Record No.: _____

Study Medication Calendar for Tivozanib

You should swallow your medication, unchewed, with a large glass of water (about 8 oz.) in the morning every day with or without food. On days of a scheduled clinic visit, you should take your dose at the clinic after visit procedures are completed. If a dose is vomited or if you miss your dose for any reason, the dose should not be made up.

Please complete this calendar on a daily basis immediately after you take your pills. Fill in the date for each day and write the total number of pills you take each day.

Start Date: _____

Take 1 pill each time.

Cycle Day	Day 1**(dose1)	Day 2	Day3(dose2)	Day 4	Day5(dose3)	Day 6	Day7(dose4)
Time							
Date							
Number of pills taken							

Cycle Day	Day 8	Day 9(dose5)	Day 10	Day11(dose6)	Day 12	Day13(dose7)	Day 14
Time							
Date							
Number of pills taken							

Cycle Day	Day15(dose8)	Day 16	Day17(dose9)	Day 18	Day19(dose10)	Day 20	Day 21
Time							
Date							
Number of pills taken							

Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date	No medication is to be taken during this time.						
Number of pills taken							

****Do NOT take your pill on Day 1 until your blood is drawn and the study staff instructs you to take it.**

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$\_\_\_\_\%\ Compliance = \left(\frac{\_\_\_\_\_}{\_\_\_\_\_}\right) \times 100$$

Patient's Signature:_____ Date:_____
CRC Signature:_____ Date:_____
Investigator Signature:_____ Date:_____

Appendix G for the SOP for PBMC isolation.

Sample collection will be obtained on: Tube station 611

- Predose on Cycle 1, Day 1
- Cycle 3, Day 1
- When the patient progresses/comes off study drug or at 1 year whichever occurs first.

For patients enrolled at RPCI, four 10 mL green-top heparinized blood collection tubes will be sent to tube station 611 for processing on the same day. PBMCs from the tubes will be isolated by members of the Thanavala laboratory according to the SOP in Appendix F (Section 1). Cells will be stored in liquid nitrogen at RPCI as described in Appendix F Section 2 & 3. PMBCs will be analyzed by members of the Thanavala laboratory when all three samples from an individual patient are available in order to minimize assay variability.

For patients enrolled at Network Sites, four 10 ml green-top heparinized blood collection tubes will be processed on the same day at the Network Site. PBMCs will be isolated by trained, designated staff at the Network Site according to the SOP in Appendix F (Section 1). Cells will be stored in liquid nitrogen at the Network Site as described in Appendix F (Section 2 & 3). PMBCs will be shipped to RPCI via Federal Express overnight delivery on dry ice (Monday – Thursday only) when all three samples from an individual patient are available.

For Network sites, batched samples must be shipped overnight on dry ice Monday – Thursday only via Federal Express delivery. There is NO SATURDAY DELIVERY. Do not ship on a Friday or the day before a holiday.

Yasmin Thanavala, PhD
Roswell Park Cancer Institute
Department of Immunology, Room L5-302
Center for Genetics and Pharmacology
Elm & Carlton Streets
Buffalo, New York 14263

For additional information regarding the handling of laboratory samples please contact RPCI's Thanavala Laboratory at 716-845-8536, 716-845-8535, 716-845-4592 or Yasmin.Thanavala@RoswellPark.org.

Isolated PBMCs will be stored in liquid nitrogen in 90% human AB serum plus 10% DMSO. All 3 samples from an individual patient will be analyzed simultaneously to minimize assay variability. Extensive testing has been performed to compare fresh and frozen thawed samples for reproducibility and to ensure that the process of freezing does not influence the viability of cells types being evaluated. FACS analysis on the various cell subsets will be performed as

described in detail in the study methodology section. Plasma samples will be stored at −80˚C for future measurement of inflammatory and immunosuppressive cytokines.

Clinical therapy, outcome and baseline demographic data will be recorded and stored in the RPCI clinical research database. These will be merged with immune data analyzed in blinded fashion and provided to the statistician for analysis.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

8 EFFICACY EVALUATIONS

8.1 Objective Tumor Response

All protocol-defined imaging studies must be performed at the investigative site or sponsor-approved facility using protocol-defined parameters. 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. RECIST 1.1 will be used to assess objective tumor response.

8.2 Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size. Lesions with the longest diameter (short axis for lymph nodes) and are ≥ 10 mm (CT and MRI), ≥ 15 mm lymph nodes, > 20 mm CXR and are for accurate repetitive measurements (either by imaging techniques or clinically) will be chosen. A sum of the longest diameter (short axis for lymph nodes) of all target lesions will be calculated and reported as the baseline sum diameters. This will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

- **Complete Response (CR):** Disappearance of all target lesions. Any lymph nodes must have a reduction in short axis to < 10 mm. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Progressive Disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as references 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 5 mm. 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 diameter while on study. Subjects having a documented response with no confirmation of the response will be listed with stable disease.

8.3 Non-Target Lesions

All other small lesions (longest diameter < 10 mm or lymph nodes ≥ 10 mm to < 15 mm short axis) and non-measurable lesions (i.e., leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, blastic bone lesions, or abdominal masses / abdominal organomegaly identified by physical exam that is not measurable by imaging) should be identified as non-target lesions and indicated as present in the source documents at baseline. The general location will also be documented on the images drawing a regularly-shaped Region of Interest. Measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation.

- **Complete Response:** Disappearance of all non-target lesions and normalization of tumor marker level, if applicable. All lymph nodes must be non-pathological in size (< 10 mm short axis).
- **Non-Complete Response/Non-Progressive Disease:** Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the upper limits of normal.
- **Progressive Disease:** Appearance of 1 or more new lesions or the unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed at a later time.

8.4 Evaluation of Response

Time point response assessments will be performed every 8 weeks (timed to coincide with the end of a cycle) with a confirmatory assessment (required for non-randomized trials) within 6 weeks after a PR or CR is deemed. To determine time point response, refer to **Table 5** and below.

Table 5. Time Point Response Criteria (+/- non-target disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 6. Time Point Response Criteria (non-target disease only)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[1]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

1 Non-CR/non-PD is preferred over SD for non-target disease since SD is used as endpoint for assessment of efficacy in trials so to assign this category when no lesions can be measured is not advised.

The best overall response is the best response recorded from the start of study treatment until the end of treatment taking into account any requirement for confirmation. In general, the subject's best response assignment will depend on the achievement of both measurement and confirmation criteria and will be determined by combining the subject's status of target lesions, non-target lesions, and new lesions.

- **Residual Disease:** Any measurable tumor that was considered a target or non-target or new lesion ≥ 1 cm.
- **Symptomatic Deterioration:** Subjects with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not related to study treatment or other medical conditions should be reported as progressive disease due to "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment due to symptomatic deterioration. Symptomatic deterioration that may lead to discontinuation of treatment include, but is not limited to, symptoms such as:
    - Weight loss > 10% of body weight.

- Worsening of disease-related symptoms (e.g., worsening dyspnea, increasing pain/increasing requirement for narcotic analgesics).
- Decline in performance status of > 1 level on ECOG scale.

8.5 Confirmation Measurement

Response will be confirmed within 6 weeks.

8.6 Guidelines 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 when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm 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 5 mm or less. If CT scans have slice thickness greater than 5 mm, 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 which 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 subject to be considered in complete clinical response.
- **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:
- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- 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.
- 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.

9 SAFETY EVALUATION

9.1 Adverse Events

9.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be **ANY** unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

9.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

9.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

9.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

9.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

9.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant drugs administered to the patient.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the patient's clinical state, other therapeutic interventions, or concomitant drugs.

- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

9.1.3 Reporting Adverse Events (Network Sites see Appendix A)

Table 7. Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Table 8. Guidelines for Routine Adverse Event Reporting for Phase 2 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, the study patient is lost to follow up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

9.2 Serious Adverse Events

9.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a patient or patients, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.2.2 Reporting Serious Adverse Events (Network Sites see Appendix A)

All new SAEs occurring from the date the patient signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported.

The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 9.3.2 for details on reporting Unanticipated Problems.

9.2.3 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study patient is lost to follow up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

9.3 Unanticipated Problems

9.3.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data.
    - The characteristics of the patient population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 9.2**.

9.3.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. An Unanticipated Problem shall be submitted to the CRS Compliance Office as "Reportable New Information" in the Click system within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the UP to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance as "Reportable New Information" in the Click system.

Network Sites, see Appendix (A) for information regarding, Unanticipated Problem reporting.

9.4 Investigator Reporting: Notifying the Study Sponsor

Adverse events, both serious and non-serious, and deaths that occur during the patient's study participation (from time of the first dose of tivozanib, written informed consent through 30 days after receiving the last dose of tivozanib hydrochloride) will be documented. All subjects receiving at least 1 dose of tivozanib hydrochloride, whether completing the treatment period or not, should be followed for 30 days after the last dose of tivozanib hydrochloride. New AEs and changes in ongoing AEs or those with an unknown outcome must be followed for 30 days after the last dose of tivozanib hydrochloride. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es). The Investigator will determine the seriousness, intensity, and causality of an adverse

event associated with the use of the study drug (i.e., events where there is a reasonable possibility that the event may have been caused by the study drug).

All SAEs should be recorded on a Medwatch Form 3500A and sent to the FDA (if applicable), to AVEO Pharmacovigilance/Drug Safety, to the NCCN, and to the Principal Investigator/ CRS Network Coordinators at CRSNetworkCoordinators@RoswellPark.org.

SAE Reporting Contact Information: please submit SAE Reports to Parexel GPPG via email (AVEOsafety@parexel.com). If submission via email is not possible or delayed then submission via fax (NA +1-781-434-5957) is acceptable.

Pharmacovigilance/Drug Safety
AVEO Pharmaceuticals, Inc
One Broadway, 14th Floor
Cambridge, MA 02142
Fax: 1- 781 434 5957
E-mail: AVEOsafety@parexel.com

SAE Reporting Contact Information
National Comprehensive Cancer Network (NCCN)
Fax: (215)358-7699

9.5 FDA Reporting

This protocol is being conducted under a RPCI, IND and it is the responsibility of the study IND holder to report certain AEs or Unanticipated Problems to the FDA.

RPCI's Compliance Office will report Network Site reports to the FDA.

The following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening.

Or meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email: CRSCompliance@Roswellpark.org. The Compliance office will send the form to the Administrator of ORSP.

9.6 Procedures for Reporting Subject Death

Any death experienced by a subject after enrollment through 30 days of receiving the last dose of tivozanib hydrochloride, regardless of relationship to study drug, or any death that occurs more than 30 days after receiving study drug that is believed to be study drug-related must be promptly reported (within 24 hours of the investigator becoming aware of the event) by telephone, telefax, or e-mail transmission to AVEO Pharmacovigilance/Drug Safety. Reports of all on-study deaths must also be communicated promptly to the FDA (if applicable) and the appropriate Institutional Review Board (IRB) and/or reported in accordance with local law and regulations.

9.7 Procedures for Reporting Study Drug Overdose

An overdose of tivozanib hydrochloride should be reported if the dose of tivozanib hydrochloride administered is greater than the assigned dose for the subject.

Should a subject experience an overdose during the course of the study (whether symptomatic or not, and regardless of seriousness), the Sponsor-Investigator or qualified designee must complete a Medwatch Form 3500A to report the overdose to AVEO Pharmacovigilance/Drug Safety within 24 hours of first becoming aware of the overdose. Follow-up information on the outcome of the overdose should be forwarded to the AVEO Pharmacovigilance/Drug Safety.

Any event associated with, or observed in conjunction with, a product overdose (whether accidental or intentional) or a product abuse and/or withdrawal is considered an AE and should

be reported as such. If a SAE occurs in conjunction with the overdose, then the reporting time frame for a SAE should be followed.

9.8 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

All pregnancies, including pregnancies in female partners of male study participants, occurring from the date of ICF signature until 45 days following tivozanib hydrochloride administration must be documented. If it is confirmed that a study participant has become pregnant while participating in this trial, study drug administration will be discontinued immediately. Any pregnancy occurring during this study will be reported immediately to AVEO Pharmacovigilance/Drug Safety.

The Sponsor-Investigator must actively follow-up, document and report the outcome of any pregnancy even if the subject has withdrawn from the study. The Sponsor-Investigator will then report follow-up information to AVEO Pharmacovigilance/Drug Safety regarding the course of the pregnancy, including perinatal and neonatal outcome. Timelines vary according to the nature of the pregnancy outcome:

- For normal outcomes, AVEO Pharmacovigilance/Drug Safety should be notified within 45 days from birth/delivery.
- For abnormal outcomes, the fully completed Medwatch Form 3500A must be sent to AVEO Pharmacovigilance/Drug Safety according to the same procedures and timelines described for expedited AE reporting.

For questions related to safety reporting, contact: AVEO Pharmacovigilance/Drug Safety.

10 DATA AND SAFETY MONITORING

Phase 1 studies will be reviewed at the scheduled RPCI Phase 1 meetings and the minutes are forwarded to the IRB for review.

The RPCI Data and Safety Monitoring Board will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMB will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

11 STATISTICAL METHODOLOGY

This is a Phase 1b/2 study to assess the safety and efficacy of tivozanib therapy in HCC patients. The Phase 1b portion follows a modified 3+3 design (refer to **Section 3.3**), and provides limited toxicity information about tivozanib therapy in this patient population. The Phase 2 portion is a two-stage, single arm, unblinded trial allowing for early termination in case of futility. Phase 1b patients treated at the recommended Phase 2 dose will be included in the Phase 2 trial.

11.1 Phase 1b

To compensate for the current lack of toxicity information about tivozanib treatment in this patient population, the safety cohort begins by enrolling 3 patients at Dose Level 1. Then

modified 3 + 3 decision rules are followed to determine the recommended Phase 2 dose. Dose decision rules are specified in **Section 3.3**. A schematic of the Phase 1b design is shown in **Figure 1**. The recommended Phase 2 dose will be considered tolerable if DLT(s) are observed in at most 1 of 6 patients completing at least 1 treatment cycles at that dose level.

The Phase 1b design requires between 6 and 18 patients. Available information about tivozanib treatment suggests Dose Level 2 will be the recommended Phase 2 dose. Under this scenario, the safety cohort will consist of 9 patients. The 6 safety cohort patients treated at the recommended Phase 2 dose will be included in Stage 1 of the Phase 2 study.

Phase 1b patients who do not complete at least 80% of the prescribed dose in Cycle 1 for reasons other than DLT will be replaced.

11.2 Phase 2

The Phase 2 study proceeds in 2 stages, and allows for early stopping for futility. The primary endpoint is the percentage of PFS responders, defined as patients who survive without disease progression (per RECIST criteria) for least 24 weeks past enrollment.

Stage 1: Enroll $n_1 = 19$ patients, including the safety cohort patients treated at the Phase 2 dose. If 9 or fewer PFS responses are observed, the therapy will be deemed ineffective and the study will end. Otherwise, the study will proceed to the second stage.

Stage 2: Enroll an additional $n_2 = 18$ patients. If $T = 23$ or fewer PFS responses are observed among the total $n_1 + n_2 = 37$ patients, the therapy will be deemed ineffective; otherwise, it will be concluded that the therapy is promising. Under the null hypothesis of a 50% PFS rate at 24 weeks, the probability of stopping early for futility is 50%. Under the alternative of a 70% PFS rate, this probability is 3.3%. Patients who die or withdraw from the study prior to the 24th week will be counted as events. These patients will not be replaced. The nominal significance level of this design is $\alpha = 0.05$.

11.3 Sample Size Determination

Between 6 and 18 patients will be enrolled in Phase 1b portion. Six Phase 1b patients treated at the recommended Phase 2 dose will be included in the Phase 2 portion of this study. An additional 31 patients will be enrolled for the Phase 2 portion of this study, for a total Phase 2 sample size of 37. This study is expected to accrue 9 patients to the Phase 1b, for a combined sample of size 40 (=9+37-6). If 18 patients are enrolled in the Phase 1b, the maximum combined sample size is 49 (=18+37-6).

The sample size calculation is based on testing hypotheses concerning the proportion of the treated population alive and disease free 24 weeks after enrollment. Let p represent the proportion of patients surviving with no evidence of disease progression by RECIST at 24 weeks after enrollment. A true progression rate of less than p0 = 0.50 is considered unacceptable and evidence of such will deem the treatment not worthy of further study. The null and alternative hypotheses to be tested are H0:p = p0 versus H1: p > p0.

This 2-stage design requires a potential total of 37 patients to achieve approximately $1-\beta$ power to detect a difference of at least Δ percentage points (p0 versus p0 + Δ). For calculations in this study, p0 = 0.50, $\alpha = 0.05$, $1 - \beta = 0.80$ and $\Delta = 0.20$. With these design parameters, the sample size was calculated using the web calculator located at http://cryptnet.net/kepner/.[17]

11.4 Randomization

This is a nonrandomized study.

11.5 Demographics and Baseline Characteristics

Descriptive statistics and statistical plots will be used to summarize changes in biomarkers levels in blood and changes in viral titers. The paired t-test, Wilcoxon signed-rank test, and McNemar's test will be used for within-patient comparisons of changes in biomarker levels across times. The repeated measures modelling may be used to explore the effects of exogenous factors (i.e., diabetes, hepatitis, cirrhosis, etc.) on the changes in biomarkers and viral titers. The effects of biomarkers on clinical benefit rate (CR+PR) will be assessed using logistic regression and the effects on time-to-event data (overall survival and progression free survival) will be estimated by Cox model.

11.6 Efficacy Analysis

The efficacy sample includes all patients enrolled in the Phase 2 study, plus Phase 1b patients treated at the recommended Phase 2 dose. Patients removed from the study for non-compliance will be counted as events in the efficacy analyses.

The primary endpoint will be progression free survival at 24 weeks using standard RECIST criteria. All enrolled patients will be included in assessment of the primary endpoint. Time-to-progression will be descriptively analyzed using standard Kaplan-Meier estimation along with the corresponding descriptive statistics and 95% confidence intervals.

Secondary endpoints will include, overall survival, toxicity, clinical benefit rate (CR+ PR+ SD). Correlative endpoints will also include AFP response, antiviral effect (if any in those with HBV or HCV associated HCC) and exploration for any correlation between drug exposure (assessed by steady state PK) and response/ survival and toxicity by quartiles of drug exposure will be exploratory.

11.7 Safety Analysis

The safety sample will include all patients exposed to the study drug.

Toxicity frequency will be tabulated by grade across all dose levels and cycles for all patients in the safety sample, and for the subset treated at the recommended Phase 2 dose. These tables will be produced at 3 time points:

1. At the end of the Phase 1b study.
2. At the Phase 2 interim analysis.
3. At the Phase 2 final analysis.

11.8 Interim Analysis and Criteria for Early Termination of the Study

No explicit interim analysis is planned for the Phase 1b dose-finding portion of the study. However, during this phase, observed toxicities will be monitored and discussed weekly by RPCI's Phase 1 Committee. Potential early termination decisions are inherent in the Phase 1 study monitoring.

The Phase 2 design includes a formal interim analysis, allowing for early termination if the experimental treatment is deemed ineffective. Early termination rules are detailed in **Section 11.2**.

12 CORRELATIVE DATA ANALYSIS

12.1 Pharmacokinetic/Pharmacodynamic Analysis

Using data collected from PK and PD samples, a population PK/PD model will be developed to characterize the time course of tivozanib concentrations in relation to target increases in VEGF-A and decreases in sVEGFR-2. Subsequently, diagnostic plots will be created to ascertain whether a trend exists in VEGF-A and sVEGFR-2 changes, immunological biomarker response, viral load and progression free survival (PFS). If trends exists, these endpoints will also be included in the PK/PD model. A variety of compartmental population pharmacokinetic structural models will be evaluated using a nonlinear mixed effects modeling approach, NONMEM®. The physiologic pharmacokinetic models explored will be described by the estimation of mean structural model parameters (e.g., plasma volumes of distribution and clearances), the magnitude of inter-individual variability (IIV) in these parameters, and the magnitude of residual variability (RV). The model for IIV will be assumed that the variance is proportional with respect to the typical value of the pharmacokinetic parameter, and the estimates are presented as percent coefficients of variation. This analysis will include evaluation of the influence of a limited number of patient covariates, such as patient demographics along with other patient cofactors, on the variability in select PK/PD parameters. Based on our sparse sampling approach, prior information on PK parameter estimates and variances can be used with a Bayesian estimation method to drive the model analysis. This allows for a smaller number of samples to be taken while still providing enough information to achieve sufficient model fitting. For each model, the fit will be assessed by examination of several diagnostics. For comparisons of hierarchical models, the change in the minimum value of the objective function (MVOF), a statistic that is proportional to minus twice the log likelihood of the data, will be examined. This change represents a statistic which is asymptotically distributed like χ^2, with degrees of freedom equal to the number of parameters added to or deleted from the model. For example, a change in the MVOF of ≥ 7.88 between two hierarchical models represents a statistically significant difference at an α-level of 0.005 for the addition of one parameter ($df = 1$). The goodness-of-fit of NONMEM® analyses will be additionally assessed by examination of the following: (1) scatterplots of measured concentrations and weighted residuals versus population predicted concentrations, and weighted residuals versus time since first and last dose; (2) scatterplots of measured concentrations, individual weighted residuals, and absolute individual weighted residuals versus individual predicted concentrations; (3) the precisions of the parameter estimates

as measured by the percent standard error of the mean (%SEM = standard error/parameter estimate x 100%); (4) changes in the estimates of interindividual (IIV) and residual variability (RV); and (5) histograms, boxplots, and plots of quantiles of individual and population weighted residuals versus quantiles of the normal distribution (QQ plots).

12.2 Immune Biomarker

Interest is in the effect of tivozanib on several tumor-associated immune response markers. One pre- and two post-treatment measurements will be obtained from each tivozanib-treated patient who completes at least two treatment cycles. Post-treatment measurements will be obtained at Day 56, and at the end of follow up (either documented disease progression, death or one year of progression-free survival). These immune marker measurements will be presented as continuous variables.

Primary interest is in the change in immune system measurements obtained at baseline and the end of Cycle 2 (Day 56). Using (linear) mixed model methods, the change in expression measurement outcomes will be modeled against a random patient effect, and fixed-effects for time (pre vs post), patient status at Day 56 (progression/death or not) and the status-by-time interaction. Fixed effects for other exogenous effects (e.g. presence of hepatitis B/C, cirrhosis, diabetes, etc) may also be included. Mixed model methods account for the hierarchical (within patient) covariance in the outcome measurements, and missing outcome observations for patients who die/withdraw before finishing Cycle 2.

The magnitude, direction and reliability of the change estimate will be described using the fitted parameter estimate for time and its 95% confidence limits. If the immune measurements change estimate, depends on the patient status (i.e., if the status-by-time interaction is statistically significant), the change will be estimated and tested within progression strata using contrast statements.

For power calculations, we assume paired (pre/post) standardized observations are available for 37 patients, and significance threshold of 0.05. If the change in immune measurements does not depend on progression status, then this experiment can be considered as a paired t-test. This test has 80% power to detect a 0.47 standard deviation shift in mean immune measurement.

If the change in immune measurements depends on progression status, we further assume a within-patient correlation coefficient of 0.2. This ANCOVA-type model combination gives 80% power to detect a 1.6 standard deviation difference in the progression strata specific regression line slopes.

Appropriate functional forms for the mean function will be explored using means by time box plots, scatter plots, and other methods. Within-patient and time correlations will be explored using profile plots. Compliance with modeling assumptions will be assessed by examination of residual plots. PRESS residuals will be utilized to identify possible influential observations. The marker measurements may be transformed to satisfy distributional assumptions. Correlations between marker values will be tested using Spearman Rank correlation methods, and visualized using spline-augmented scatter plots.

In all, we expect a total of 8 immune measurements for each patient at each time point (four marker measurements, and four phosphorylation measurements). Methods proposed by Hochberg will be used to control the overall (family-wise) Type I error rate to 0.05.

In exploratory analyses, the three expression measurement outcomes for each patient will be modeled using (linear) mixed model methods with fixed-effects for time, cumulative dose, patient status (progressed, died, other), exogenous factors and the second order interactions with patient status. The effects of baseline expression measures on progression free and overall survival will be assessed using multivariable proportional hazards models, with adjustment for possible confounding factors. Survival distributions will be obtained using the product-limit based Kaplan Meier methods, with descriptive quantities such as median survival time and corresponding 95% confidence intervals.

13 ETHICAL AND REGULATORY STANDARDS

13.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each subject (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the subject is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the subject log and subject records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining subject authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the subject is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices.

13.2 Informed Consent

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each subject or the subject's legally authorized representative in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the subject according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional

information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The subject should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the information sheet and of the signed consent form to the subject and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the subject file. At any stage, the subject may withdraw from the study and such a decision will not affect any further treatment options.

14 STUDY RESPONSIBILITIES

14.1 Data Collection

Data entry into the database is to be completed in a timely fashion (within 30 days) after the subject's clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Form, which is handled in an expedited fashion.

Data management activities will be performed using EXPeRT. EXPeRT is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the EXPeRT Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs. EXPeRT is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

14.2 Maintenance of Study Documents

Essential documents will be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

15 ADMINISTRATIVE RULES

15.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

15.2 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of subjects enrolled in the study.

15.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

15.4 Compliance with Laws and Regulations

This study will be conducted in accordance with current US Food and Drug Administration (FDA) Good Clinical Practices (GCPs) and local ethical and legal requirements.

15.5 Record Retention

Retain all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for at least 6 years after the investigation is completed.

15.6 Publication Statement

The results of this study will be used for papers, abstracts, posters, or other informational materials to be presented at scientific meetings or published in professional journals, or as part of an academic thesis by an Investigator. Publications by the clinical trial site (s) of any data from this study will be carried out in accordance with the Clinical Trial Agreement.

16 APPENDICES

Appendix A. Network Sites

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:

Roswell Park Cancer Institute
CRS Network Office
ASB K 104
Buffalo, New York 14263

Telephone:
Monday - Friday; 8:00 AM to 4:30 PM EST
716-845-3155; 716-845-8360

After hours, weekends, and holidays request the RPCI Investigator
716-845-2300; **Fax:** 716-845-8743

2. INFORMED CONSENT

- Informed consent must be obtained by the site Investigator/designee from any subjects wishing to participate, prior to any procedures or change in treatment.
- An informed consent template is provided by RPCI and can be amended to reflect institutional requirements.
- All consent changes must be reviewed by RPCI Network Office prior to submission to the site IRB.
- The informed consent must be IRB approved.
- Always check that the most up to date version of the IRB approved consent is being used.
- Within 5 business days, notify the RPCI Network Office of all participant withdrawals or consent to limited study participation and appropriately document the discontinuation and the reason(s) why.

3. SUBJECT REGISTRATION

The subject completes the **Gender, Race, and Ethnicity Form** and this is placed in the study binder.

RPCI does not grant exceptions to eligibility criteria.

Phase 2 Protocol Registration Instructions

The **Subject Screening and Enrollment Log** must be faxed or emailed to the RPCI Network Office within one business day of the date the subject is consented. Once the Investigator has determined that eligibility has been met, complete the Subject Registration Form and fax or email it to the RPCI Network Monitor at 716-845-8743.

4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this must be reported to the RPCI Network, site IRB and any other regulatory authority involved in the trial.
- ALL study deviation will be recorded on the Study Deviation Log.
- Notify the RPCI Network Office of any early subject withdrawal and appropriately document the discontinuation and the reason why.

5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The RPCI Network Monitor must be able to read what has been deleted.
    - Do **NOT** use white-out, magic marker, scratch-outs.
    - Do **NOT** erase entries.
- Use only black ink for documentation on the accountability form and any other study forms.
- It is the responsibility of RPCI to inform the Investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to RPCI upon written agreement between the Investigator and RPCI.

6. DRUG ACCOUNTABILITY

Drug accountability must be strictly maintained.

- Responsibility rests solely with the Investigator but can be delegated as appropriate (e.g., to pharmacy personnel).
- A drug accountability record form (DARF) will record quantities of study drug received, dispensed to subjects and wasted, lot number, date dispensed, subject ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.
- Study drug supply will only be used in accordance with the IRB approved study.
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.
- An inventory count must be performed with each transaction. Any discrepancies shall be documented and explained.
- Drug accountability forms must be stored with study related documents.
- Each medication provided for this study and each dosage form and strength must have its own DARF.
- Dispensing the wrong study supply is considered a **medication error**.
    - **NEVER** replace investigational agents with commercial product.

- Do **NOT** "transfer", "borrow" or "replace" supplies between studies.

7. SERIOUS ADVERSE EVENT REPORTING

The site Investigator or designated research personnel will report all SAEs, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**. The site will notify the RPCI Network Monitor within 1 business day of being made aware of the SAE. A preliminary written report must follow within 1 business day of the first notification using the following forms:

- RPCI SAE Source report form
- MedWatch 3500A
- Drug Supplier is Aveo Pharmaceuticals send to: (please submit SAE Reports to Parexel GPPG via email (AVEOsafety@parexel.com). If submission via email is not possible or delayed then submission via fax (NA +1-781-434-5957) is acceptable)

  Pharmacovigilance/Drug Safety
  Aveo Pharmaceuticals, Inc.
  One Broadway, 14th Floor
  Cambridge, MA 02142Fax: 1-781-434-5957
  E-mail: AVEOsafety@parexel.com

- NCCN National Comprehensive Cancer Network FAX to (215)358-7699

A complete follow-up report must be sent to the RPCI Network Monitor within 10 working days.

8. UNANTICIPATED PROBLEM REPORTING

An unanticipated problem (UP) is any incident, experience, or outcome that meets **all** of the criteria in **Section 9.3.1.**

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention, the participating physician or delegated research staff from each site will notify **their local IRB in accordance with their local institutional guidelines**. The site must also notify the RPCI Network Monitor within 1 business day of being made aware of the Unanticipated Problem by completing the **RPCI Unanticipated Problem Report Form** and faxing or emailing it to the RPCI Network Monitor.

9. ADDITIONAL REQUIRED REPORTING

a. Refer to protocol **Section 9.6** for **Procedures for Reporting Subject Death**.

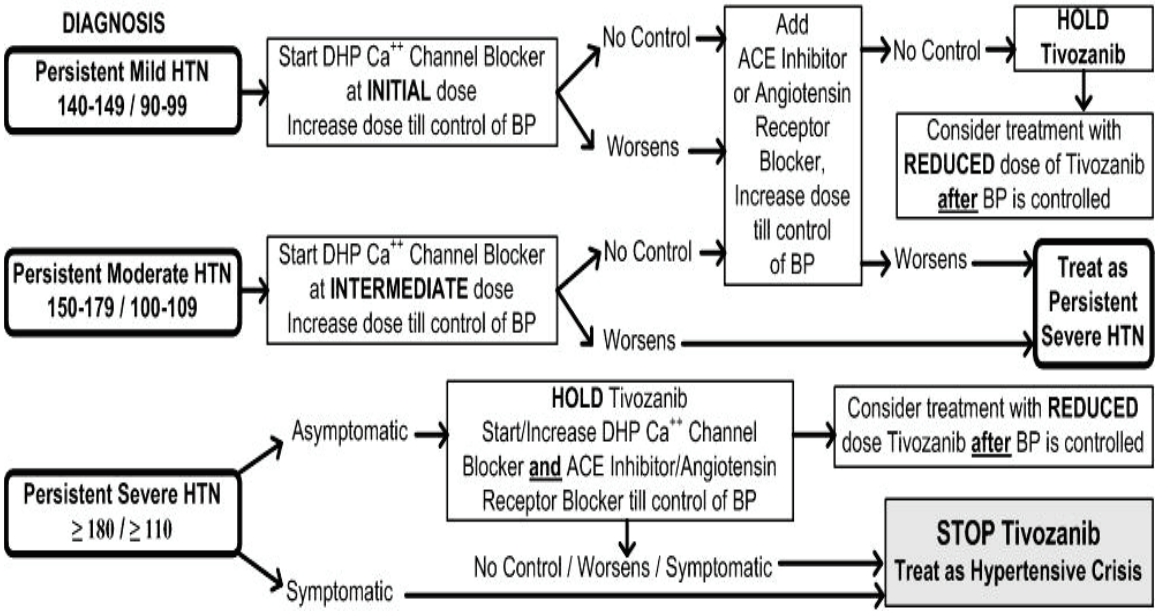
b. Refer to protocol **Section 9.7** for **Procedures for Reporting Study Drug Overdose**.

c. Refer to protocol **Section 9.8** for **Procedures for Reporting Drug Exposure during Pregnancy and Birth Events**.

Appendix B. ECOG Performance Status Scores

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

Appendix C. Recommended Management for Tivozanib Hydrochloride-Related Hypertension



Appendix D. Recommended Anti-Hypertensive Medications

Agent	Initial Dose	Intermediate Dose	Maximum Dose
Dihydropyridine (DHP) Calcium Channel Blockers			
Nifedipine XL	30 mg po qd	60 mg po qd	90 mg po qd
Amlodipine	2.5 mg po qd	5 mg po qd	10 mg po qd
Felodipine	2.5 mg po qd	5 mg po qd	10 mg po qd
Angiotensin Converting Enzyme (ACE) Inhibitors			
Captopril	12.5 mg po tid	25 mg po tid	50 mg po tid
Enalapril	5 mg po qd	10–20 mg po qd	40 mg po qd
Ramipril	2.5 mg po qd	5 mg po qd	10 mg po qd
Lisinopril	5 mg po qd	10–20 mg po qd	40 mg po qd
Fosinopril	10 mg po qd	20 mg po qd	40 mg po qd
Perindopril	4 mg po qd	None	8 mg po qd
Quinapril	10 mg po qd	20 mg po qd	20 mg po qd
Angiotensin II Receptor Blockers			
Losartan	25 mg po qd	50 mg po qd	100 mg po qd
Candesartan	4 mg po qd	8–16 mg po qd	32 mg po qd
Ibresartan	75 mg po qd	150 mg po qd	300 mg po qd
Telmisartan	40 mg po qd	None	80 mg po qd
Valsartan	80 mg po qd	None	160 mg po qd

Appendix E1. Patient Pill Diary(Full Dose)

Study No.: _____ **Patient's Name: _____**

Drug Name: _____ **Cycle:_____**

Medical Record No.: _____

Study Medication Calendar for Tivozanib

You should swallow your medication, unchewed, with a large glass of water (about 8 oz.) in the morning every day with or without food. On days of a scheduled clinic visit, you should take your dose at the clinic after visit procedures are completed. If a dose is vomited or if you miss your dose for any reason, the dose should not be made up.

Please complete this calendar on a daily basis immediately after you take your pills. Fill in the date for each day and write the total number of pills you take each day.

Start Date: _____

Take 1 pill each time.

Cycle Day	Day 1**	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Time							
Date							
Number of pills taken							

Cycle Day	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Time							
Date							
Number of pills taken							

Cycle Day	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Time							
Date							
Number of pills taken							

Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date	No medication is to be taken during this time.						
Number of pills taken							

****Do NOT take your pill on Day 1 until your blood is drawn and the study staff instructs you to take it.**

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$\_\_\_\%\ Compliance = \left(\frac{\_\_\_}{\_\_\_}\right) \times 100$$

Patient's Signature:_____ Date:_____

CRC Signature:_____ Date:_____

Investigator Signature:_____ Date:_____

Appendix F2. Patient Pill Diary(Dose Reduced)

Study No.: _____ **Patient's Name:** _____

Drug Name: _____ **Cycle:**_____

Medical Record No.: _____

Study Medication Calendar for Tivozanib

You should swallow your medication, unchewed, with a large glass of water (about 8 oz.) in the morning every day with or without food. On days of a scheduled clinic visit, you should take your dose at the clinic after visit procedures are completed. If a dose is vomited or if you miss your dose for any reason, the dose should not be made up.

Please complete this calendar on a daily basis immediately after you take your pills. Fill in the date for each day and write the total number of pills you take each day.

Start Date: _____

Take 1 pill each time.

Cycle Day	Day 1**(dose1)	Day 2	Day3(dose2)	Day 4	Day5(dose3)	Day 6	Day7(dose4)
Time							
Date							
Number of pills taken							

Cycle Day	Day 8	Day 9(dose5)	Day 10	Day11(dose6)	Day 12	Day13(dose7)	Day 14
Time							
Date							
Number of pills taken							

Cycle Day	Day15(dose8)	Day 16	Day17(dose9)	Day 18	Day19(dose10)	Day 20	Day 21
Time							
Date							
Number of pills taken							

Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date							
Number of pills taken	No medication is to be taken during this time.						

****Do NOT take your pill on Day 1 until your blood is drawn and the study staff instructs you to take it.**

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$\_\_\_\%\ Compliance = \left(\frac{\quad}{\quad}\right) \times 100$$

Patient's Signature:_____ Date:_____

CRC Signature:_____ Date:_____

Investigator Signature:_____ Date:_____

Appendix G. Separation of PBMC on FICOLL Hypaque Gradient

1. Collect peripheral blood in heparinized green top tubes (BD vacutainer^{R} Sodium Heparin, REF 366480). **Process blood immediately as detailed below.**

   1.1. Dilute 40 mL of blood with equal volume of 1 x sterile PBS (Cellgro^{r}, Mediatech, Inc. Cat. No. 2 appent1.031-CV) and mix gently using sterile pipette (**Do not vortex**).

   1.2. Put 15 mL of Ficoll (Ficoll-Paque^{TM} Plus, GE Healthcare, 17-1440-42) into a 50 mL sterile centrifuge tube (Corning^{R} 430828, Corning Incorporated).

   1.3. Using a sterile pipette, gently layer 30 mL of the diluted blood over 15 mL Ficoll in 50 mL sterile centrifuge tube, without disturbing the Ficoll layer.

   1.4. Centrifuge the tubes at settings of 2500 rpm, room temperature for 15 minutes, **centrifuge break must be off**.

   1.5. After centrifugation aspirate 8 mL of diluted plasma without disturbing the mononuclear cell layer and transfer to 2 polypropylene tubes (4 mL each) and freeze the tubes at –80˚C.

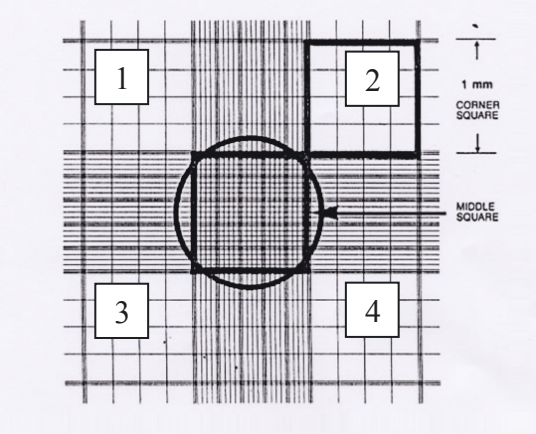
   1.6. After the removal of plasma, carefully transfer the mononuclear cell layer (ring), using a sterile pipette into one 50 mL sterile centrifuge tube containing 10 mL: PBS. Top up the tube to 50 mL with PBS.

   1.7. Gently invert the tube containing mononuclear cells few times to mix, and centrifuge the tubes at 1,500 rpm, room temperature for 5 minutes.

   1.8. After the centrifugation step, carefully remove the supernatant and discard and gently resuspend each cell pellet in 2 mL of PBS and combine the cell suspensions from all centrifuge tubes into a single tube. Dilute cell suspension to 50 mL with PBS, invert tube gently to mix, and centrifuge at 1500 rpm, room temperature for 5 minutes.

   1.9. After centrifugation remove the supernatant and gently resuspend the cell pellet in 10 mL PBS. Mix the cells by gently inverting the tubes. Remove 100 µL sample for cell count and viability. Dilute the cells appropriately, so that you are able to count a minimum 75 cells to maximum 100 cells.

1.10. Count the cells in a Hemocytometer (Neubauer) after mixing with equal volume 0.4% Trypan blue stain in PBS. Count all cells in the four large corner squares as shown below.



Squares		Cell Counts				Average Cell Count (1+2+3+4)/4	Final Cell Concentration
		1	2	3	4		
PBMC	Total						
	Viable						

Percentage of viable cells = Average viable cells / Average total cells x 100

Cell concentration = Average cell count x 10^4 x dilution factor _____

Total amount of cells = Cells/mL x volume (10 mL) _____

2. **Cryopreservation of PBMC in Freezing Medium**

2.1. Preparation of freezing medium. Prepare appropriate volume of freezing medium based on the total number of PBCs to be frozen.

2.2. Thaw frozen tube of heat inactivated human AB (HAB) serum (CellgroR, Mediatech, Inc. Cat. No. 35-060-CL) in a 37˚C water bath.

2.3. Add one part of DMSO (Sigma Cat. No. D5879) to 9 parts of heat inactivated HAB serum (90% HAB serum+10% DMSO) and mix well.

2.4. Centrifuge the PBMC at 1500 rpm for 5 minutes, decant the supernatant and gently resuspend the pellet in freezing medium at a concentration of 10^7 cells/mL of freezing medium.

2.5. Dispense 1 mL of the cell suspension into cryovials and keep overnight in -80˚C. Next day transfer the vials to liquid nitrogen.

3. **Preservation of Cells in RNA Later**

3.1. Take a volume of cell suspension (equivalent to 4 x 10^6 cells) in a 50 mL centrifuge tube.

3.2. Centrifuge at 1500 rpm for 5 minutes, decant the supernatant, and gently resuspend the cells in 1 mL PBS.

3.3. Pipette out 0.5 mL of cell suspension into each of 2 sterile eppendorf tubes containing 1 mL of RNA later (Ambion Cat. No. AM7021) and mix well before freezing the tubes at -20˚C overnight and then transfer to -80˚C.

Appendix H. Child-Pugh liver function Class A

CHILD-PUGH CLASSIFICATION OF SEVERITY OF LIVER DISEASE

Parameter	Points Assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin (mg/dL)	< 2	2 to 3	> 3
Albumin (g/dL)	> 3.5	2.8 to 3.5	< 2.8
Prothrombin Time			
Seconds over control	1 to 3	4 to 6	>6
INR	< 1.7	1.8 to 2.3	> 2.3
Encephalopathy	None	Grade 1 to 2	Grade 3 to 4

Modified Child-Pugh Classification of the severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the Prothrombin time, and the degree of encephalopathy. A total score of 5 to 6 is considered Grade A (well-compensated disease); 7-9 is Grade B (significant functional compromise); and 10 to 15 is Grade C (decompensated disease). These grades correlate with one and two year survival; Grade A – 100% and 65%; Grade B: 80% and 60%; and Grade C – 45% and 35%.

Appendix I. Procedure for Obtaining a Urine Protein /Creatinine Ratio

1) Obtain at least 4 ml of a random urine sample (does not have to be a 24-hour urine)
2) Determine protein concentration (mg/dL)
3) Determine creatinine concentration (mg/dL)
4) Divide #2 by #3 above: urine protein / creatinine ratio = protein concentration (mg /dL) / Creatinine concentration (mg/*dL)*

The UPC directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of I should be equivalent to 1 g protein in a 24hr urine collection)

Protein and creatinine concentrations should be available on standard reports of urinalyses, not dipsticks. If protein and creatinine concentrations are not routinely reported at an Institution, their measurements and reports may need to be requested.

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