STUDY TITLE

Regorafenib in Metastatic Colorectal Cancer: An Exploratory Biomarker Study

Test drug(s):	Regorafenib
[Study purpose:]	Biomarker Study
Clinical study phase:	II
Version no.:	2.3 Date: July 17, 2017
Amendment no.:	Date: N/A
NCT	NCT02402036
Institution Study no.:	2014-0133

Principal Investigator:
John Marshall, MD
3800 Reservoir Rd NW
Washington DC, 20007
P: (202) 444-3958

Co-Principal Investigator:
Anton Wellstein MD, PhD
Aiwu R. He MD, PhD
Michael Pishvaian MD, PhD
Anne Noonan MD
Emanuel Petricoin PhD

Statistician:

Hongkun Wang
4000 Reservoir Rd NW
Building D
Washington DC 20007
202-687-4146

Participating Institutions:

Angela Alistar, MD
Section on Hematology and Oncology
Wake Forest School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157
(P) 336-716-0457
aalistar@wakehealth.edu

Emil Lou, M.D., Ph.D
Assistant Professor of Medicine
University of Minnesota
Division of Hematology, Oncology and Transplantation
Mayo Mail Code 480
420 Delaware Street SE
Minneapolis, MN 55455
Office: 612-625-7667
E-mail: emil-lou@umn.edu

The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

Synopsis

Title	Regorafenib in Metastatic Colorectal Cancer: An Exploratory Biomarker Study
Clinical study phase	Exploratory biomarker study
Study objective(s)	**Primary objective:** To prospectively identify and prioritize leading biomarkers in tumor tissue and/or serum that are potentially predictive of response to regorafenib in patients with metastatic colorectal cancer, and can be further validated in subsequent larger clinical trials. **Secondary objective:** To prospectively determine the molecular mechanisms by which Regorafenib can control colorectal tumors refractory to other treatments.
[Background treatment]	Regorafenib 120 mg orally (40 mg tablets x 3) daily for 21 days out of a 28 day cycle
Indication	Patients with a histological or cytological diagnosis of adenocarcinoma of the colon or rectum who have progressed on, or are ineligible for, standard treatment options other than regorafenib
Diagnosis and main criteria for inclusion	1. Patients with metastatic colorectal cancer who have progressed on, or are ineligible for, standard therapy such as fluoropyrimidines, oxaliplatin, irinotecan, bevacizumab and anti-EGFR antibodies (where appropriate) but are suitable candidates for regorafenib treatment. 2. Patients with radiographically measurable biopsy-accessible disease. 3. Patients who are aged 18 years or older and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. 4. Patients with a life expectancy of at least 3 months. 5. Patients with adequate bone marrow, liver, and renal function. 6. Patient signed consent form.

Study design	The study is an exploratory biomarker study conducted at the Georgetown Lombardi Comprehensive Cancer Center. Patients with metastatic colorectal cancer will receive regorafenib therapy, a treatment that is FDA approved for this setting, on day 1-21 of every consecutive 28 day cycle. Patients will be asked to undergo tumor biopsy in addition to providing peripheral blood samples prior to starting Regorafenib (at baseline). A second tumor biopsy will be obtained 2 weeks (14-21 days) after starting regorafenib. Tumor tissue will be analyzed for phosphoproteins and RNA. Peripheral blood samples will be collected at baseline, 2 weeks after starting regorafenib therapy and then, after initiation of cycle 3, every 4 weeks for the duration of regorafenib therapy. Specifically, peripheral blood will be collected and banked for protein, miRNA, and mutated DNA analysis. A sample will also be obtained upon disease progression. Circulating biomarker (miRNA and mutated DNA) will be analyzed in the laboratory of Dr. Anton Wellstein at the Georgetown Lombardi Comprehensive Cancer Center. Caris Molecular Intelligence and Topologic Oligonucleotide Profiling will be performed by Caris Life Sciences.
Type of control	Uncontrolled study
Number of subjects	40
Plan for statistical analysis	For clinically relevant biomarker discovery, exploratory analyses of biomarkers will be performed to determine if a correlation can be established between the presence or change in the level of a marker species (“yes” or “no”) and the clinical response. Descriptive statistics, graphical methods, and statistical modeling, whichever is appropriate, may be used. Chi-square test or Fisher’s exact test will be used to explore the association between categorical variables and the clinical response. Kaplan-Meier method will be used to explore the association between categorical variables and time-to-event response variables. The analysis will be exploratory in nature.

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List of abbreviations

ADL	Activities of Daily Living
ALT	Alanine aminotransferase
Ang	Angiopoietin
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BID	*bis in die*, twice daily
B-Raf	B isoform of Rapidly Accelerated Fibrosarcoma protein
BUN	Blood Urea Nitrogen
c-KIT	Stem Cell Factor Receptor Tyrosine Kinase
CR	Complete Response
C-RAF	C isoform of Rapidly Accelerated Fibrosarcoma protein
CTCAE	Common Terminology Criteria for Adverse Events
DCE	Dynamic Contrast Enhanced
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
ERK	Extracellular Signal-regulated Kinases
FDA	Food and Drug Administration
FGFR	Fibroblast Growth Factor Receptor
FLT3	FMS-like Tyrosine Kinase 3
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HCC	Hepatocellular Carcinoma
HFSR	Hand-foot-skin reaction
IB	Investigator's Brochure
IC_{50}	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IR	Immediate Release
IRB	Institutional Review Board
MAPK	Mitogen Activated Protein Kinase
MEK	MAP Kinase / ERK Kinase 1
NM	Nano molar

NYHA	New York Heart Association
PD	Progressive Disease
PDGFR-β	Platelet Derived Growth Factor Receptor-beta
PFS	Progression free survival
PO	*per oris*, oral
PR	Partial Response
PS	Performance Status
PTT	Partial thromboplastin time
QD	*quaque die*, once daily
RAF	Rapidly Accelerated Fibrosarcoma
RAS	Rat sarcoma
RCC	Renal Cell Carcinoma
RECIST	Response Evaluation Criteria for Solid Tumors
RET	Rearranged during transfection
RTK	Receptor Tyrosine Kinase
SAE	Serious Adverse Event
SD	Stable Disease
SUSARs	Suspected Unexpected Serious Adverse Reactions
TIE2	Tyrosine kinase with Immunoglobulin and Epidermal Growth Factor (EGF) homology domain 2
TK	Tyrosine Kinase
TTP	Time to Progression
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor

1. Background

Colorectal cancer (CRC) is the third leading cause of cancer death in the United States *(1,2)*. Surgery is the primary treatment modality is stage I-III CRC *(2)*. In spite of surgery and adjuvant chemotherapy, 34% of patients with resected stage II and 27% of patients with resected stage III colorectal cancer will experience a recurrence within 5 years. Moreover, 19% of patients with CRC will present with metastatic disease *(3)*. Despite of the advances in chemotherapy and surgery, metastatic CRC continues to be a dreadful disease with a 5 year survival of approximately 11% *(2)*. Chemotherapy is the mainstay of treatment of metastatic CRC. Chemotherapy treatment options include 5-fluorouracil (5-FU) based regimens such as the combinations of irinotecan or oxaliplatin with bolus and infusional 5-FU (FOLFIRI or FOLFOX, respectively) (4). The addition of monoclonal antibodies targeting vascular endothelial growth factor receptors (VEGFR) and epidermal growth factor receptors (EGFR; in KRAS wild-type tumors) has lead to clinically-relevant improvements in patient survival. Recently, bevacizumab was approved by the FDA for the treatment of patients with metastatic CRC (4). Bevacizumab is a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), which is an essential factor in controlling tumor-associated angiogenesis *(5-8)*. Bevacizumab when combined with chemotherapy is associated with a 3-5 month improvement in overall survival compared to chemotherapy without bevacizumab *(4)*. Regorafenib was also approved recently by the FDA and is associated with an improved in overall survival compared to best supportive care *(9)*. Regorafenib has antiangiogenic activity in addition to activity again multiple other cellular targets *(9)*. Angiogenesis directed therapy has provided a new therapeutic strategy for patients with metastatic colorectal cancer but is associated with side effects such as gastrointestinal perforation, arterial and possibly venous thromboembolism, proteinuria, nephrotic syndrome, hypertension, bleeding, and hypertension *(4)*. Therefore, there is considerable interest in predicting subgroups of patients who will respond to anti-angiogenic therapy in order to better individualize treatment and avoid unnecessary toxicity for patients who are resistant to treatment. A few studies have attempted to identify biomarkers that predict response to anti-angiogenic therapy, mostly bevacizumab. Bates et al *(10)* evaluated a VEGF splice isoform that binds bevacizumab, VEGF(165)b to determine its predictive value. High VEGF (165)b appeared to predict resistance to bevacizumab therapy but this finding was not statistically significant. An exploratory analysis by Etienne-Grimaldi et al *(11)* also suggested that VEGF gene polymorphism might influence VEGF-A expression and serve as a marker of time to progression (TTP) in patients with metastatic breast cancer receiving bevacizumab containing therapy. Similarly Gerger et al *(12)* suggested that germline variants in VEGF-dependent and -independent angiogenesis genes can predict survival and tumor response in patients with mCRC treated with first-line bevacizumab and oxaliplatin-based chemotherapy. Additionally, Goede et al *(13)* demonstrated that serum angiopoietin -2, a key regular of vascular remodeling in conjunction with VEGF, may be predictive of RR, PFS and survival. Angiopoietin-2 originates in the stroma and was significantly elevated in patients with metastatic CRC compared to healthy controls. An exploratory analysis by Duda et al *(14)* evaluated VEGF, placental growth factor (PIGF), soluble VEGF receptor 1 (sVEGFR-1) and s VEGFR-1 suggested that sVEGFR-1 may predict response and toxicity to neoadjuvant bevacizumab in combination with chemotherapy. Taken together, these studies suggest that the search for predictive biomarkers for regorafenib is feasible and may identify subgroups that are may be particularly sensitive to it.

1.1 Rationale of the study

A phase III multinational trial (CORRECT trial) was conducted recently to evaluate the use of regorafenib versus placebo in patients with metastatic CRC who had progressed on all standard treatment options (9). Regorafenib was associated with a 1.4 month improvement in overall survival compared to placebo (hazard ratio, 0.77; 95% CI, 0.64–0.94; one-sided p=0.0052). These results were well received as they provided an

additional treatment option for patients with metastatic colorectal cancer. However, a few questions remained following the well-designed CORRECT trial. Kaplan-Meier curves for progression-free survival suggest that different patient subgroups may respond differently to treatment with regorafenib. Further exploration into the mechanism of action of regorafenib in patients with CRC is, therefore, necessary in order to be able to use the drug to its full potential. A handful of human CRC xenograft animal model studies have described regorafenib's mechanism of action but these mechanisms have not been described clinically. It is increasingly obvious that biomarkers for targeted cancer therapies, especially anti-angiogenesis compounds, are necessary. Therefore, we need to identify patient subgroups that may be particularly sensitive to regorafenib therapy and elucidate the mechanism of action of regorafenib in these subgroups. **We are performing this pilot clinical trial in order to prospectively identify and prioritize leading biomarkers of activity for subsequent validation studies.**

1.2 Regorafenib

Regorafenib has potent preclinical antitumor activity and long-lasting anti-angiogenic activity as measured by dynamic contrast enhanced (DCE) – magnetic resonance imaging (MRI) (1).

Regorafenib is a small molecule inhibitor of multiple membrane-bound and intracellular kinases involved in normal cellular functions and in pathologic processes such as oncogenesis, tumor angiogenesis, and maintenance of the tumor microenvironment. In vitro biochemical or cellular assay results showed that regorafenib or its major human active metabolites M-2 and M-5 inhibited the activity of RET, VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-alpha, PDGFR-beta, FGFR1, FGFR2, TIE2, DDR2, Trk2A, Eph2A, RAF-1, BRAF, BRAFV600E, SAPK2, PTK5, and Ab1 at concentrations of regorafenib that have been achieved clinically. Results from in vivo animal model studies demonstrated that regorafenib had anti-angiogenic activity in a rat tumor model, and inhibition of tumor growth as well as anti-metastatic activity in several mouse xenograft models, including some for human colorectal carcinoma.

1.2.1 Preclinical

In vivo, regorafenib exhibited anti-angiogenic and anti-proliferative effects in human colon and breast xenografts as demonstrated by a reduction in microvessel area, reduced Ki-67 staining, and reduced pERK1/2 staining in tissue sections from tumor xenografts, and dose-dependent inhibition of growth in multiple xenograft models (breast, colon, renal, NSCLC, melanoma, pancreatic, thyroid, ovarian). (1) Immunohistochemical ex-vivo studies with a phospho –specific monoclonal anti-ERK 1 / 2 antibody demonstrated inhibition of the MAPK pathway five days after treatment with regorafenib in 2 of 3 tumor models examined (MDA-MB 231 and BxPC-3), but not in NSCLC (H460).

In addition, all tested human tumor xenografts (MDA-MB-231, H460, BxPC-3 and Colo-205) demonstrated a significant reduction in new blood vessels by histomorphometry as detected in tumor samples using a murine CD31 antibody. (1) These data suggest that regorafenib can target the tumor cell MAPK pathway (tumor cell survival) and tumor vasculature in some but not all tumors.

1.2.2 Clinical experience

Two phase III global randomized studies have evaluated the efficacy of regorafenib. The CORRECT (Patients with metastatic colorectal cancer treated with regorafenib or placebo after failure of standard therapy) trial is an international, multicenter, randomized, double-blind, placebo-controlled study that enrolled 760 patients with mCRC whose disease has progressed after approved standard therapies. Metastatic colorectal cancer patients were randomized to regorafenib plus best supportive care (BSC) or placebo plus BSC. Treatment cycles consisted of 160 mg of regorafenib (or matching placebo) once daily for three weeks on / one week off plus BSC. The primary endpoint of this trial was overall survival. Secondary endpoints included progression-free

survival, objective tumor response rate and disease control rate. The safety and tolerability of the two treatment groups were also compared.

At a preplanned second interim analysis, there was a statistically significant survival benefit for regorafenib. The estimated hazard ratio for overall survival was 0.773 (95% confidence interval [CI], 0.635 to 0.941; 1-sided $p = .0051$). Patients treated with regorafenib had a median overall survival of 6.4 months, compared with 5.0 months for placebo — a 29% increase in survival. In addition to improved overall survival, progression-free survival was superior; median progression-free survival was 1.9 months (95% CI, 1.88 to 2.17) for regorafenib and 1.7 months (95% CI, 1.68 to 1.74) for placebo. The estimated hazard ratio for progression-free survival was 0.493 (95% CI, 0.418 to 0.581; 1-sided $p< .000001$). There was a substantial difference in disease control rate in the regorafenib and placebo groups (44% vs. 15%; $p<.000001$). Regorafenib demonstrated comparable efficacy benefits across patient subgroups analyzed including age, number of metastases, number of lines of prior therapy, and kras status.

The most frequent grade 3+ adverse events in the regorafenib group were hand–foot skin reaction (17%), fatigue (15%), diarrhea (8%), hyperbilirubinemia (8%), and hypertension (7%). The efficacy and safety from the CORRECT study supported FDA approval in September 2012.

The efficacy and safety of regorafenib were examined in the Phase III GRID trial in patients with gastrointestinal stromal tumors (GISTs) who had exhausted all other treatment options. The study involved 199 patients with metastatic and/or unresectable GIST that had become resistant to imatinib and sunitinib. Patients were randomized 2:1 to regorafenib (160 mg orally once daily on a 3 weeks on/1 week off cycle) or placebo, plus best supportive care.

The results showed that treatment with regorafenib led to a statistically significant 3.9-month improvement in progression-free survival (PFS), compared with placebo (4.8 months vs. 0.9 months; hazard ratio [HR] = 0.27; $p <.0001$). Overall survival was statistically similar between groups as expected due to a trial design that allowed crossover to regorafenib for disease progression (85% for placebo and 31% regorafenib randomized patients). The median survival period without tumor growth among patients on regorafenib was 4.8 months while for the control group on placebo it was less than a month. The overall disease control rate combining partial responses with durable stable disease for at least 12 weeks was 53% with regorafenib compared with 9% in the control group. The most common grade ≥3 adverse events associated with regorafenib were hand-foot skin reaction (56.1%), hypertension (48.5%), and diarrhea (40.9%). The efficacy and safety of the GRID study data supported FDA approval February 2013.

2. Study objectives

2.1 Primary objective:

To prospectively identify and prioritize leading biomarkers in tumor tissue and/or serum that are potentially predictive of response to regorafenib in patients with metastatic colorectal cancer, and can be further validated in subsequent larger clinical trials.

2.2 Secondary objective:

To prospectively determine the molecular mechanisms by which regorafenib can control colorectal tumors refractory to other treatments.

3. Investigator[s] and other study participants

Principal Investigator

John Marshall MD
3800 Reservoir Rd NW
Washington DC, 20007
Email: marshal@georgetown.edu

Co-Principal Investigators:

Anton Wellstein MD, PhD
3970 Reservoir Rd NW E501
Washington, DC, 20007
Email: wellstea@georgetown.edu
Tel: 202-687-3672

Aiwu R. He MD, PhD
3800 Reservoir Rd NW
Washington DC, 20007
Email: Aiwu.r.he@gunet.georgetown.edu
Tel: 202-444-2144

Michael Pishvaian MD, PhD
3800 Reservoir Rd NW
Washington DC, 20007
Email: pishvaim@gunet.georgetown.edu
Tel: 202-444-2144

Emanuel Petricoin PhD
George Mason University
10900 University Blvd
Room 324 Bull Run Hall

Manassas VA 20110
Email: epetrico@gmu.edu
Tel: 703-993-8646

Dr. Mariaelena Pierobon MD MPH
George Mason University
10900 University Blvd
Room 324 Bull Run Hall
Manassas VA 20110
Email: mpierobo@gmu.edu

Dr. Lance Liotta, MD PhD
George Mason University
10900 University Blvd
Room 324 Bull Run Hall
Manassas VA 20110
Email: lliotta@gmu.edu

Investigative Site
Caris Life Sciences
4610 South 44th Place
Phoenix, AZ 85040
Email: sreddy@carisls.com

4. Correlative Study design

4.1 Study method overview:

The study will be conducted at the Georgetown Lombardi Comprehensive Cancer Center, Wake Forest University, and Masonic Cancer Center, University of Minnesota, and Caris Life Sciences. Patients will each be allocated a unique anonymous study number which will be noted on all tumor and peripheral blood samples.

Tumor Collection

Patient will undergo tumor biopsy prior to starting Regorafenib (at baseline). A second tumor biopsy will be obtained 2-3 weeks after starting Regorafenib. The timing of the biopsy was selected to allow for its completion before patients undergo dose reduction of regorafenib. Second biopsy can be waived in case of patient refusal, complications after the first biopsy or if the investigator deems the biopsy unsafe. All tumor samples will be labeled with the date, protocol number, and protocol assigned patient number. Tumor samples obtained at Georgetown Hospital and other sites will be collected by the staff of the biospecimen bank. Two specimens will be placed in biopsy cassettes embedded in optimal cutting temperature compound (OCT) and snap frozen within 15 min in dry ice or liquid nitrogen and subsequently stored at -80 until further use. Once study is completed, t**he OCT embedded frozen core needle biopsy samples will be sent to the laboratory of Dr. Emanuel Petricoin at George Mason University for tissue-based laser capture microdissection and protein pathway activation mapping via reverse phase protein array analysis.** The rest of the samples will be placed in formalin and then paraffin blocks will be formed to **be sent to Caris for CMI testing**. Only the baseline tumor samples should be sent to Caris (unless the baseline tumor sample did not have enough tissue to be analyzed, the second biopsy could be sent). Please see Appendix 14.1 for specimen requirements.

Blood Collection

Peripheral blood samples (7 EDTA tubes) will be collected at baseline, 2 weeks (14-21 days) after starting regorafenib (the same day as the tumor biopsy), at the initiation of cycle 2and at the beginning of every cycle thereafter for the duration of regorafenib therapy, up to a total of 8 blood draws. Blood samples will also be obtained upon disease progression. Samples will be collected in lavender-topped K2 EDTA tubes. **The peripheral blood samples from** all participating sites **will be sent to Georgetown for storage and then distribution to Dr. Wellstein's laboratory and Caris Life Sciences .** Samples will be stored in a -80°C freezer. Analysis will be performed only after the last sample has been obtained, Samples from all participating sites will be shipped in a dry ice box to the Indvumid laboratory of **then distribution to Dr**. Anton Wellstein and **Caris Life Sciences**. All blood samples will be labeled with the date, protocol number, and protocol assigned patient number. Circulating biomarker studies and TOP will be performed in all patients.

4.1.1 Serum-based correlative

4.1.1.1 Serum-based correlative studies: Anton Wellstein MD, PhD, Narayan Shivapurkar PhD

Hypothesis for correlative studies: Regorafenib has antiangiogenic activity in addition to activity again multiple other cellular targets (9). Angiogenesis directed therapy has provided a new therapeutic strategy for patients with metastatic colorectal cancer but is associated with side effects such as gastrointestinal perforation, arterial and possibly venous thromboembolism, proteinuria, nephrotic syndrome,

hypertension, bleeding, and hypertension (15). Therefore, there is considerable interest in predicting subgroups of patients who will respond to anti-angiogenic therapy in order to better individualize treatment and avoid unnecessary toxicity for patients who are resistant to treatment. A few studies have attempted to identify biomarkers that predict response to anti-angiogenic therapy, mostly bevacizumab. Bates et al (10) evaluated a VEGF splice isoform that binds bevacizumab, VEGF(165)b to determine its predictive value. High VEGF (165)b appeared to predict resistance to bevacizumab therapy but this finding was not statistically significant. An exploratory analysis by Etienne-Grimaldi et al (11) suggested that VEGF gene polymorphism may influence VEGF-A expression and serve as a marker of time to progression (TTP) in patients with metastatic breast cancer receiving bevacizumab containing therapy. Similarly Gerger et al (12) suggested that germline variants in VEGF-dependent and -independent angiogenesis genes can predict survival and tumor response in patients with mCRC treated with first-line bevacizumab and oxaliplatin-based chemotherapy. Additionally, Goede et al (13) demonstrated that serum angiopoietin -2, a key regular of vascular remodeling in conjunction with VEGF, may be predictive of RR, PFS and survival. Angiopoietin-2 originates in the stroma and was significantly elevated in patients with metastatic CRC compared to healthy controls.

An exploratory analysis by Duda et al *(*14*)* that evaluated VEGF, placental growth factor (PIGF), soluble VEGF receptor 1 (sVEGFR-1) and s VEGFR-1 suggested that sVEGFR-1 might predict response and toxicity to neoadjuvant bevacizumab in combination with chemotherapy. This data indicate that biomarkers can potentially help in predicting patients who will respond to anti-angiogenic therapy. Regorafenib will be associated with a 1.4 month improvement in overall survival compared to placebo (hazard ratio, 0.77; 95% CI, 0.64–0.94; one-sided p=0.0052). These results were well received as they provided an additional treatment option for patients with metastatic colorectal cancer. However, a few questions remained following the well-designed CORRECT trial. Kaplan-Meier curves for progression-free survival suggest that different patient subgroups may respond differently to treatment with regorafenib. Further exploration into the mechanism of action of regorafenib in patients with CRC is, therefore, necessary in order to be able to use the drug to its full potential. A handful of human CRC xenograft animal model studies have described regorafenib's mechanism of action but these mechanisms have not been described clinically. It is increasingly obvious that biomarkers for targeted cancer therapies, especially anti-angiogenesis compounds, are necessary. Therefore, we need to identify patient subgroups that may be particularly sensitive to regorafenib therapy and elucidate the mechanism of action of regorafenib in these subgroups. We are performing this pilot clinical trial in order to prospectively identify and prioritize leading biomarkers of activity for subsequent validation studies. We aim to identify biomarkers that can be assayed prior to starting regorafenib to predict if patients will respond to it. Additionally, we plan to measure those biomarkers serially to better define their role and describe the exact mechanism of action of regorafenib. We hypothesize that serum and tissue biomarkers can help predict which subgroups of patients that will respond to regorafenib monotherapy.

4.1.1.2 Topologic Oligonucleotide Profiling (TOP): Caris Life Sciences

Exosomes (EXOs) are cell-specific, small double membrane extracellular microvesicles heterogeneous in size, ranging from 30 to 200 nm that are produced by different cell type (Schorey, Bhatnagar, 2008). Their classification has been based in their size, density, morphology (typical "cup-shaped" observed by electron microscopy) and the presence of common surface markers such as the CD63, CD81 and CD9 tetraspanins, fusion proteins (Flotilin, Annexins, GTPases), endosome-associated proteins (Alix,TSG101) and heat shock proteins (Hsc70, Hsp 90). These markers are reported in all biological fluids analyzed in different organisms and are released to media by most cells when cultured. The mechanism proposed for EXOs production is the fusion of multivesicular bodies with the plasma membrane in an exocytic manner. This route could serve as a directional packaging system of signaling molecules, in fact growing evidence indicate there is enrichment of certain RNA species such as some mRNAs, miRNAs and other ncRNAs with exclusion of rRNAs. Also, proteomic analysis suggests the presence of particular

compositions related to tissue of origin and to physiological state. In this sense, the presence of certain surface markers has been linked to directed cell-cell communication processes and to the development of disease. Therefore, particular EXO cargos represent molecular signatures of pathological processes (Garcia-Contreeras, Ricordi, Robbins, Oltar, 2014).

EXOs and microvesicles have been identified as potential sources for clinical disease biomarkers. They are particularly useful for this application because EXOs can be isolated from different body fluids collected by non-invasive (urine, saliva, breast milk, etc) or minimal-invasive (blood) methods (Hoorn, et al., 2014). In addition, EXOs provide advantages versus classical methods for the identification of biomarkers such as the quantification of soluble proteins or RNA molecules in plasma for several reasons: 1) they provide protease/nuclease controlled environment increasing molecule stability at the time, 2) allow for concentration of specific molecules of interest in easy to isolate particles, and 3) a subset can be isolated using a specific anti-cell surface marker antibody followed by analysis of specific cargo proteins/RNAs. In fact, the EXO cargo of proteins, mRNAs, miRNAs and other ncRNAs is determined by the state of the cell-type of origin and has been associated with some pathological condition with changes that correlate with specific stages of the disease. EXOs not only serve as carriers of biomarkers of the disease, but can serve as methods for monitoring prognosis and early diagnosis of a disease.

Exosome components present in the plasma from defined patient populations shall be processed with an oligonucleotide library to identify differentially binding oligonucleotides that are isolated using various techniques. The recovered oligos are then mixed with the plasma from a control population and non-binding oligos are retained. The initial library has the potential to measure 10^{12} biological features, though after multiple rounds of exposing the library to selected positive and negative control input samples, an informative subset library may be obtained capable of characterizing a smaller number of biological features, e.g., 10^5 or 10^6. The oligonucleotides that bind to the exosomes in the desired population are identified using sequencing resulting in a quantitative measure of molecular complexes for patients profiled. Common trends between patients can be identified and bio-signatures can be defined across populations or subpopulation groups. The oligonucleotide signature provides a unique and powerful method to stratify and characterize complex biological differences between patients.

4.1.2 Tissue-based correlative

4.1.2.1 Tissue-based correlative studies: Emanuel F Petricoin PhD, Mariaelena Pierobon, MD, MPH

As described previously, regorafenib is a small molecule inhibitor of multiple membrane-bound and intracellular kinases. Moreover, in vitro biochemical or cellular assay results indicated that regorafenib or its major human active metabolites M-2 and M-5 inhibited the activity of RET, VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-alpha, PDGFR-beta, FGFR1, FGFR2, TIE2, DDR2, Trk2A, Eph2A, RAF-1, BRAF, BRAFV600E, SAPK2, PTK5, and Ab1 at concentrations of regorafenib that have been achieved clinically. Preclinical studies have demonstrated in vivo that regorafenib exhibited anti-angiogenic and anti-proliferative effects in human colon and breast xenografts, reduced Ki-67 staining, and reduced phosphorylated ERK1/2 staining in tissue sections from tumor xenografts, Given that the mechanism of action of regorafenib is directed at protein kinase activity modulation, a direct analysis of protein phosphorylation and thus the protein signaling activation networks in patient tumor cells would be postulated to be highly predictive for drug response since protein phosphorylation is the major way that kinase-based cell signaling events occur. Given the large number of drug targets for regorafenib, assays that can quantify the activation/phosphorylation levels of many proteins simultaneously from microscopic amounts of cellular input are critical. Consequently, we will utilize the reverse phase protein

microarray (RPPA) technology that we (Petricoin) invented in our laboratories to map the activated signaling architecture of the regorafenib drug target portfolio and other immune cell/stroma signaling networks in laser capture microdissected tumor epithelium and patient-matched liver parenchyma in baseline and week-2 core needle biopsy samples. We are performing this pilot clinical trial in order to prospectively identify and prioritize leading biomarkers of activity for subsequent validation studies. We aim to identify biomarkers that can be assayed prior to starting regorafenib to predict if patients will respond to it. Additionally, we plan to measure those biomarkers serially to better define their role and describe the exact mechanism of action of regorafenib. We hypothesize that serum and tissue biomarkers can help predict which subgroups of patients that will respond to regorafenib monotherapy.

4.1.2.2 Caris Molecular Intelligence: Caris Life Sciences

Molecular profiling has been used successfully to guide therapy selection in patients with advanced solid tumors.

- Von Hoff et al. found a 27% response rate with molecularly selected therapies in a prospective trial of chemotherapy refractory cancer patients. [2]
- Tsimberidou et al. at MD Anderson utilized molecular profiling to guide clinical trial enrolment to appropriate targeted therapies, based on the presence of a single genetic aberration, leading to a response rate of 27% in a population enrolled into matching clinical trials for which tumors expressed a molecular target, compared to 5% in those patients who were not treated according to a molecularly- defined targeted therapy [3]. This molecularly-targeted group, however, represented only 175 of the 1144 patients screened were treated with a molecularly targeted drug.
- In a similar approach undertaken at the Princess Margaret Cancer Centre as part of the IMPACT trial, 678 patients were screened, leading to 43 patients receiving a treatment matched to their genotype – this represented 24% of the total number of patients with tumors that harbored mutations. [4]

Caris Molecular Intelligence (CMI) is a CLIA certified biomedical laboratory service. Utilizing a recent FFPE tumor sample, Caris performs a broad biomarker profile, using state of the art molecular diagnostic tests and technologies, including immunohistochemistry, next-generation sequencing and in situ hybridization.

The biomarker profile is assessed in relation to the latest and strongest published clinical evidence of oncology drug-biomarker associations. For each patient an individual list of drugs with more likely benefit or less likely benefit is produced. Drugs available in clinical trials, which could benefit the patient, are also listed. Linking the biomarker analyses with the available published clinical evidence enables the treating physicians to tailor evidence-based treatment plans for each patient in an efficient way.

CMI is intended to increase the likelihood with which the physician can identify an effective therapy and avoid exposing the patient to unnecessary side effects. By using a multi-platform approach, CMI identifies more potential targets than sequencing alone and should therefore yield at least an equivalent degree of benefit in profile-matched therapy patients as the previous studies described earlier.

4.2 Methodology according to individual objectives

4.2.1 Primary Objective 1:

To determine the value of serum miRNA in predicting clinical benefit from regorafenib. MicroRNA (miRNA) is a new class of small, non-coding RNA that can regulate the expression of multiple genes.

miRNAs have been implicated in a diverse number of cellular processes including cell proliferation, apoptosis, regulation of embryonic stem cell development, and cancer cell invasion. Recent studies have shown that, unlike other types of biomarkers, miRNAs in the circulation are remarkably stable making them robust and reliable biomarkers of cancer. Our group has published previously on the use of miRNA to predict response to therapy (16) miRNA can be assayed in serum and therefore is an attractive new investigational tool that can prove to be very helpful. We plan to correlate the changes in serum miRNA levels with the clinical benefit of regorafenib. MicroRNA level may identify patients that are more susceptible to the beneficial effect of regorafenib. We will conduct an exploratory analysis using a panel of 380 miRNA to determine if there is a lead miRNA/s that may offer predictive value in patients who are candidates for regorafenib.

Methodology: We will use serum for miRNA quantification**.** 0.5 mL serum will be mixed with 10 vol of Qiazol lysis reagent and mixed by vortexing. The lysate will be extracted with $CHCl_3$ and the aqueous phase will be further enriched for miRNA using the miRNeasy kit (Qiagen, Valencia, CA). The miRNA-enriched fraction was eluted in RNase free water. The miRNA will be converted to cDNA using miScriptII RT kit and miRNA expression profiling will be conducted using miScript miRNA PCR arrays in combination with the miScript SYBRgreen PCR kit on an ABI 7900 HT Real-Time PCR system (Applied Biosystems, Foster City, CA). We will assay miRNA at baseline, 4 and 8 weeks. Changes in the expression between the samples will be calculated using [delta][delta]Ct method with commercial software. The data will be expressed as fold up-regulation or down-regulation in miRNA expression compared to that at base level (time 0) serum sample.

4.2.2 Primary Objective 2:

To evaluate the predictive value of resignaling pathways and angiogenesis using reverse phase microarray (RPPA) technology. Changes in protein levels and structure have also been shown to play critical roles in tumor development and progression, which are not reflected by genetic changes. Studying the levels and activation status of multiple protein pathways through changes such as protein phosphorylation or cleavage can greatly aid in understanding the causes and determining effective treatment of cancers. Since regorafenib acts on protein kinase activity (the proteins are the drug target), measuring the activation levels of regorafenib drug targets and interconnected signaling pathways could provide a highly predictive marker set. This study will allow us to identify pathways that are inhibited or upregulated upon exposure to regorafenib by measuring signaling at baseline and at 2 week (14-21 days) post drug. Moreover, evaluation of the stroma signaling and immune checkpoint pattern in the surrounding liver parenchyma could provide a new opportunity to uncover new predictive markers. This knowledge will be correlated with clinical endpoints and will allow us to better understand potential mechanisms of action and resistance. For this study in particular, we will use the reverse phase protein microarray assay (RPPA) developed by our collaborator, Emanuel F Petricoin at George Mason University. RPPA is a high-throughput antibody-based technique that currently allows for the analysis of regorafenib targets and linked signaling. Examples of protein signaling that would be measured in this study (examples from 100-125 protein/phosphoproteins) include:

a. RAS-RAF-ERK signaling:
pARAF, pBRAF, pCRAF, pJNK/SPAK, pp38, pMEK, pERK, pELK, pCREB, and P90RSK;

b. PI3K-AKT-mTOR signaling:
pAKT, pmTOR, p4EBP1, p70S6K, pRSK, pGSK3, pFOXO, pSGK, and pPRAS;

c. RTK signaling:
pSHC, pFGFR, pVEGFR, pKIT, pRET, pTIE2, Tie2, pEphaA, pAbl, pPDGFR, pTYK2, pROS

pRON, pIGF, pEGFR, pHER2, and pHER3;

d. JAK-STAT signaling:
pJAK1, pJAK2, pSTAT3, pSTAT5, pSTAT2, and pSTAT1; and

e. immune cell biology:
pZAP70, pLCK, pCD3zeta, PDL-1, PD1, PDL2, B7-H3, B7-H4, LAG3, CX40L, CS40, CD3, and CD4.

Tumor processing and phosphoprotein analysis will be performed by Emanuel Petricoin at George Mason University using LCM coupled to RPPA. **Samples from** all participating sites **will be sent to Georgetown for storage and then distribution to George Mason University via FedEx medical shipment service after the last sample has been obtained or two years after the first sample has been obtained whichever occurs first.**

Methodology: An 18-20 gauge needle will be used to collect at least four 1-2 cm core tumor biopsies from each patient at each of the allocated time-points (baseline and at 2 weeks (14-21 days) post-regorafenib treatment initiation), as per interventional radiology guidelines, Georgetown Department of Radiology. Within 15 minutes, 2 of the 4 tumor samples taken will be placed into a biopsy cassette, embedding in optimal cutting temperature compound (OCT) and snap frozen on dry ice and stored at -80 until processed further. The OCT embedded biopsy material will be sent to George Mason University where the tissue will sectioned onto uncharged glass slides with a cryostat. The slides (7 μM) will be fixed in 70% ethanol; washed in purified water (Milli-Q); stained with Mayer's hematoxylin, followed by Scott's tap water substitute; and dehydrated by successive washes with increasing concentrations of ethanol, followed by final dehydration in xylene. Proteinase inhibitors (Roche, Indianapolis, IN) will be added to the water and 70% ethanol to minimize protein degradation. Approximately 20,000 cells from each sample will be captured onto caps (CapSure Macro LCM; Arcturus, Life Technologies, Carlsbad, CA) using a laser capture microscope (PixCell II; Arcturus, Life Technologies), and captured cells will stored at −80°C before extracting protein with a lysis buffer containing a 1:1 dilution of tissue protein extraction reagent (Pierce Biochemicals, Rockford, IL) and ×2 SDS loading buffer (Invitrogen, Carlsbad, CA) supplemented with 2.5% mercaptoethanol. An H and E FFPE slide from each sample will be provided to review the histological features. All samples will be collected and processed in accordance with approved Institutional Review Board tissue banking and use protocols at Georgetown University. RPPA analysis will be performed as previously described (17) and background subtracted, total protein normalized data will be generated as a continuous variable and reported via Excel.

Statistical Analysis: Parametric and Non-parametric statistical analysis will be performed based on RPPA data distribution normalcy check based on responder vs. non-responder clinical designation. Analysis will be done using the both baseline and week 2 generated data. Moreover, changes in protein/phosphoprotein levels will be calculated between baseline and week 2 and the relative change in expression/activation will also be correlated with response. Spearman and Pearson correlations between proteins will be determined for both the responding and non-responding population and de novo network reconstruction produced from this data.

4.2.3 Secondary Objective 1:

To determine if mutated DNA in serum could serve a predictive marker for clinical benefit to regorafenib. Quantitative assessment of mutated DNA in serum represents a new intriguing modality to

predict response to therapy. Several publications have described the use of mutated DNA in serum and its value as a predictive biomarker. Mutated DNA can be analyzed in 1-2 ml of serum and represents a non-invasive modality to predict therapeutic benefit. At least mutated BRAF and KRAS will be assayed as described by Pinzani et al 2010 and Diehl et al. The results of such assays will be correlated with treatment benefit. Additionally, the time course change of these mutations will be followed to determine if they can be used as a method to early detect resistance when it occurs.

Methodology: DNA will be purified from of 1µml of banked serum using the QIAamp circulating nucleic acid kit (Qiagen, catalogue no. 55114) following the manufacturer's recommendations. Amplifiable DNA was quantified with quantitative PCR, using primers and conditions. The ligation assays will be performed as previously described (11) using the primers and probes described by Diehl et al (Nature Methods 2006). KRAS fragments containing codons 12 and 13 will be amplified with primers designed to yield a small PCR product (106 base pairs) to accommodate the degraded DNA found in serum. To confirm and further quantify samples containing KRAS mutations, BEAMing assays were used with the primers and probes described by Diehl et al 2006. The number of mutant fragments per ml of serum will be determined from the fraction of alleles containing the mutant allele (determined by BEAMing) and the number of alleles assessed per ml of serum will be determined by qPCR and correlated with the clinical benefit from regorafenib.

4.2.4 Secondary Objective 2:

To correlate the presence of baseline gene expression with clinical outcomes of regorafenib: Given the genetic complexity of colorectal cancer, accurate identification of the somatic mutations that occur in a cancer cell, may be a useful tool in predicting response to various therapies. Han et al has demonstrated that targeted sequencing of cancer genes in colorectal cancer was feasible. A total of 526 somatic variations in 113 genes were found. The most commonly altered genes were the APC, TP3, KRAS, TTN, FVXW &, SMAD, MAFB, GNAS and SRC genes. While knowledge of these mutations is important, taking the next step to determine if the mutational profile of these tumors can be used to predict response to therapies is essential. To this end we will send FFPE tissue for a commercially available predefined illumina TruSight tumor sequencing panel. The TruSight Tumor Sequencing panel is a comprehensive assay for examining the most relevant cancer genes involved in solid tumors. Combined with illumina's proven next generation technology, the TruSight Tumor panel delivers unmatched SNP, insertion, and deletion across the broadest regions of variation to enable a better understanding of biology leading to improved clinical studies. The genes involved with solid tumors will be carefully selected panel of 26 genes from relevant publications and late phase pharmaceutical clinical trials. It looks beyond hot spots with 174 amplicons tiled over 82 exons in 26 genes associated with solid tumor progression, prognosis and targeted therapy for a more comprehensive view of tumor heterogeneity. These genes include AKT, ALK, APC, BRAF, CDH1, CTNNB1, EGFR, ERBB2, FBXW7, FGFR2, FOXL2, GNAQ, GNAS, KIT, KRAS, MAP2K1, MET, MSH6, NRAS PDGFRA, PIK3CA, PTEN, SMAD4, SRC, STK11, and TP53. This provides a more comprehensive view of somatic variation in solid tumors enabling researchers to look beyond point mutations within hot spots in single genes. The protocol involves DNA extraction; Amplicon based library preparation and automated sequencing as recommended by illumina (www.illumina.com/trusight-tumor). The portion of the biopsies will be sent to the vendor for TruSight Tumor sequencing.

5. Study population

5.1 Eligibility

5.1.1 Inclusion criteria

- Patients with metastatic colorectal cancer who have progressed on, or are ineligible for standard therapy such as fluoropyrimidines, oxaliplatin, irinotecan, endotherlial growth factor (VEGF) inhibitors such as bevacizumab, and anti-EGFR antibodies where appropriate, but are suitable candidates for regorafenib treatment.
- Patients with radiographically measurable biopsy-accessible disease.
- Patients who have an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1.
- Age ≥ 18 years.
- Life expectancy of at least 12 weeks (3 months).
- Subjects must be able to understand and be willing to sign the written informed consent form. Ideally, a signed informed consent form will be appropriately obtained prior to the conduct of any trial-specific procedure. However, if patients have had standard of care assessments performed within the screening period, but before the consent was signed, these will be admissible.
- Adequate bone marrow, liver and renal function as assessed by the following laboratory requirements:
    - Total bilirubin ≤ 1.5 x the upper limits of normal (ULN)
    - Alanine aminotransferase (ALT) and aspartate amino-transferase (AST) ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
    - Alkaline phosphatase limit ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
    - Serum creatinine ≤ 1.5 x the ULN
    - For subjects with liver metastases, AST and ALT ≤ 5 X the upper normal limit of institution's normal range, and Non-fasting bilirubin 1.5 - 3.0 X the upper normal limit of institution's normal range are acceptable
    - As patients will undergo tissue biopsy, their international normalized ratio (INR)/ Partial thromboplastin time (PTT) has to be ≤ 1.5 x ULN. In addition, a platelet count > 100000 /mm3 and absolute neutrophil count (ANC) ≥ 1500/mm3 is necessary.
-
- Women of childbearing potential must have a negative serum pregnancy test performed within 7 days prior to the start of study drug. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not required to undergo a pregnancy test. Subjects (men and women) of childbearing potential must agree to use adequate contraception beginning at the signing of the ICF until at least 3 months after the last dose of study drug. The definition of adequate contraception will be based on the judgment of the principal investigator or a designated associate but is generally defined as a hormonal or barrier method of birth control, or abstinence.
- Subject must be able to swallow and retain oral medication.

5.1.2 Exclusion criteria

- Previous assignment to treatment during this study. Subjects permanently withdrawn from study participation will not be allowed to re-enter study.
- Uncontrolled hypertension (systolic pressure >140 mm Hg or diastolic pressure > 90 mm Hg [NCI-CTCAE v4.0] on repeated measurement) despite optimal medical management.
- Active or clinically significant cardiac disease including:
  - Congestive heart failure – New York Heart Association (NYHA) > Class II.
  - Active coronary artery disease.
  - Cardiac arrhythmias requiring anti-arrhythmic therapy other than beta blockers or digoxin.
  - Unstable angina (anginal symptoms at rest), new-onset angina within 3 months before randomization, or myocardial infarction within 6 months before randomization.
- Evidence or history of bleeding diathesis or coagulopathy.
- Any hemorrhage or bleeding event ≥ NCI CTCAE Grade 3 within 4 weeks prior to start of study medication.
- Subjects with any previously untreated or concurrent cancer that is distinct in primary site or histology from breast cancer except cervical cancer in-situ, treated basal cell carcinoma, or superficial bladder tumor. Subjects surviving a cancer that was curatively treated and without evidence of disease for more than 3 years before randomization are allowed. All cancer treatments must be completed at least 3 years prior to study entry (i.e., signature date of the informed consent form).
- Patients with pheochromocytoma.
- Known history of human immunodeficiency virus (HIV) infection or current chronic or active hepatitis B or C infection requiring treatment with antiviral therapy.
- Ongoing infection > Grade 2 NCI-CTCAE v4.0.
- Symptomatic metastatic brain or meningeal tumors. NB. If a patient does not have known or symptomatic CNS metastases at screening, then brain imaging is not required for the purpose of this trial.
- Presence of a non-healing wound, non-healing ulcer, or bone fracture.
- Renal failure requiring hemo-or peritoneal dialysis.
- Interstitial lung disease with ongoing signs and symptoms at the time of informed consent.
- Pleural effusion or ascites that causes respiratory compromise (≥ NCI-CTCAE version 4.0 Grade 2 dyspnea).
- History of organ allograft (including corneal transplant).
- Known or suspected allergy or hypersensitivity to any of the study drugs, study drug classes, or excipients of the formulations given during the course of this trial.
- Any malabsorption condition.
- Women who are pregnant or breast-feeding.
- Any condition which, in the investigator's opinion, makes the subject unsuitable for trial participation.

- Substance abuse, medical, psychological or social conditions that may interfere with the subject's participation in the study or evaluation of the study results.
- Excluded therapies and medications: See section 5.1.3.

5.1.3 Excluded therapies and medications, previous and concomitant

- Concurrent anti-cancer therapy (chemotherapy, radiation therapy, surgery, immunotherapy, biologic therapy, or tumor embolization) other than study treatment. The washout period between radiation therapy or tumor embolization is two weeks. Given the refractory nature of the study population, the washout period between study treatment and prior chemotherapy or biological therapy, will be 2 weeks prior to use of regorafenib.
- Concurrent use of another investigational drug or device therapy (i.e., outside of study treatment) during, or within 4 weeks of trial entry .
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days before start of study medication.
- Therapeutic anticoagulation with Vitamin-K antagonists (e.g., warfarin) .
    - However, prophylactic anticoagulation as described below is allowed:
        - Low dose warfarin (1 mg orally, once daily) with PT-INR ≤ 1.5 x ULN is permitted. Infrequent bleeding or elevations in PT-INR have been reported in some subjects taking warfarin while on regorafenib therapy. Therefore, subjects taking concomitant warfarin should be monitored regularly for changes in PT, PT-INR or clinical bleeding episodes.
        - Prophylactic doses of heparin.
- Use of any herbal remedy (e.g. St. John's wort [Hypericum perforatum])

5.2 Withdrawal of subjects from study

5.2.1 Withdrawal

Subjects **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Pregnancy. Pregnancy will be reported as an SAE. (Note: subjects who have been withdrawn from treatment with study drug because of pregnancy should not undergo CT scans [with contrast]/MRI or bone scans while pregnant.)
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- Subject is lost to follow-up.
- Death.

Subjects **may be** withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol.

- Severe allergic reaction to regorafenib (such as exfoliative erythroderma or Grade 3 or 4 hypersensitivity reaction).
- The development of a second cancer.
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 4.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

Details for the premature termination of the study as a whole (or components thereof [e.g. centers, treatment arms, dose steps]) are provided in Section 11 (Premature termination of the study).

5.2.2 Screen Failures/Dropouts

A subject who discontinues study participation prematurely for any reason is defined as a “dropout” if the subject has already been randomized; assigned to treatment/run-in/wash-out; or had at least one dose of study drug administered.

A subject who, for any reason (e.g. failure to satisfy the selection criteria) terminates the study before the time point used for the definition of “dropout” (see above) is regarded a “screening failure”.

5.2.3 Replacement

Subjects who withdraw from the study prior to undergoing the first biopsy will be replaced.

5.3 Collaboration with Caris Life Sciences

The study will be conducted in collaboration with Wake Forest University, Masonic Cancer Center, University of Minnesota, , and Caris Life Sciences. Dr. John Marshall is the Principal Investigator for the study and will oversee the study including the data gathering, safety and reporting. Data will be shared amongst the collaborators for further analysis.

Dr. Angela Alistar, and Dr. Emil Lou, will be the main study contacts at the perspective sites. Dr. Sandeep Reddy will be the main study contact at Caris Life Sciences. Patients will be recruited at all institutions for the study. The tissue and blood samples obtained at the collaborative sites will be mailed to Georgetown University as follows:

1) Peripheral blood samples will be shipped on dry ice using Fed Ex medical shipments to Georgetown University

   Indivumed Laboratory
   Georgetown University Medical Center
   Georgetown Lombardi Cancer Center
   Preclinical Science Building, Room LC6
   3900 Reservoir RD, NW
   Washington, D.C. 20016
   Tel: 202-687-1265

Fax: 202-687-5351

2) Formalin-embedded tissue blocks will be shipped using Fed Ex medical shipments to Caris Life Sciences in Phoenix, Arizona for the molecular profile an.

3)

Monthly conference calls will be conducted between all institutions to follow up on the progress of the trial. Dr. Marshall will be responsible for coordination of the trial between all institutions. Study coordinators will notify Dr. Marshall upon the accrual of any patients in any institution.

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5.3.1 Personnel

At each site, personnel dedicated to this protocol will be:

- A study PI
- A research coordinator
- A data manager

In addition, at Lombardi-Georgetown, there will be a dedicated "multi-institutional" research coordinator who will play the primary role in coordinating the trial between Lombardi-Georgetown and additional sites. This coordinator will be the main point of contact for Dr. Marshall and the other site PIs for any study related concerns, and to screen each patient being considered for enrollment (Including "remote" screening for the patients being screened at other sites). This coordinator will also be the point of contact for the data managers for data entry questions. Finally, this coordinator will play a major role in regulatory coordination of the study, specifically by: 1) Reviewing and confirming all study-related adverse events; 2) Submitting all SAE reports to the Georgetown IRB (The research coordinators at the other sites will prepare SAE reports for patients treated at their respective sites, but the "multi-institutional" coordinator will submit the final report); 3) Gathering and preparing all primary source data for review/audit by Theradex, Inc.

5.3.2 Patient Enrollment

Enrollment at the sites will be competitive. If a patient is being screened for enrollment, the local research coordinator must send an email within 24 - 48 hours containing the subject number, to the PI, and to the multi-institutional coordinator. If a patient is successfully screened, the local research coordinator must send all supporting documentation to the multi-institutional research coordinator (by email [hka10@georgetown.edu]). Patients should not start therapy until both PI and the multi-institutional coordinator have reviewed the patient's records and confirmed that the patient is indeed eligible for enrollment.

5.3.3 Data Collection and Management

Patient data will be entered into the on-line accessible database. This database is housed at Lombardi-Georgetown, but is accessible anywhere there is internet access. The data manager and research coordinator at each site will attend an on-line training session so that they may learn how to enroll data into the database. All screening data should be entered prior to starting therapy, and all ongoing patient data should be entered within one week of each patient visit.

5.3.4 Conference Calls

A monthly conference call will be held between Lombardi-Georgetown and the other sites to review patient enrollment, toxicity, and response assessment.

6. Treatment[s]

6.1 Blinding

Given the nature of this trial, patient or investigator blinding will not be necessary.

6.2 Study Treatment

As Regorafenib is commercially available, patients will be provided a prescription and will fill it from retail or specialty pharmacies.

Regorafenib is administered as monotherapy during the study; 120 mg qd will be administered for 3 weeks on /1 week off. One cycle is 28 days.

Three 40-mg regorafenib tables should be taken in the morning with approximately 8 fluid ounces (240 mL) of water after a low-fat (<30% fat) breakfast. Some examples of low fat breakfasts are:

- Two slices of white toast with 1 tablespoon of low-fat margarine and 1 tablespoon of jelly and 8 ounces (240 mL) of skim milk (approximately 319 calories and 8.2 g of fat).
- One cup of cereal (i.e. Special K), 8 ounces (240 mL) of skim milk, one piece of toast with jam (no butter or marmalade), apple juice, and one cup of coffee or tea (2 g fat, 17 g protein, 93 g of carbohydrate, 520 calories.

6.2.1 Dose Reduction Levels

The starting dose of regorafenib is 120 mg once daily. Patients will be asked to take regorafenib at their allocated dose on a 3 weeks on/1 week off schedule [3 weeks out of every 4].

Doses will be delayed or reduced for clinically significant hematologic and non-hematologic toxicities that are related to protocol therapy according to the guidelines shown in the Dose Delays/Dose Modifications table that follows. Dose modifications will follow predefined dose levels. Dose adjustments for hematologic toxicity are based on the blood counts obtained in preparation for the day of treatment.

The modifications of regorafenib will follow the following predefined dose levels:		
Dose level 0 (standard starting dose)	120 mg po qd	Four 40-mg tablets of regorafenib
Dose level - 1	80 mg po qd	Three 40-mg tablets of regorafenib
Dose level - 2	40 mg po qd	Two 40-mg tablets of regorafenib

If a subject experiences more than one toxicity, dose reduction should be carried out according to the toxicity with the highest grade

In the case of two or more toxicities of the same grade, the investigator may dose reduce according to that deemed most causally related to study treatment

If more than 2 dose reductions are required, regorafenib only will be discontinued and the rest of the study treatment may be continued. If a dose reduction has been performed, intra-subject dose re-escalation can be considered (up to the maximal 120 mg daily dose) at the discretion of the treating physician provided that the toxicity(ies) has resolved to baseline.

The following tables outline dose adjustments for toxicities related to study drug except hand-foot skin reaction, hypertension and liver function test abnormalities.

Table 6-1: Recommended dose modification for toxicities except hand-foot-skin reaction, hypertension and ALT/ST/bilirubin

NCI-CTCAE v4.0[a]	Dose Interruption	Dose Modification[b]	Dose for Subsequent Cycles
Grade 0-2	Treat on time	No change	No change
Grade 3	Delay until ≤ Grade 2[c]	Reduce by 1 dose level	If toxicity remains < Grade 2, dose re-escalation can be considered at the discretion of the treating investigator. If dose is re-escalated and toxicity (≥ Grade 3) recurs, institute permanent dose reduction.
Grade 4	Delay until ≤ Grade 2[c]	Reduce by 1 dose level. Permanent discontinuation can be considered at treating investigator's discretion	

a. NCI-CTCAE = National Cancer Institute - Common Terminology Criteria for Adverse Events, version 4.0
b. Excludes alopecia, non-refractory nausea/vomiting, non-refractory hypersensitivity and nonclinical and asymptomatic laboratory abnormalities.
c. If no recovery after a 4 week delay*, treatment should be permanently discontinued unless subject is deriving clinical benefit.

The table above outlines dose adjustments for hematologic and non-hematologic toxicities related to regorafenib except HFSR and hypertension.

In addition to these recommended dose modifications, subjects who develop diarrhea, mucositis, anorexia or other events predisposing to fluid loss or inadequate fluid intake should be carefully monitored and rehydrated as clinically necessary. This is in order to minimize the risk of postural hypotension and renal failure.

Table 6-2: Grading for Hand-Foot-Skin-Reaction

	Grade 1	**Grade 2**	**Grade 3**
NCI-CTCAE v4.0 Palmar-plantar erythrodysesthesia syndrome	Minimal skin changes or dermatitis (e.g., erythema, edema, or hyperkeratosis) without pain	Skin changes (e.g., peeling, blisters bleeding, edema, or hyperkeratosis) with pain	Severe skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain
Further description / examples of skin changes	Numbness, dysesthesia / paresthesia tingling, painless swelling, or erythema of the hands and/or feet	Painful erythema and swelling of the hands and/or feet	Moist desquamation, ulceration, blistering, or severe pain of the hands and/or feet
Effect on activities	Does not disrupt normal activities	Limiting instrumental activities of daily life (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money)	Limiting self-care activities of daily life (e.g., bathing, dressing and undressing, feeding self, using the toilet, taking medications) and not bedridden

a. Palmer-planter erythrodysesthesia syndrome is a disorder characterized by redness, marked discomfort, swelling, and tingling in the palms of hands or the soles of the feet.

Table 6.3 Recommended dose modification for hand-foot-skin reaction[a]

Grade of event (NCI-CTCAE v4.0)	Occurrence	Suggested Dose Modification
Grade 1	Any	Maintain dose level and immediately institute supportive measures for symptomatic relief
Grade 2	1st occurrence	Consider decreasing dose by one dose level and immediately institute supportive measures. If no improvement, interrupt therapy for a minimum of 7 days, until toxicity resolves to Grade 0-1 [b, c]
	No improvement within 7 days or 2nd occurrence	Interrupt therapy until toxicity resolves to Grade 0-1.[c] When resuming treatment, treat at reduced dose level [b]
	3rd occurrence	Interrupt therapy until toxicity resolves to Grade 0-1. [c] When resuming treatment, decrease dose by one dose level. [b, d]
	4th occurrence	Discontinue therapy
Grade 3	1st occurrence	Institute supportive measures immediately. Interrupt therapy for a minimum of 7 days until toxicity resolves to Grade 0-1. [c] When resuming treatment, decrease dose by one dose level. [b, d]
	2nd occurrence	Institute supportive measures immediately. Interrupt therapy for a minimum of 7 days until toxicity resolves to Grade 0-1. [c] When resuming treatment, decrease dose by one additional dose level [b, d]
	3rd occurrence	Discontinue treatment permanently.

a. More conservative management is allowed if judged medically appropriate by the investigator.
b. If toxicity returns to Grade 0-1 after dose reduction, dose re-escalation is permitted at the discretion of the investigator if subject has completed one cycle at reduced dose without recurrence of event.
c. If there is no recovery after a 4-week delay, treatment with regorafenib will be discontinued permanently.
d. Subjects requiring > 2 dose reductions should go off protocol therapy.
e. The maximum daily dose is 120 mg.

(For studies with combination therapy, consider including the following statement "The other study treatment may be continued").

At first occurrence of HFSR, independent of grade, prompt institution of supportive measures such as topical emollients, low potency steroids, or urea-containing creams should be administered.

Recommended prevention/management strategies for skin toxicities consistent with HFSR are summarized below:

Control of calluses

Before initiating treatment with regorafenib:

- Check condition of hands and feet.
- Suggest a manicure/pedicure, when indicated.
- Recommend pumice stone use for callus or 'rough spot' removal.

During regorafenib treatment:

- Avoid pressure points.
- Avoid items that rub, pinch or create friction.

Use of creams

- Non-urea based creams may be applied liberally.
- Keratolytic creams (e.g. urea-based creams, salicylic acid 6%) may be used sparingly and only to affected (hyperkeratotic) areas.
- Alpha hydroxyl acids (AHA) based creams may be applied liberally 2 times a day. Approximately 5% to 8% provides gentle chemical exfoliation.
- Topical analgesics (e.g. lidocaine 2%) are to be considered for pain control.
- Topical corticosteroids like clobetasol 0.05% should be considered for subjects with Grade 2 or 3 HFSR. Avoid systemic steroids.

Tender areas should be protected as follows:

- Use socks/gloves to cover moisturizing creams
- Wear well-padded footwear
- Use insole cushions or inserts (e.g. silicon, gel)
- Foot soaks with tepid water and Epson salts

Hypertension

Hypertension is a known AE associated with regorafenib treatment. Subject will have their blood pressure measured at least weekly at the study site during the first 6 weeks of treatment. If additional blood pressure measurements are done outside the study site, and the blood pressure is > 140 mm Hg systolic or > 90 mm Hg diastolic (NCI CTCAE v4.0), then the subject must contact study personnel. The management of hypertension, including the choice of antihypertensive medication, will be performed according to local standards and to the usual practice of the investigator. Every effort should be made to control blood pressure by medical means other than study drug dose modification. If necessary, Table 6-4 outlines suggested dose reductions.

Grade (CTCAE v4.0)	**Antihypertensive Therapy**	**Regorafenib Dosing**
1 Prehypertension (systolic BP 120 - 139 mmHg or diastolic BP 80 - 89 mmHg)	None	• Continue regorafenib • Consider increasing blood pressure (BP) monitoring
2 Systolic BP 140 - 159 mmHg or diastolic BP 90 - 99 mmHg, OR Symptomatic increase by > 20 mmHg (diastolic) if previously within normal limits	• Treat with the aim to achieve diastolic BP ≤ 90 mm Hg: • If BP previously within normal limits, start anti-hypertensive monotherapy • If patient already on anti-hypertensive medication, titrate up the dose.	• Continue regorafenib • If symptomatic, hold regorafenib until symptoms resolve AND diastolic BP ≤ 90 mm Hg[a]. When regorafenib is restarted, continue at the same dose level.
3 Systolic BP ≥ 160 mmHg or diastolic BP ≥ 100 mmHg OR More than one drug or more intensive therapy than previously used indicated	Treat with the aim to achieve diastolic BP ≤ 90 mm Hg: Start anti-hypertensive medication **AND/OR** Increase current anti-hypertensive medication **AND/OR** Add additional anti-hypertensive medications.	• Hold regorafenib until diastolic BP ≤ 90 mm Hg, and if symptomatic, until symptoms resolve.[a] • When regorafenib is restarted, continue at the same dose level. • If BP is not controlled with the addition of new or more intensive therapy, reduce by 1 dose level.[b] • If Grade 3 hypertension recurs despite dose reduction and antihypertensive therapy, reduce another dose level.[c]
4 Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis)	Per institutional guidelines	Discontinue therapy

a. Patients requiring a delay of >4 weeks should go off protocol therapy
b. If BP remains controlled for at least one cycle, dose re-escalation permitted per investigator's discretion.
c. Patients requiring >2 dose reductions should go off protocol therapy.

Table 6-4: Management of Treatment-Emergent Hypertension

Liver Function Abnormalities

For patients with observed worsening of serum liver tests considered related to regorafenib (i.e. where no alternative cause is evident, such as post-hepatic cholestasis or disease progression), the dose modification and monitoring advice in Table 6-5 should be followed.

Regorafenib is a UGT1A1 inhibitor. Mild, indirect (unconjugated) hyperbilirubinemia may occur in patients with Gilbert's syndrome.

Table 6.5: Recommended measures and dose modifications in case of drug-related liver function test abnormalities

Observed elevations of ALT and/or AST	**Occurrence**	**Recommended measures and dose modification**
≤ 5 times upper limit of normal (ULN) (maximum Grade 2)	Any occurrence	Continue Regorafenib treatment. Monitor liver function weekly until transaminases return to < 3 times ULN (Grade 1) or baseline.
>5 times ULN to ≤ 20 times ULN (Grade 3)	1st occurrence	Interrupt Regorafenib treatment. Monitor transaminases weekly until return to < 3 times ULN or baseline. Restart: If the potential benefit outweighs the risk of hepatotoxicity, re-initiate Regorafenib treatment, reduce dose by 40 mg (one tablet), and monitor liver function weekly for at least 4 weeks.
	Re-occurrence	Discontinue treatment with Regorafenib permanently.
>20 times ULN (Grade 4)	Any occurrence	Discontinue treatment with Regorafenib permanently.
>3 times ULN (Grade 2 or higher) with concurrent bilirubin >2 times ULN	Any occurrence	Discontinue treatment with Regorafenib permanently. Monitor liver function weekly until resolution or return to baseline. Exceptions: patients with Gilbert's syndrome who develop elevated transaminases should be managed as per the above outlined recommendations for the respective observed elevation of ALT and/or AST.

6.2.2 Prevention/management strategies for diarrhea

Diarrhea can be a common side effect of regorafenib . The preventive/management strategies for diarrhea should be consistent with local standards (e.g., anti-diarrheals and optimized hydration status).

Anti-diarrhea medications may be introduced if symptoms occur. Previous trials have shown that the diarrhea could be managed with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2 to 4 hours until diarrhea-free for 12 hours.

6.3 Drug logistics and accountability

Regorafenib is an FDA approved agent for metastatic colorectal cancer. It will be prescribed by the investigators or their staff. Patient will obtain it from their retail or specialty pharmacies.

6.4 Treatment compliance

An adequate record of receipt, distribution, and return of all study drugs must be kept in the form of a Drug Accountability Form.

Subject compliance with the treatment and protocol includes willingness to comply with all aspects of the protocol, and to have blood collected for all safety evaluations. At the discretion of the principal investigator, a subject may be discontinued from the trial for non-compliance with follow-up visits or study drug.

6.5 Prior and concomitant therapy

All medication that is considered necessary for the subject's welfare, and which is not expected to interfere with the evaluation of the study treatment, may be given at the discretion of the investigator. All medications (including contrast media) taken within 2 weeks prior to the start of the study and during the study must be recorded in the subject's source documentation and in the CRF (including start/stop dates, dose frequency, route of administration, and indication). Specific caution should be taken when considering or administering a concomitant medication that is metabolized by the cytochrome enzymes CYP2C8, CYP2B6 and CYP2C9. Such concomitant medication should be avoided, if possible.

Co-administration of a strong CYP3A4 inducer (rifampin) with a single 160 mg dose of Stivarga decreased the mean exposure of regorafenib, increased the mean exposure of the active metabolite M-5, and resulted in no change in the mean exposure of the active metabolite M-2. Avoid concomitant use of Stivarga with strong CYP3A4 inducers (e.g. rifampin, phenytoin, carbamazepine, phenobarbital, and St. John's Wort)

Co administration of a strong CYP3A4 inhibitor (ketoconazole) with a single 160 mg dose of Stivarga increased the mean exposure of regorafenib and decreased the mean exposure of the active metabolites M-2 and M-5. Avoid concomitant use of Stivarga with strong inhibitors of CYP3A4 activity (e.g., clarithromycin, grapefruit juice, itraconazole, ketoconazole, nefazadone, posaconazole, telithromycin, and voriconazole).

Permitted concomitant therapy includes:

- Standard therapies for concurrent medical conditions.
- Supportive care for any underlying illness.
- Palliative radiation therapy is allowed if the target lesion(s) are not included within the radiation field and no more than 10% of the bone marrow is irradiated.
- Granulocyte colony-stimulating factor (G-CSF) and other hematopoietic growth factors may be used in the management of acute toxicity, such as febrile neutropenia, when clinically indicated or at the investigator's discretion. However, they may not be substituted for a required dose reduction. Subjects are permitted to take chronic erythropoietin.
- Treatment with nonconventional therapies (such as acupuncture), and vitamin/mineral supplements are permitted provided that they do not interfere with the study endpoints, in the opinion of the investigator.
- Bisphosphonates

- As the study requires tissue biopsies, subjects that are therapeutically treated with an agent such as warfarin or heparin will not be allowed to participate in the trial. However, patients that require anticoagulation after the biopsies are obtained can continue on the study. The following are not permitted:
- Other investigational treatment during or within 30 days before starting study treatment
- Systemic antitumor therapy, including cytotoxic therapy, signal transduction inhibitors, immunotherapy, and hormonal therapy
- Bone marrow transplant or stem cell rescue
- Subjects taking narrow therapeutic index medications should be monitored proactively (e.g. warfarin, phenytoin, quinidine, carbamazepine, Phenobarbital, cycloosporin, and digoxin). Warfarin is metabolized by the cytochrome enzyme CYP2C9 and its levels may be especially affected by regorafenib
- Use of any herbal remedy (e.g. St. John's wort [Hypericum perforatum])
- Please note: Patients should be seen frequently / early during treatment as per Prescribing Information
- Liver function tests should be obtained before initiation of regorafenib and monitored at least every 2 weeks during first 2 months of treatment. Thereafter liver function should be monitored monthly or more frequently as clinically indicated
- Monitor blood pressure weekly for the first 6 weeks of treatment and every cycle or more frequently as clinically indicated.

7. Detailed Study Plan

7.1 Screening

Screening will occur within 28 days prior to administration of the first dose of chemotherapy on Day 1. Signed informed consent will be obtained from the subject or the subject's legally acceptable representative before any study-specific procedures are undertaken. For procedures performed at screening and repeated, the later procedure performed prior to dosing will serve as a baseline for clinical assessment. A complete history and physical will be obtained at the screening visit. Additionally, labs will be reviewed/ordered during the screening visit, prior to the initiation of therapy.

7.2 Study Procedures (details are provided in the events table (Page 42)

7.2.1 Subject assessments

Physical examinations (including neurological assessment), vital signs (vital sign determinations include heart rate, blood pressure and body temperature - *Vital signs should be taken after a 5-minute resting period. Labs will then be drawn once the vital signs are recorded*), performance status, chemistry, hematology, medication review, and adverse event evaluations will be conducted at screening, on C1D1, C1D14, C2D1, C2D14, D1 of every cycle starting C3, and at the off study visit. Study procedures may be performed 2 days before the scheduled visit date, due to unforeseen or unavoidable circumstances.

Body weight will be recorded during every physical exam. The subject will wear lightweight clothing and no shoes during weighing. Height will be measured at the Screening Visit only; the subject will not wear shoes.

30 days after the patient's "end of treatment" visit; a 30-day follow-up patient survival assessment will be made by phone.

7.2.1.1 Tumor Imaging and Response Evaluation

Baseline imaging (CT scan) will be carried out for all cohorts within 28 days prior to regorafenib therapy—— and then must be performed every 8 weeks (as near to Day 1 as possible; every 2 cycles) following initiation of regorafenib treatment.

7.2.1.2 Medical History

The following information will be collected during the Screening Visit:

1) Complete medical history, including documentation of any clinically significant medical conditions
2) History of tobacco and alcohol use
3) Presence and severity of any symptoms/conditions associated with metastatic colorectal cancer
4) Detailed oncology history, including:
    a. Date of primary cancer diagnosis
    b. Pathology (histology or cytology) of primary tumor
    c. Metastasis information (including the location)
    d. Surgical history
    e. Anti-cancer and radiation treatments administered (including dates and type of modality)

7.2.1.3 Research Samples

Tumor biopsies will be obtained at baseline—prior to treatment initiation—and on day 14-21 of therapy (cycle 1). Peripheral blood collection for research purposes will occur at baseline, on day 14-21 of therapy (on day of tumor biopsy; cycle 1) and then on D1 of each cycle starting C3 (i.e., every 4 weeks starting cycle 3) up to 8 blood draws while taking regorafenib on study.

7.2.1.4 Pregnancy Test

For female subjects of childbearing potential, a serum pregnancy test will be performed at the Screening Visit within 28 days of C1D1. Subjects considered not of childbearing potential must be documented as being surgically sterile or post-menopausal (for at least 1 year). The test results must be reviewed and determined to be negative prior to dosing. The test may be repeated at the discretion of the investigator at any time during the study. Should a female study subject become pregnant or suspect she is pregnant while participating in this study, she should inform the treating Investigator immediately.

7.2.1.5 Clinical Laboratory Tests

All subjects will undergo the laboratory assessments outlined in the events table.

1) Hematology and chemistry samples will be collected at the Screening Visit, on C1D1, C1D14, C2D1, C2D14, D1 of every cycle starting C3, and at the off study visit. For Day 1 of each cycle after Cycle 1, hematology and chemistry samples may be collected up to 48 hours prior to the scheduled visit.

2) A CEA will be collected on Day 1 of every cycle

Clinical laboratory samples for this study will be assessed using the certified laboratories at Georgetown University and affiliated Medstar Hospitals; OR any CLIA-certified laboratory such as LabCorp or Quest is acceptable. The PI or sub-investigator will review, initial and date all laboratory results. Any laboratory value outside the reference range that is considered clinically significant by the investigator will be followed as appropriate. Clinically significant laboratory values will be recorded as adverse events if they meet the criteria as specified in Section 9.4.

7.2.1.6 Imaging and Response Evaluation

Imaging studies should include a diagnostic CT scan of the chest, abdomen, and pelvis with PO and IV contrast (unless medically contraindicated). Patients may undergo other modalities such as an MRI instead of a CT scan at the treating physician's discretion if appropriate (such as patient allergy to CT contrast, extremity tumors, bone metastases requiring bone scans, etc).
Response evaluation will occur every 8 weeks (as measured from Cycle 1, Day 1), and at the off study visit (if not performed within the last four weeks). Tumor response and/or disease progression will be assessed by the modality(ies) used prior to treatment. Patients will continue to remain on study as long as there is no evidence of progression of disease and the therapy is adequately tolerated.

7.2.1.7 Adverse Event Evaluation

The Principal Investigator or Sub-investigators will assess adverse events, laboratory data and vital signs throughout the study. Adverse events will be assessed by NCI CTCAE Version 4.0.
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf
The investigators will monitor each subject for clinical and laboratory evidence of adverse events on a routine basis throughout the study. The investigator will assess and record any adverse event in detail including the date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome, relationship of the adverse event to study drug, and any action(s) taken. For serious adverse events not considered "probably related" to study drug, the investigator will provide an "Other" cause of the event. For adverse events to be considered intermittent, the events must be of similar nature and severity. Adverse events, whether in response to a query, observed by site personnel, or reported spontaneously by the subject will be recorded. All adverse events will be followed to a satisfactory conclusion.

7.2.1.8 Research Sample Handling Details

All tumor and blood samples will be labeled with the date, protocol number, and protocol assigned patient number.

At baseline (before starting Regorafenib), patients will each be asked to undergo

- peripheral blood draw (7 K2 EDTA lavender top tubes)
- tumor biopsies

Two –three weeks (14-21 days) after starting Regorafenib,

- Peripheral blood draw (7 K2 EDTA lavender top tubes)
- A second tumor biopsies (optional)

7.2.1.9 Peripheral blood samples

Further peripheral blood samples (7 K2 EDTA lavender top tubes) will be collected after initiation of cycle 2, and then at the beginning of every treatment cycle for the duration of regorafenib therapy, up to a total of 8 blood draws.
Specifically, peripheral blood will be collected and banked for miRNA, and mutated DNA analysis.
A peripheral blood sample (7 K2 EDTA lavender top tubes)will also be obtained upon disease progression.
The peripheral blood samples will be sent to Georgetown University Medical Center for storage then distribution to Dr. Wellstein's lab and Caris Life Sciences (4610 South 44th Place, Suite 100, Phoenix, Arizona 85040; Email: mbaker@carisls.com; Phone: 602-792-2407) for storage and distribution.

7.2.1.10 Tumor Biopsies

The second tumor biopsy (at 2-3 weeks) can be waived in case of patient refusal, complications after the first biopsy or if the investigator deems the biopsy unsafe.

Tumor samples obtained at Georgetown Hospital and other sites will be collected by the staff of the biospecimen bank. Two specimens will be placed in biopsy cassettes embedded in optimal cutting temperature compound (OCT) and snap frozen within 15 min in dry ice or liquid nitrogen and subsequently stored at -80 until further use. The OCT embedded frozen core needle biopsy samples will be Samples will be stored in the Georgetown University laboratory then shipped to the laboratory of Dr. Emanuel Petricoin (George Mason University, 10920 George Mason Circle , Room 2006 Institute of Advanced Biomedical Research , Manassas VA 20110; Email: epetrico@gmu.edu; Phone: 703-993-8646) via FedEx medical shipment service after the last sample has been obtained or two years after the first sample has been obtained whichever occurs first. Once at George Mason University tissue-based laser capture microdissection and protein pathway activation mapping via reverse phase protein array analysis will take place. The rest of the samples will be placed in formalin and then paraffin blocks will be formed to be sent to Caris for CMI testing. Only the baseline tumor samples should be sent to Caris. Please see Appendix 14.1 for specimen requirements.

EVENTS TABLE and STUDY CALENDAR

Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to the start of therapy. Unless otherwise noted, a standard window of -1 day to + 2 days will be considered acceptable for all testing and evaluations (will not be considered study deviation). In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next dose of therapy.

	Screening Day -28 to -1	C1D1 & C2D1	Day 1 - 21 of every Cycle; starting C1	C1 D 14-21	C1D14 & C2D14	Day 1 of each cycle starting cycle 2	Every 8 weeks	Disease progression or end of treatment[8]
Informed consent	X							
Demographics	X							
Medical history	X							
Concurrent meds	X	X			X	X		X
β-HCG[1]	X							
Physical Exam	X	X			X	X		X
Vital signs	X	X			X	X		X
Height	X							
Weight	X	X			X	X		X
Performance status	X	X			X	X		X
Oral Regorafenib		X	X		X	X		
CBC with diff	X	X			X	X		X
Serum chemistries [2]	X	X			X	X		X
PT/INR/PTT[7]	X							
CEA[3]	X	X				X		X
Adverse event evaluation	X	X			X	X		X
Radiological evaluation and tumor measurement [4]	X						X	X[5]
Research Biopsy	X			X				
Research serum samples[6]	X			X		X		X

[1] A serum pregnancy test (women of child bearing potential) at screening only. However, the test may be repeated at the discretion of the investigator at any time during the study.

[2] Serum chemistry will include sodium, potassium, chloride, BUN, bicarbonate creatinine, AST, ALT, total bilirubin, direct bilirubin, albumin, total protein and alkaline phosphatase

[3] CEA should be taken on D1 monthly

[4] Tumor measurements will be obtained by CT scan (or MRI at physician discretion) using RECIST criteria. If patient insurance will not cover a CT/MRI scan within 14 days of regorafenib treatment then a scan within 4 weeks of treatment is acceptable.
[5] Only if not performed within the last 4 weeks
[6] Patients will undergo peripheral blood collection for research purposes every 4 weeks for up to 8 blood draws (if still actively on study for that long)
[7] PT/INR/PTT is only performed at screening unless especially required at 4 weeks by the investigator.
[8] 30 days after the patient's "end of treatment" visit, a 30-day follow-up patient survival assessment will be made by phone.

8. Endpoints

8.1 Primary endpoint:

8.1.1 Level of serum miRNAs at baseline, 4 and 8 weeks of study treatment

8.2 Secondary endpoints:

8.2.1 Eight-week clinical benefit rate (CBR):

Defined as proportion of patients who achieve completed response (CR) any time + partial response (PR) any time + stable disease (SD) at 8-week and will be measured every eight weeks based on the RECIST criteria version 1.1.

8.2.2 Objective response rate (ORR):

Will follow the RECIST criteria version 1.1 and will be defined as partial response (PR) or complete response (CR). Patients who are lost to follow-up without a valid response assessment will be classified as non-responders.

8.2.3 Progression free survival (PFS):

Defined as the number of days from study entry until progression or death; patients who are alive and free from progression on the date of closing follow-up will be censored on that date.

8.2.4 Overall survival (OS):

Defined as the time in days from study entry until death; patients who are alive on the date of closing follow-up will be censored on that date. All events of death will be included.

8.2.5 Safety:

Assessed by determining the percent of subjects who experience unacceptable (Grade 3/4) adverse events and by describing the safety profiles; adverse events will be summarized by the common terminology criteria for adverse events (CTCAE version 4.0).

8.2.6 Activation status of phosphoproteins in tissue specimens

At baseline and 2-3 weeks into treatment; as measured by RPMA assay

8.2.7 Presence mutated DNA in serum at baseline, 4 and 8 weeks of study treatment

8.3 Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below.

8.3.1 Measurable disease

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

8.3.2 Non-measurable disease

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

8.3.3 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

8.3.4 Non-target lesions

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

8.3.5 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 when both methods have been used to assess the antitumor effect of a treatment.

Clinical Lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, 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 These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US) When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

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.

8.3.6 Response Criteria

Evaluation of target lesions:

8.3.6.1 Complete Response (CR):

Disappearance of all target lesions

8.3.6.2 Partial Response (PR):

At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

8.3.6.3 Progressive Disease (PD):

At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

8.3.6.4 Stable Disease (SD):

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

8.3.6.5 Clinical benefit rate (CBR):

Objective responses (complete or partial response [PR] as well as stable disease [SD] $\geq$ 8 weeks)

8.3.6.6 Progression free survival (PFS):

Time from randomization until objective tumor progression or death from any cause

8.3.6.7 Overall survival (OS):

Time from randomization until death from any cause

8.3.6.8 Objective response rate:

Proportion of patients with tumor size reduction of a predefined amount and for a minimum time period

8.4 Safety

All subjects who receive at least one dose of study treatment will be valid for the safety analysis.

All observations pertinent to the safety of the study treatment will be recorded and included in the final report.

Safety variables include the following: AEs, laboratory changes (complete blood counts, electrolytes, chemistry, and coagulation), changes in vital signs (blood pressure, heart rate, respiratory rate, and temperature) and ECG and, in some instances, changes in chest x-ray images, as produced at the investigator's discretion (e.g., for evaluation for pneumonia).

All AEs whether considered drug-related or not, will be reported in with a diagnosis, start/stop dates, action taken, whether treatment was discontinued, any corrective measures taken, outcome, and other possible causes. For all events, the relationship to treatment and the intensity of the event will be determined by the investigator.

This trial will use the NCI-CTCAE v4.0 criteria for assessment of toxicity and SAE reporting with regard to toxicity grade.

8.4.1 Data safety Monitoring Plan:

The Georgetown Lombardi Comprehensive Cancer Center will be responsible for the data and safety monitoring of this multi-site trial. As this study is an investigator initiated exploratory study utilizing an FDA approved drug it is considered a moderate risk study which requires real-time monitoring by the PI and study team and semi-annual reviews by the LCCC Data and Safety Monitoring Committee (DSMC).

The Principal Investigator and the Co-Investigators will review the data including safety monitoring at their weekly institution based disease group meetings and on monthly teleconferences.

All Severe Adverse Events (SAEs) are required to be reported to the IRB. Based on SAEs, the IRB retains the authority to suspend further accrual pending more detailed reporting and/or modifications to further reduce risk and maximize the safety of participating patients.

Progress on the trial and the toxicities experienced will be reviewed by the LCCC Data and Safety Monitoring Committee every 6 months from the time the first patient is enrolled on the study. Results of the DSMC meetings will be forwarded to the IRB with recommendations regarding need for study closure.

DSMC recommendations should be based not only on results for the trial being monitored as well as on data available to the DSMC from other studies. It is the responsibility of the PI to ensure that the DSMC is kept apprised of non-confidential results from related studies that become available. It is the responsibility of the DSMC to determine the extent to which this information is relevant to its decisions related to the specific trial being monitored.

A written copy of the DSMC recommendations will be given to the trial PI and the IRB. If the DSMC recommends a study change for patient safety or efficacy reasons the trial PI must act to implement the change

as expeditiously as possible. In the unlikely event that the trial PI does not concur with the DSMC recommendations, then the LCCC Associate Director of Clinical Research must be informed of the reason for the disagreement. The trial PI, DSMC Chair, and the LCCC AD for Clinical Research will be responsible for reaching a mutually acceptable decision about the study and providing details of that decision to the IRB. Confidentiality must be preserved during these discussions. However, in some cases, relevant data may be shared with other selected trial investigators and staff to seek advice to assist in reaching a mutually acceptable decision.

If a recommendation is made to change a trial for reasons other than patient safety or efficacy the DSMC will provide an adequate rationale for its decision. If the DSMC recommends that the trial be closed for any reason, the recommendation will be reviewed by the Associate Director for Clinical Research at G-LCCC. Authority to close a trial for safety reasons lies with the IRB, with the above described input from DSMC and the AD for Clinical Research.

8.4.2 Adverse events

Investigators should refer to the Safety Information section of the current IB for regorafenib , including the DCSI (development core safety information), for the expected side effects of , regorafenib. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions. The IB will be updated if any new relevant safety data are obtained.

Therapeutic monitoring should be performed following dose selection or modification of regorafenib , in a manner consistent with the local clinical standard of care. In general, subjects should be closely monitored for side effects of all concomitant medications regardless of the path of drug elimination.

All concomitant medications must be recorded in the subject's source documentation.

Subjects must be carefully monitored for AEs. This monitoring also includes clinical laboratory tests. Adverse events should be assessed in terms of their seriousness, intensity, and relationship to the study drug, or other chemotherapy/treatment.

8.4.3 Definitions

Definition of adverse event (AE)

> In a clinical study, an AE is any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as AE (however, the condition for which the surgery is-required may be an AE if worsens compared to baseline).

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history (e.g. allergic pollinosis).

- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events.

Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death.

b. Is life-threatening.

The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization.

A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours.
- The admission is pre-planned.
  (i.e. elective or scheduled surgery arranged prior to the start of the study)
- The admission is not associated with an AE.
  (e.g., social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity.

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect.

f. Is another medically important serious event as judged by the investigator

8.4.4 Classifications for adverse event assessment

All AEs will be assessed and documented by the investigator according to the categories detailed below.

8.4.4.1 Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 8.4.3.

8.4.4.2 Intensity

The intensity of the AE is classified according to the CTCAE v4.0. Grade refers to the severity (intensity) of the AE:

CTCAE v4 Grade 1: mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.

CTCAE v4 Grade 2: moderate; minimal, local, or noninvasive intervention is indicated; limiting to age-appropriate instrumental activities of daily living (ADL; instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).

CTCAE v4 Grade 3: Severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting to self care ADL (self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

CTCAE v4 Grade 4: life-threatening consequences; urgent intervention is indicated.

CTCAE v4 Grade 5: death due to an AE.

8.4.4.3 Causal relationship

The assessment of the causal relationship between an AE and the administration of treatment is a clinical decision based on all available information.

The assessment is based on the question whether there was a "reasonable causal relationship" to the study treatment in question.

Possible answers are "yes" or "no".

An assessment of "no" would include:

1. The existence of a clear alternative explanation, e.g. mechanical bleeding at surgical site.

Or

2. Non-plausibility, e.g. the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration.

An assessment of "yes" indicates that there is a reasonable suspicion that the AE is associated with the use of the study treatment.

Factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge):
- Subject's response after de-challenge or subjects response after re-challenge should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases:
  Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment:
  The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study treatment:
  The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual subject's pharmacodynamics should be considered.

[Causal relationship to protocol-required procedure(s)]

The assessment of a possible causal relationship between the AE and protocol-required procedure(s) is based on the question whether there was a "reasonable causal relationship" to protocol-required procedure(s).

Possible answers are "yes" or "no".

8.4.4.4 Action taken with study treatment

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

8.4.4.5 Other specific treatment(s) of adverse events

- None
- Remedial drug therapy

8.4.4.6 Outcome

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

8.4.5 Assessments and documentation of adverse events

8.4.5.1 Reporting of serious adverse events

The definition of serious adverse events (SAEs) is given in Section 8.4.3.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

All serious adverse events should be reported to Bayer within 24 hours. In the event of such an event, the investigator should refer to the Pharmacovigilance section of the contract for reporting procedures.

The Investigator may report serious adverse drug reactions (SADRs) using either:

An ADEERS form (Adverse Event Expedited Reporting System) available at
http://ctep.cancer.gov/reporting/adeers.html

OR

A MedWatch form available at http://www.fda.gov/medwatch/

All reports shall be sent electronically to:

Electronic Mailbox: DrugSafety.GPV.US@bayer.com

Facsimile: (973) 709-2185

Address:
Mail only:
Global Pharmacovigilance - USA
Bayer HealthCare Pharmaceuticals Inc.
P.O. Box 915
Whippany, NJ 07981-0915

Address:
FDX or UPS only
100 Bayer Boulevard, Whippany, NJ 07981

Reports for all Bayer products can also be phoned in via our Clinical Communications Dept:

Phone: 1-888-842-2937

8.4.5.2 Expected adverse events

For this study, the applicable reference document is the most current version of the investigator's brochure (IB) / summary of product characteristics.

Overview listings of frequent events that have occurred so far in the clinical development are shown in the current IB. If relevant new safety information is identified, the information will be integrated into an update of the IB and distributed to all participating sites.

The expectedness of AEs will be determined by Bayer according to the applicable reference document and according to all local regulations.

8.4.5.3 Adverse events of special safety interest

As with any new chemical entity, there is always potential for unexpected adverse events, including hypersensitivity reactions.

Based on data studies with regorafenib and from current knowledge of the pharmacological properties of other small molecule tyrosine kinase inhibitors in this drug class, as soon as there is reasonable suspicion of any of the following AEs, the investigator should immediately notify the sponsor as outlined in Section 7.4.1.4.

Reportable adverse events include:

- Acute renal failure (NCI-CTCAE version 4.0 ≥ grade 3) or severe proteinuria (NCI-CTCAE version 4.0 ≥ grade 3)
- Interstitial lung disease
- Acute cardiac failure
- Clinically significant bleeding (NCI-CTCAE version 4.0 ≥ grade 3)
- Stevens-Johnson Syndrome and erythema multiforme

- Hepatic failure
- Reversible posterior leukoencephalopathy syndrome
- Gastrointestinal perforation or fistula

8.4.6 Pregnancies/Contraception

The effects of regorafenib on the developing human fetus at the recommended therapeutic dose are unknown. Regorafenib was embryo-lethal and teratogenic in rats and rabbits at exposures lower than human exposures at the recommended dose, with increased incidences of cardiovascular, genitourinary, and skeletal malformations. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

The investigator must report to Bayer any pregnancy occurring in a study subject, or in his partner, during the subject's participation in this study. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

For a study subject, the outcome of the pregnancy should be followed up carefully, and any abnormal outcome of the mother or the child should be reported.

For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent.

For all reports, the forms provided are to be used.

9. Statistical methods and determination of sample size

9.1 Analysis sets

9.1.1 Safety analysis set.

The safety analysis set includes all enrolled patients who receive at least one dose of study medication.

9.1.1.1 Full analysis set.

The full analysis set includes all enrolled patients

9.1.1.2 Response analysis set

All enrolled patients who are eligible, receive study treatment, have baseline assessments and at least 1 on-study tumor assessment will be considered evaluable for response. Patients who are treated and removed from study prior to on-study tumor assessment because of disease progression will be considered evaluable for efficacy and counted as failures.

9.2 Variables

9.2.1 The primary endpoints for biomarker discovery:

1) Level of serum miRNAs at baseline, 4 and 8 weeks of study treatment

9.2.2 The secondary endpoints are:

1) Eight-week clinical benefit rate (CBR), defined as proportion of patients who achieve completed response (CR) any time + partial response (PR) any time + stable disease (SD) at 8-week.
2) Objective response rate (ORR), will follow the RECIST criteria and will be defined as partial response (PR) or complete response (CR). Patients who are lost to follow-up without a valid response assessment will be classified as non-responders.
3) Progression free survival (PFS), defined as the number of days from study entry until progression or death. Patients who are alive and free from progression on the date of closing follow-up will be censored on that date.
4) Overall survival (OS), defined as the time in days from study entry until death. Patients who are alive on the date of closing follow-up will be censored on that date. All events of death will be included.
5) Safety will be assessed by determining the percent of subjects who experience unacceptable (Grade 3/4) adverse events and by describing the safety profiles. Adverse events will be summarized by the common terminology criteria for adverse events (CTCAE version 4.0).
6) Activation status of phosphoproteins in tissue specimens at baseline and 2-3 weeks into treatment as measured by RPMA assay.
7) Presence and level of mutated DNA in serum at baseline, 4 and 8 weeks of study treatment.

9.3 Statistical and analytical plans

Demographic and baseline characteristics will be summarized in all subjects using descriptive statistics.

9.3.1 Analysis for the Primary endpoints:

The analysis will be exploratory in nature. There will be no adjustment for multiplicity to be utilized.

For clinically relevant biomarker discovery, exploratory analyses of biomarkers will be performed to determine if a correlation can be established between the presence or change in the level of a marker species ("yes" or "no") and the clinical response. Our main goal is to identify biomarkers that, when assayed at baseline, can predict the patient's response to regorafenib. Descriptive statistics, graphical methods, and statistical modeling including repeated measure models, whichever is appropriate, may be used. The expression of biomarker levels within patients at different time points will be analyzed using paired t-test or the non-parametric Wilcoxon Signed Rank test to see whether any changes between different time points are apparent. Chi-square test or Fisher's exact test will be used to explore the association between categorical variables and the clinical response. We may also use Cox models for association of biomarker variables (categorical or continuous) and time-to-event variables. Kaplan-Meier method will be used to explore the association between categorical variables and time-to-event response variables. ROC (Receiver Operating Characteristic) curves will also be plotted to examine the sensitivity and specificity of the identified biomarkers.

9.3.2 Analysis for the secondary endpoints:

The clinical benefit rate (CBR) will be estimated with 95% exact binomial confidence interval.

The objective response rate (ORR) will be computed for all subjects with at least one measurable lesion at baseline.

The progression free survival (PFS), as well as overall survival (OS), will be estimated and presented by the method of Kaplan and Meier (1958) and 95% confidence interval will be presented for the estimated PFS survival rate and for the median.

Safety analyses will be performed in all treated subjects. Descriptive statistics of safety will be presented using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All on-study AEs, treatment-related AEs, SAEs, and treatment-related SAEs will be tabulated using worst grade per NCI CTCAE v 4.0 criteria by system organ class and preferred term. On-study lab parameters including hematology, chemistry, liver function, and renal function will be summarized using worst grade NCI CTCAE v 4.0 criteria. For continuous safety variables, mean, standard deviation, and 95% confidence interval will be calculated at baseline and each visit.

Descriptive statistics (N, percentage for categorical variables and N, mean, std, median, min, max for continuous variables) will be used to summarize the activation status of phosphoproteins, presence and level of mutated DNA in serum at baseline, 4 and 8 weeks of study treatment. Repeated measures models may be used to explore the relationship between the level of mutated DNA in serum with clinical response.

9.4 Determination of sample size

The study is exploratory in nature. The sample size is not based on certain statistical power for significance testing. However, a sample size of 40 could demonstrate an effect size of 0.5 using a 1 degree of freedom Chi-Square test with 80% power at a significance level of 5%. If the biomarkers we are looking for exhibit big changes/big-effect size, then this sample size would be sufficient for us to perform the analysis. It is estimated 40 patients will be enrolled at Georgetown University.

10. Data handling and quality assurance

10.1 Data recording

Clinical and laboratory data will be collected at the Georgetown Lombardi Cancer Center and entered into electronic case report forms (CRF) using LCCC's web-based CRFs.

10.2 Monitoring

The study will be monitored by the Georgetown Lombardi Comprehensive Cancer Center multi-site coordinator and data safety monitoring board (DSMC).

10.3 Data processing

Data will be submitted to the Georgetown Lombardi Cancer Center for final analysis. Since the Georgetown Lombardi Cancer Center is the lead institution in the trial, it will be responsible for accuracy of the data processing and presentation.

10.4 Audit and inspection

Inspections by regulatory health authority representatives i.e. FDA and IEC(s)/IRB(s) are possible. The investigator should notify Bayer immediately of any such inspection.

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10.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

11. Premature termination of the study

- If risk-benefit ratio becomes unacceptable owing to, for example,
  - Safety findings from this study (e.g. SAEs)
  - Results of any interim analysis
  - Results of parallel clinical studies
  - Results of parallel animal studies
    (on e.g. toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions (e.g. IEC(s)/IRB(s); competent authority(ies); study center; head of study center) must be informed as applicable according to local law.
- In case of a partial study closure, ongoing subjects, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section 5.2.1.

12. Ethical and legal aspects

12.1 Ethical and legal conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the

guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to Bayer.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by Bayer. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IEC/IRB/Bayer approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and properly documented.

12.2 Subject information and consent

Each subject / legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the subject / legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject / legal representative or proxy consenter will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of Bayer and the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.

The informed consent form and any other written information provided to subjects / legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and / or the written informed consent form. The investigator will inform the subject / legal representative or proxy consenter of changes in a timely manner and will ask the subject to confirm his/her

participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB`s approval / favorable opinion in advance of use.

12.3 Publication policy

Bayer recognizes the right of the investigator to publish results upon completion of the study. However, the investigator must send a draft manuscript of the publication or abstract to Bayer at least thirty days in advance of submission in order to obtain approval prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and approval will not be withheld unreasonably. In case of a difference of opinion between Bayer and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties. All relevant aspects regarding data reporting and publication will be part of the contract between Bayer and the investigator/institution

The Principal Investigator should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

12.4 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

13. Reference list

(1) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010 Sep-Oct;60(5):277-300.

(2) Siegel R, Desantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012 Jul;62(4):220-241.

(3) Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 2010 Feb 1;116(3):544-573.

(4) Benson AB,3rd, Bekaii-Saab T, Chan E, Chen YJ, Choti MA, Cooper HS, et al. Metastatic colon cancer, version 3.2013: featured updates to the NCCN Guidelines. J Natl Compr Canc Netw 2013 Feb 1;11(2):141-52; quiz 152.

(5) Gerger A, El-Khoueiry A, Zhang W, Yang D, Singh H, Bohanes P, et al. Pharmacogenetic angiogenesis profiling for first-line Bevacizumab plus oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. Clin Cancer Res 2011 Sep 1;17(17):5783-5792.

(6) Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004 Jun 3;350(23):2335-2342.

(7) Hurwitz HI, Yi J, Ince W, Novotny WF, Rosen O. The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. Oncologist 2009 Jan;14(1):22-28.

(8) Suenaga M, Matsusaka S, Ueno M, Yamamoto N, Shinozaki E, Mizunuma N, et al. Predictors of the efficacy of FOLFIRI plus bevacizumab as second-line treatment in metastatic colorectal cancer patients. Surg Today 2011 Aug;41(8):1067-1074.

(9) Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013 Jan 26;381(9863):303-312.

(10) Bates DO, Catalano PJ, Symonds KE, Varey AH, Ramani P, O'Dwyer PJ, et al. Association between VEGF splice isoforms and progression-free survival in metastatic colorectal cancer patients treated with bevacizumab. Clin Cancer Res 2012 Nov 15;18(22):6384-6391.

(11) Etienne-Grimaldi MC, Formento P, Degeorges A, Pierga JY, Delva R, Pivot X, et al. Prospective analysis of the impact of VEGF-A gene polymorphisms on the pharmacodynamics of bevacizumab-based therapy in metastatic breast cancer patients. Br J Clin Pharmacol 2011 Jun;71(6):921-928.

(12) Gerger A, LaBonte M, Lenz HJ. Molecular predictors of response to antiangiogenesis therapies. Cancer J 2011 Mar-Apr;17(2):134-141.

(13) Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, et al. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. Br J Cancer 2010 Oct 26;103(9):1407-1414.

(14) Duda DG, Willett CG, Ancukiewicz M, di Tomaso E, Shah M, Czito BG, et al. Plasma soluble VEGFR-1 is a potential dual biomarker of response and toxicity for bevacizumab with chemoradiation in locally advanced rectal cancer. Oncologist 2010;15(6):577-583.

(15) Chung YS, Maeda K, Sowa M. Prognostic value of angiogenesis in gastro-intestinal tumours. Eur J Cancer 1996 Dec;32A(14):2501-2505.

(16) Shivapurkar N, Mikhail S, Navarro R, Bai W, Marshall J, Hwang J, et al. Decrease in blood miR-296 predicts chemotherapy resistance and poor clinical outcome in patients receiving systemic chemotherapy for metastatic colon cancer. Int J Colorectal Dis 2012 Aug 15.

(17) Wulfkuhle JD, Berg D, Wolff C, Langer R, Tran K, Illi J, et al. Molecular analysis of HER2 signaling in human breast cancer by functional protein pathway activation mapping. **Clin Cancer Res. 2012** Dec 1;18(23):6426-35.

14. Appendices

14.1 Examples of a low fat breakfast

Two slices of white toast with 1 tablespoon of low-fat margarine and 1 tablespoon of jelly and 8 ounces of skim milk. (Approximately 319 calories and 8.2 grams of fat)

One cup of cereal (i.e. Special K), 8 ounces of skimmed milk, one piece of toast with jam (no butter or marmalade), apple juice, and one cup of coffee or tea (2 g fat, 17 g protein, 93 g of carbohydrate, 520 calories).

14.2 Caris Specimen Requirements

Formalin Fixed Paraffin Embedded (FFPE) Samples

Sufficient tumor must be present to complete all analysis. If you have any questions, please contact Client Services at (888) 979-8669.

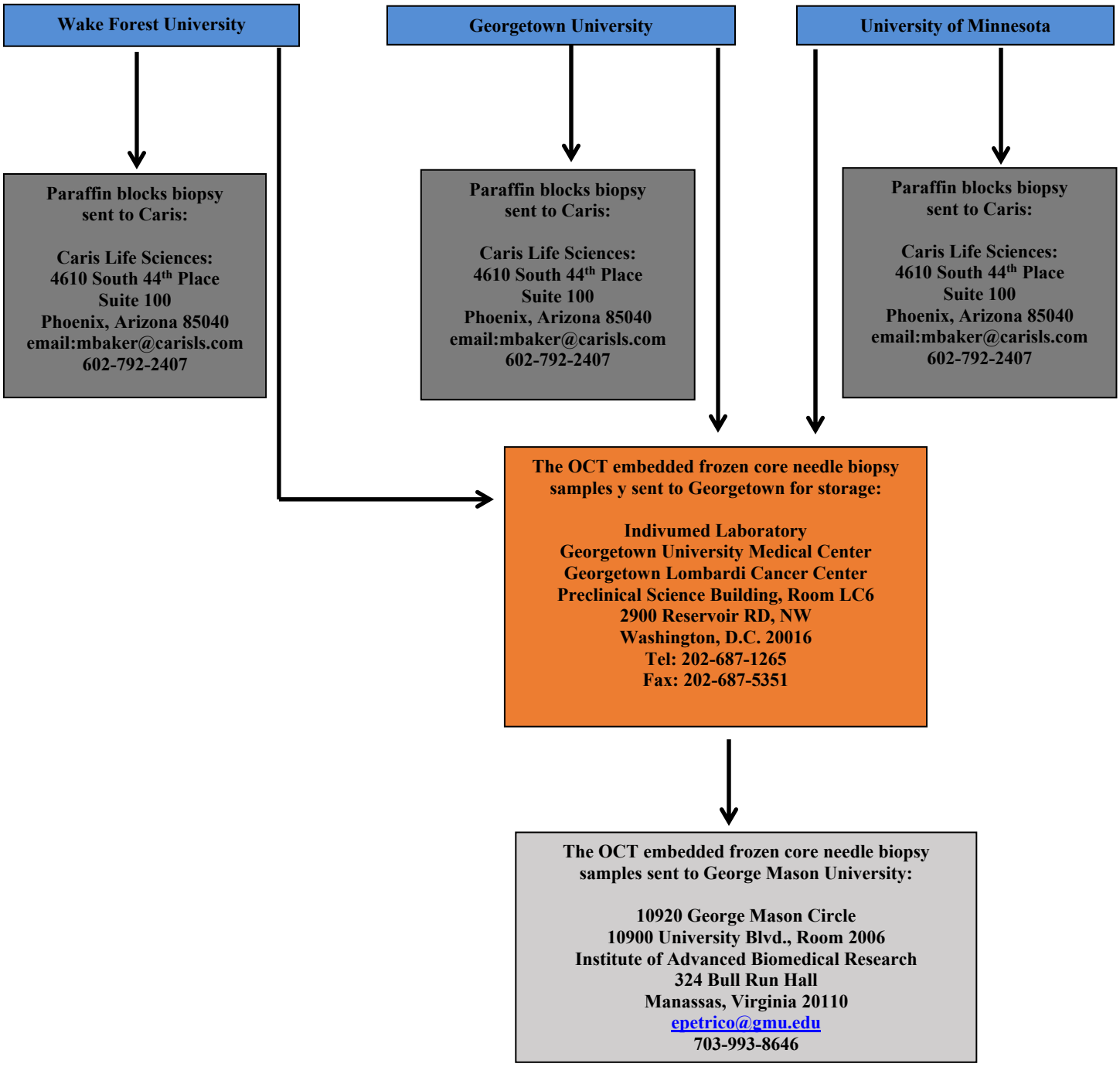
SPECIMEN TYPE	SPECIMEN REQUIREMENTS
Fixed Tissue	One (1) tumor-containing formalin fixed paraffin embedded block (FFPE) from most recent surgery or biopsy. Successive four (4) micron sections will be created from the block until sufficient material for the testing orders is obtained. For the molecular analysis, tumor cells will be excised by microdissection until a total area of at least 50mm^2 is obtained.
Core Needle Biopsy	Four to six (4-6) biopsies formalin fixed paraffin embedded • 18 gauge needle preferred
Fine Needle Aspirate (FNA)	One (1) formalin fixed paraffin embedded block containing sufficient tumor
Unstained Slides	Unstained, positively charged, unbaked slides from one single, tumor-containing formalin fixed paraffin embedded block; 4 micron sections • MI Profile™ - 55 slides • Next-Generation Sequencing only - 15 slides Note: At least a 5mm x 5mm section of tissue per slide is required. For small biopsies (tissue area < 5 mm x 5 mm) please cut two sections per slide for at least one half of the slides to ensure sufficient material for molecular assays.
Malignant Fluid	One (1) formalin fixed paraffin embedded cell block containing sufficient tumor.

Formalin Samples

Sufficient tumor must be present to complete all analysis. If you have any questions, please contact Client Services at (888) 979-8669.

SPECIMEN TYPE	SPECIMEN REQUIREMENTS
Fresh Tissue	Two (2) or more samples with a minimum thickness of ~3mm (height, width, length) and submit in 10% neutral buffered formalin.
Core Needle Biopsy	Four to six (4-6) biopsies • 18 gauge needle preferred
Bone/Bone Metastasis	Two (2) or more samples with minimum thickness of 3mm (height, width, length) and submit in 10% neutral buffered formalin **(DO NOT DECALCIFY)**

14.3 Biopsy Flowchart



14.4 Blood Flowchart