Study of Regorafenib in Combination With Oral Methotrexate for KRAS Mutated Non-Small Cell Lung Cancer (NSCLC)

Study Protocol and Statistical Analysis Plan

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Study of Regorafenib in combination with Oral Methotrexate for *KRAS* Mutated Non-Small Cell Lung Cancer (NSCLC)

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07 Jan 2022	Corrected administrative discrepancies on sample size and exclusion criteria

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PROTOCOL SYNOPSIS

TITLE	Study of Regorafenib in combination with Oral Methotrexate for *KRAS* Mutated Non-Small Cell Lung Cancer (NSCLC)
STUDY PHASE	Phase 2
INDICATION	KRAS mutated NSCLC Patients who have previously received one line of systemic therapy
INVESTIGATION-AL PRODUCT	Regorafenib in combination with Oral Methotrexate
PRIMARY OBJECTIVE	•To determine the progression-free survival (PFS) of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy
SECONDARY OBJECTIVE(S)	•To determine the objective response rate (ORR) of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy •To determine the disease control rate (DCR) at 8 weeks of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy •To determine the safety of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy •To determine the pharmacokinetic parameters of methotrexate when combined with regorafenib (i.e., trough and C_{max})
EXPLORATORY OBJECTIVES	•To correlate circulating tumor DNA (ctDNA) using CAncer Personalized Profiling by Deep Sequencing (CAPP-Seq)[1] pre-treatment and throughout treatment with clinical outcomes •To correlate computational simulation model prediction of sensitivity to the combination of regorafenib and methotrexate based on genomic data with clinical outcomes
INCLUSION/ EXCLUSION CRITERIA (Appendix E)	**Inclusion Criteria** In order to be eligible for participation in this trial, the patient must meet *ALL* of the following criteria (i.e., mark "yes" or "N/A" to all criteria): 1. Histologic or cytologic confirmed diagnosis of non-squamous non-small cell lung cancer that is recurrent or metastatic. Adenosquamous is allowed provided the patient has confirmed adenocarcinoma component. 2. Documentation of pathogenic *KRAS* mutation

	3. Previous receipt of at least one systemic therapy for recurrent or metastatic disease **OR** previous receipt of adjuvant systemic therapy within 6 months of enrollment. There is no limit on number of prior therapies allowed. 4. Prior systemic therapy must be completed at least 2 weeks prior to study treatment, with either improvement of clinically significant treatment-related toxicities to grade 0 to 1 **OR** stabilized to a new baseline. 5. Previously treated **OR** asymptomatic non-progressing < 1 cm untreated brain metastases are allowed 6. Measurable disease based on RECIST version 1.1 criteria (Appendix B) 7. Ability to understand and the willingness to sign a written informed consent document 8. Age ≥ 18 years-old 9. ECOG performance status of 0 or 1 (Appendix A) 10. Adequate bone marrow, liver, and renal function as assessed by the following laboratory requirements: a. Absolute neutrophil count (ANC) ≥ 1500/mm^3 b. Platelet count ≥ 100,000 /mm^3 c. Hemoglobin (Hb) ≥ 9 g/dL d. Serum creatinine ≤ 1.5x upper limit of normal (ULN) **OR** calculated (Cockcroft-Gault formula) *or* measured creatinine clearance ≥ 50 mL/min for patients with creatinine levels > 1.5x ULN e. Total bilirubin ≤ 1.5x ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5x ULN f. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3x ULN (≤ 5x ULN for patients with liver involvement of their cancer) 11. Must be able to swallow and retain oral medication. 12. Women patients of childbearing potential and men patients with women partners of childbearing potential must agree to use adequate contraception or agree to abstain from heterosexual activity beginning at the time of signing informed consent until at least 3 months after the last dose of study treatment. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not considered childbearing.

	Exclusion Criteria In order to be eligible for participation in this trial, the patient must <u>*NOT*</u> meet the following criteria (i.e., mark "no" or "N/A" to all criteria): 1. Previously treated with regorafenib 2. Known allergy to regorafenib or methotrexate 3. Currently receiving another systemic standard or investigational anti-cancer therapy. Prior investigational therapy must be completed within 4 half-lives (if known) or 2 weeks, whichever is longer. The maximal washout of investigational therapy will not exceed 4 weeks prior to study treatment. Bone medications such as bisphosphonates and RANK ligand inhibitors permitted. 4. Leptomeningeal disease as documented by CSF cytology. 5. Clinically significant cardiovascular-related disease including: a. Uncontrolled hypertension (systolic pressure >150 mm Hg or diastolic pressure > 90 mm Hg on *repeated* measurements that does not resolve prior to study treatment on C1D1 despite optimal medical management) b. Congestive heart failure – New York Heart Association (NYHA) Class III or greater c. Active coronary artery disease (i.e., unstable or new onset angina within 3 months of study treatment; myocardial infarction within 6 months of study treatment) d. Clinically significant cardiac arrhythmias other than atrial flutter/fibrillation e. Stroke, including transient ischemic attacks, within 6 months of study treatment f. Other clinically significant arterial events, *except* for controlled asymptomatic pulmonary embolism, within 6 months of study treatment 6. Clinically significant hemorrhage or bleeding event within 1 month of study treatment 7. Uncontrolled symptomatic pleural effusion or ascites 8. Known active additional malignancy that is undergoing or expected to undergo systemic treatment during duration of study participation.

	9. Known history of human immunodeficiency virus (HIV) infection or known current active hepatitis B (i.e., Hep B DNA positive in prior 3 months) or hepatitis C infection (i.e., Hep C RNA positive in prior 3 months, with the exception of patients who have completed curative therapy and are Hep C RNA negative on retest). 10. Major surgical procedure (e.g., involving the opening of a major body cavity) within 4 weeks of study treatment. This does *not* apply to low-risk procedures (i.e., thoracentesis; dparacentesis; chest tube/PleurX catheter placement; line placement; needle biopsy of tumor; and bronchoscopy). 11. Presence of a clinically significant non-healing wound or non-healing ulcer. 12. Concomitant therapy required at time of first dose of study treatment, including: a. Strong CYP3A4 inhibitors and CYP3A4 inducers (Appendix C) a. Regular use of NSAIDs, proton pump inhibitors, and probenecid 13. Women who are pregnant or breast-feeding. 14. Any condition which, in the investigator's opinion, including substance abuse, medical, psychological or social conditions that makes the patient unsuitable for trial participation or may interfere with the patient's participation in the study.
TREATMENT SUMMARY	**Treatment:** Study treatment (regorafenib and methotrexate) will be administered 3 weeks ON/1 week OFF of each 28-day cycle, ongoing until disease progression or intolerable toxicity. <u>Regorafenib</u> will begin on Cycle 1 Day 1 and will be self-administered at 80 mg oral daily 3 weeks on/1 week off of the 28-day cycle with a low-fat meal. Regorafenib will NOT be dose escalated during Cycle 1. If a patient requires a dose reduction below 80 mg daily due to a treatment-related toxicity, the patient should discontinue study treatment. If a patient requires >4 week delay for treatment-related toxicity, it is encouraged that the patient discontinue study treatment, unless the patient may derive ongoing benefit by resuming treatment as per investigator discretion and after discussion with the study PI.

	Regorafenib can be dose escalated to 120 mg oral daily (same schedule of 3 weeks on/1 week off of the 28-day cycle) according to strict criteria beginning at Cycle 2 Day 1. The strict criteria for escalation include no evidence of significant drug-related toxicities (SDRT), defined as any event that would require a dose modification of regorafenib (i.e., interruption only, reduction only, or interruption followed by reduction) according to the toxicity guidelines/tables in Section 6.2.1 (regorafenib toxicities) and Section 6.2.3 (overlapping regorafenib and methotrexate toxicities). If after the first two cycles a patient has not yet escalated or is considering re-escalation to regorafenib 120 mg, this is permitted as long as all treatment-related toxicities have resolved to grade 0-1 or to the patient's baseline and at the discretion of the investigator. **However, a patient is not required to dose escalate regorafenib to 120 mg during the course of study treatment and is permitted to remain at the 80 mg dose if it is better tolerated and per investigator discretion.** Methotrexate will begin on Cycle 1 Day 1 and is self-administered during the same weeks as regorafenib (i.e., 3 weeks on /1 week off each 28-day cycle). The initial starting dose during Cycle 1 Week 1 of methotrexate is 10 mg oral twice weekly. Methotrexate doses will be separated by at least 2 days each week (doses separated by 3 days preferred). Methotrexate will be dose escalated in the following manner as tolerated weekly (+/- 1 day): Cycle 1 Week 1: 10 mg oral twice weekly, Cycle 1 Week 2: 15 mg oral twice weekly, Cycle 1 Week 3: 20 mg oral twice weekly. Beginning in Cycle 2, methotrexate will be self-administered at doses ranging from 10 to 20 mg oral twice weekly, depending on tolerability, with 2-3 days between doses. Dose escalation of methotrexate can continue after Cycle 1 depending on the patient's tolerability, if the patient has not dose escalated to 20 mg by the end of Cycle 1. Methotrexate will be taken within 30 minutes of regorafenib, if feasible, during Cycle 1 for ease of pharmacokinetic studies. Beginning in Cycle 2, patients may take regorafenib and methotrexate at different times of day depending on their preference. Self-administration with food is encouraged to improve tolerability.

	Schedule of Events: **Please see Study Calendar in Section 9 for further details such as windows allowed for study procedures.** Screening: Performed within 30 days of Cycle 1 Day 1 or on Cycle 1 Day 1 if *prior* to the first dose of study treatment. Exception includes brain MRI, which can be performed within 45 days of Cycle 1 Day 1. Screening studies include: informed consent, documentation of patient demographics and medical history, beta-HCG (for women of childbearing potential), EKG (as indicated), review of concurrent medications, physical examination, vital signs (including height and weight), ECOG performance status, complete blood count with differential, serum chemistry, random urine protein:creatinine ratio (as a baseline, not for eligibility), CT chest ± abdomen/pelvis with contrast (unless contraindicated), brain MRI (or CT head), archival tumor tissue (if available). Clinic Visit with Physical Exam/Vitals: Patients will be evaluated in clinic weekly during Cycle 1, and during Week 1 and Week 3 of Cycle 2. Starting with Cycle 3, patients will be evaluated in clinic once per cycle. During these scheduled clinic visits, a physical exam will be performed and vital signs will be documented. During Cycle 2 Week 2, patients will document blood pressure locally and report to investigator team if blood pressure is >140/90. Weight/ECOG Performance Status: Patient's weight and ECOG Performance Status will be recorded once per cycle. Medications/Adverse Events/Pill Count: Concurrent medications and adverse events will be documented throughout study. Pill count will be performed on remaining patient supplies of regorafenib and methotrexate weekly during Cycle 1, and thereafter once per cycle. Labs: Complete blood count with differential and serum chemistry will be measured weekly during Cycle 1, and Week 1 and Week 3 during Cycle 2. Thereafter, these studies will be performed once per cycle. Serum amylase/lipase and thyroid studies (TSH/free T4) will be measured once per cycle. Pharmacokinetic (PK) Studies: Blood samples for methotrexate PK studies will be measured during Cycle 1 *prior* to self-administration of study treatment and one hour *post*-self administration of methotrexate during Weeks

	1, 2, and 3. Cycle 1 Week 4 will only include a single methotrexate measurement (to check the trough since this is an off week). Circulating Tumor DNA (ctDNA) Studies: Blood samples for ctDNA will be collected at baseline (Week 1) and Week 3 of Cycle 1, and thereafter, once per cycle for Cycles 2 and 3. Thereafter, ctDNA samples will be collected at a timepoint that is closest to time of radiologic imaging. Imaging: *CT chest ± abdomen/pelvis with contrast* (unless contraindicated): Image all known baseline sites of disease. Perform every 8 weeks ± 1 week for the first 1 year and then after 1 year, every 12 weeks ± 1 week. For patients who come off study for toxicity or any other reason, obtaining interval imaging (i.e., unscheduled scan) will be strongly encouraged to monitor response. *Brain MRI*: Screening study for all patients. For patients with untreated brain metastases at baseline, perform every 8 weeks ± 1 week at baseline for the first 1 year and then after 1 year, every 12 weeks ± 1 week. If patient has history of treated brain metastases or no history of brain metastases, frequency of brain imaging follow-up is per the investigator discretion, although it is encouraged to perform the imaging every 8-16 weeks (± 1 week). *Chest X-ray (2 view)*: For patients with a history of pleural effusion, perform once per cycle because methotrexate can accumulate in pleural effusions. For patients with adequate chest imaging with re-staging CT prior to cycle, it is permitted to omit Chest X-ray imaging. C1D1 chest X-ray may be omitted by the treating physician if baseline chest imaging (completed within 30 days of C1D1) is deemed adequate. Refer to Section 9 Study Calendar for further details. **Dose Limiting Toxicities (DLT) will be monitored during Cycle 1 (first 28 days).** During Cycle 1, patients will be on regorafenib 80 mg oral daily 3 weeks on/1 week off and methotrexate with doses ranging three dose levels between 10-20 mg oral twice weekly 3 weeks on/1 week off. A patient will be eligible for DLT assessments if he/she has received at least 80% of the planned doses of study treatment, which will be calculated by dosing days of regorafenib in case a patient is off treatment during Cycle 1 for a reason other than toxicity.

	A DLT will be generally defined as any Grade 3 or 4 toxicity occurring during Cycle 1 (first 28 days), which is regarded as clinically significant and related to study treatment with clarifications and exceptions for adverse event terms noted below. The NCI-CTCAE version 4.03 will be used to assess toxicities. <u>Only the following hematologic toxicities will be considered a DLT</u>: • Absolute Neutrophil Count (ANC) < 500/mm^3 for > 7 days despite dose interruption • Febrile neutropenia [ANC <1000/mm^3 with a single temperature of > 38.3 degrees C (101 degrees F) or a sustained temperature of ≥ 38 degrees C (100.4 degrees F) for more than one hour] • Platelets < 25,000/mm^3 or ≥ Grade 3 thrombocytopenia associated with clinically significant bleeding <u>Non-hematologic Grade 3 or Grade 4 toxicity considered clinically significant and study treatment-related is a DLT, except for the following, *permitting protocol therapy can resume according to the toxicity guidelines in Section 6.2*. *(Note: If patient declines to resume protocol therapy despite being able to, this will not be considered a DLT).*</u> • Grade 3 nausea, vomiting, or diarrhea that improves to grade 2 or less with dose interruption and appropriate supportive care within 5 days • Grade 3 mucositis or stomatitis that improves to grade 0-1 with dose interruption and appropriate supportive care within 28 days • Grade 3 hypertension that improves to grade 2 or less with dose interruption and appropriate supportive care within 7 days • Grade 3-4 electrolyte abnormalities that are asymptomatic and can be corrected with supplementation and/or appropriate supportive care (e.g., hypomagnesemia, hypokalemia, hypocalcemia, hyponatremia, hypophosphatemia) or any grade of electrolyte abnormality that is related to another adverse event such as diarrhea, nausea, or vomiting • Grade 3 asymptomatic maculo-papular rash that improves to grade 2 or less with dose interruption and appropriate supportive care within 28 days The following will not be considered a DLT: • ≥ Grade 3 lipase and amylase that is asymptomatic • Grade 3 unconjugated/indirect bilirubin secondary to Gilbert's disease or equivalent

	Miscellaneous: In case an unexpected drug-related toxicity (even at a lower grade) is seen more frequently than expected or beyond the DLT time period, this toxicity may be declared as a DLT for the remainder of the study after consultation with the PI.
SAMPLE SIZE	18 patients, with at least 15 evaluable for PFS
STATISTICAL CONSIDERATIONS	**Efficacy:** Our null hypothesis is that the median PFS is 2 months. We wish to have 80% power at the alternative hypothesis of a median PFS of 8 months. Assuming a horizon for PFS of 2.0 months and exponential PFS, the probability of being PFS-free at that time is 50.0% under the null hypothesis and 8% under the alternative hypothesis. With 18 informative patients, we would have approximately 80% power to reject the null hypothesis at a one-sided significance level of 5%. The null hypothesis for this study is based on two major references: 1) Median PFS with regorafenib alone in pre-treated metastatic NSCLC (KRAS mutation not specified) was 84 days (approximately 2-3 months), and 2) Median PFS with a similar agent to regorafenib, sorafenib, in pre-treated metastatic KRAS mutated NSCLC was 2.3 months (95% CI 1.6-3.0)[2]. A clinically meaningful alternative hypothesis would be 8 months. **Safety Stopping Rule:** We will consider the number of patients with dose limiting toxicity (DLT), defined in section 6.1, to ensure there is not excessive toxicity**. If 2 or more of the first 6 patients have a DLT, or if 3 or more out of the first 12 patients have a DLT,** we will suspend enrollment to the trial and reconsider the dosing strategy (including dose and schedule of regorafenib and/or methotrexate). Finally, if at the final analysis, 3 or more patients have DLT, we will take this as an indication that the regimen may be too toxic and additional dose and schedule may be considered for future development of the combination. The table below shows the probability of stopping early (pstop) and the probability of declaring the regimen too toxic at an interim look or at the final analysis (pcross), for various value of the probability of DLT (ptox); the expected sample size under each scenario is also shown (ess). Ptox pcross pstop ess 0.05 0.012 0.004 18.0 0.10 0.102 0.033 17.7

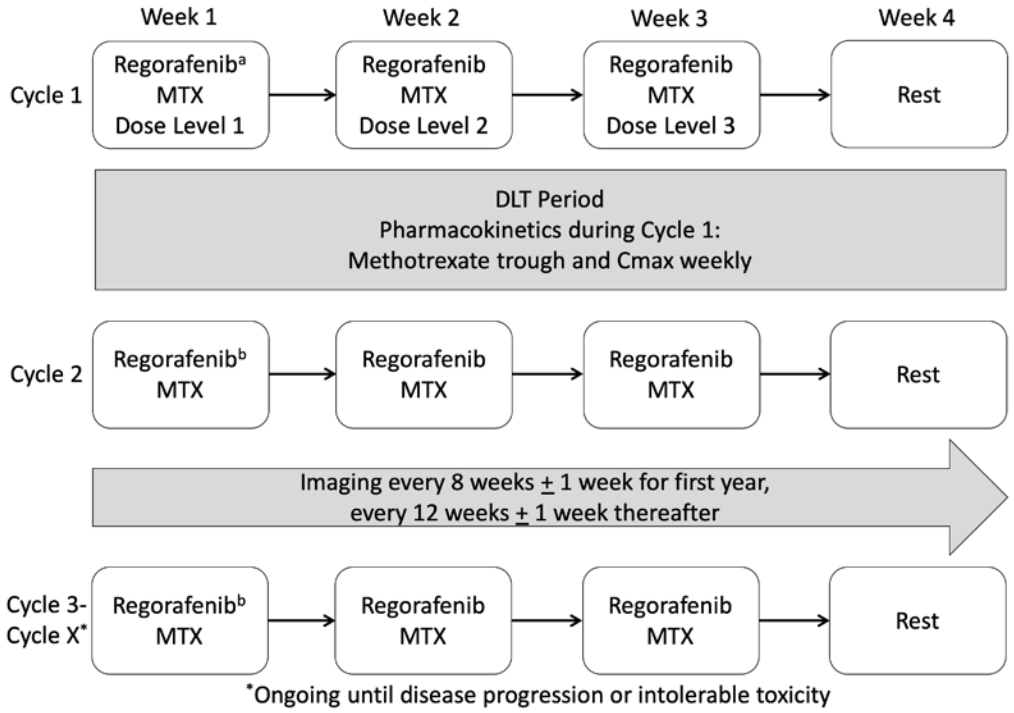
	0.14 0.243 0.089 17.2 0.18 0.417 0.175 16.5 0.22 0.588 0.284 15.5 0.26 0.731 0.406 14.5 0.30 0.838 0.530 13.3

SCHEMA

PATIENTS

18 KRAS mutated metastatic and/or recurrent NSCLC patients who received at least 1 prior systemic therapy

TREATMENT



Dosing Strategies (3 Weeks ON/1 Week OFF of 28-Day Cycle)		
Regorafenib[a]	80 mg	Daily
Regorafenib[b]	80 mg or 120 mg as tolerated	Daily
MTX Dose Level 1*	10 mg	Twice Weekly
MTX Dose Level 2	15 mg (escalate as tolerated)	Twice Weekly
MTX Dose Level 3	20 mg (escalate as tolerated)	Twice Weekly

OBJECTIVES

Primary:

- Progression Free Survival

Secondary:

- Objective response rate (CR plus PR; RECIST v1.1)
- Disease control rate at 8 weeks (CR+PR+SD at 8 weeks; RECIST v1.1);
- Safety (dose limiting toxicities; CTCAE v4.03)
- Pharmacokinetic parameters of methotrexate (trough, Cmax)

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
AHA	Alpha hydroxyl acid
ALK	Anaplastic lymphoma kinase
ALT	Alanine aminotransferase
ANR	Absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
AUC	Plasma concentration vs. time curve
BCRP	Breast Cancer Resistance Protein
Beta-HCG	Beta human chorionic gonadotropin
BP	Blood pressure
CAPP-Seq	Cancer Personalized Profiling by Deep Sequencing
C	Celsius
CI	Confidence interval
Cmax	Maximum serum concentration
CRF	Case report form
CR	Complete response
CSF	Cerebrospinal fluid
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumor DNA
Cys	Cysteine
DCE	Dynamic contrast enhanced
DCR	Disease control rate
DHFR	Dihydrofolate reductase
DLT	Dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
EGFR	Epidermal growth factor receptor
EKG	Electrocardiogram
F	Fahrenheit
FDA	Food and Drug Administration
FOLT	Folate transporter 1
FPGS	Folylpolyglutamyl synthetase
G-CSF	Granulocyte colony-stimulating factor
GCP	Good Clinical Practice
GIST	Gastrointestinal solid tumor
GMP	Good Manufacturing Practices
GTPase	Guanosine triphosphatases
Hb	Hemoglobin
HFSR	Hand-foot skin reaction
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
IB	Investigator's brochure

IM	Intramuscular
IRB	Institutional Review Board
KRAS	Kirsten rat sarcoma
mCRC	Metastatic colorectal cancer
mg	milligrams
MRI	Magnetic resonance imaging
MTX	Methotrexate
N/A	Not applicable
NCI	National Cancer Institute
NSAID	Nonsteroidal anti-inflammatory drug
NSCLC	Non-small cell lung cancer
NYHA	New York Heart Association
ORR	Objective response rate
OS	Overall survival
PD-L1	Programmed death-ligand 1
PFS	Progression free survival
PI	Principal Investigator
PK	Pharmacokinetic
PPE	Palmar-plantar erythrodysesthesia
PPI	Proton pump inhibitor
PR	Partial response
RANKL	Receptor activator of nuclear factor kappa-B ligand
RECIST	Response evaluation criteria in solid tumors
RFC	Reduced folate carrier
RPLS	Reversible Posterior Leukoencephalopathy Syndrome
SAE	Serious adverse event
SD	Stable disease
SDRT	Significant drug-related toxicities
TKI	Tyrosine kinase inhibitor
ULN	Upper limit of normal
VEGFR	Vascular endothelial growth factor receptor

OBJECTIVES

1.1. Primary Objective

- To determine the progression-free survival (PFS) of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy

1.2. Secondary Objectives

- To determine the objective response rate (ORR) of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy
- To determine the disease control rate (DCR) at 8 weeks of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy
- To determine the safety of the combination of regorafenib and methotrexate for metastatic *KRAS* mutated NSCLC patients who have received at least 1 prior systemic therapy
- To determine the pharmacokinetic parameters of methotrexate when combined with regorafenib (i.e., trough and Cmax)

1.3 Exploratory Objectives

- To correlate circulating tumor DNA (ctDNA) using CAncer Personalized Profiling by Deep Sequencing (CAPP-Seq)[1] pre-treatment and throughout treatment with clinical outcomes
- To correlate computational simulation model prediction of sensitivity to the combination of regorafenib and methotrexate based on genomic data with clinical outcomes

2. BACKGROUND

In the United States, lung cancer is the number one cause of cancer-related deaths, with an estimated 222,500 new cases of lung cancer and 155,870 lung cancer-related deaths in 2017[3,4]. Non-small cell lung cancer (NSCLC) accounts for over 85% of all lung cancer cases, and includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. With the rise of precision medicine, NSCLC care has transitioned from treatment based on histology to a focus on targetable genetic driver mutations[5], with the most successful stories to date including epidermal growth factor receptor (*EGFR*)-mutated and anaplastic lymphoma kinase (*ALK*)-gene rearranged NSCLC. However, for the most common oncogenic driver mutation, *KRAS* (Kirsten murine sarcoma viruses), targeted therapy has been elusive[6,7].

2.1 *KRAS* Mutated Non-Small Cell Lung Cancer

KRAS-mutant NSCLC comprises approximately 30% of NSCLC adenocarcinomas and 5% of squamous carcinomas[8,9]. It is significantly associated with a history of smoking, an older age of diagnosis, and a higher prevalence among Caucasians[8]. To date, the impact of *KRAS* on prognosis is unclear. Initially, *KRAS* mutations were identified as negative prognostic factors for patients with adenocarcinoma[10]. Subsequent studies, however, have reported mixed results[11-16].

One recent meta-analysis concluded that although the association was weak, *KRAS* mutation remained a valid predictor for poor prognosis and treatment outcomes in NSCLC[17].

KRAS belongs to the family of *RAS* genes that encode small guanosine triphosphatases (GTPases) located at the inner surface of the cellular membrane[18]. The KRAS enzyme functions primarily to relay signaling from activated transmembrane receptors, such as EGFR, into the cytoplasm. Important downstream signaling pathways include RAF-MEK-ERK and PI3K-AKT-mTOR, both of which mediate cellular growth, proliferation, motility, and survival[19,20]. Mutations in the *KRAS* gene constitutively activate these pathways, and are thought to occur as an early event in lung cancer tumorigenesis[21]. The most common *KRAS* mutations are found on codons 12, 13, and, less frequently, on 61[22-24]. Notably, a group of irreversible oral allosteric inhibitors for *KRAS* G12C have been developed that selectively bind to the mutant Cys 12, leaving the wild-type protein unaffected[25]. Experiments have shown that these cysteine-reactive molecules successfully impair KRAS(G12C) function and reduce the viability of *KRAS*(G12C)-mutant lung cancer cell lines, thus representing one promising area of development for *KRAS*-specific therapies. Given that *KRAS* mutations are easily identifiable and highly prevalent in NSCLC, they are an appealing target for therapeutic intervention.

Current management of metastatic *KRAS*-mutant NSCLC resembles that for NSCLC without known currently "actionable" mutations (i.e., EGFR/ALK/ROS1 negative). First-line therapies include immunotherapy with pembrolizumab for patients with PD-L1 expression on at least 50% of tumor cells[26], and platinum-based doublet chemotherapy for patients with less than 50% PD-L1 expression[27]. Second-line immunotherapy options include pembrolizumab for patients with PD-L1 expression greater than or equal to 1%[28], as well as nivolumab[29,30] and atezolizumab[31], irrespective of PD-L1 expression. Second-line FDA-approved chemotherapeutics include single-agent docetaxel +/- ramucirumab[32] and pemetrexed[33,34], among other non-FDA approved chemotherapeutics (i.e., gemcitabine, irinotecan). Active investigation for combination therapies in the second-line setting for EGFR/ALK negative NSCLC is currently ongoing, although there have yet to be any major changes in the standard of care based on these studies[35].

Considerable attention has turned to the search for an intervention that offers patients with *KRAS*-mutated NSCLC a targeted therapy of their own. Since KRAS itself is not currently clinically targetable, efforts have focused largely on modulating the activity of its downstream effectors. Inhibitors of mTOR[36], BRAF[37], and MEK[38] are currently being evaluated in clinical trials. However, limitations of MEK inhibition have been revealed through a lack of specificity and sub-optimal responses[39-42]. Unfortunately, the phase III randomized SELECT-1 study examining docetaxel versus docetaxel plus selumetinib failed to show benefit in *KRAS*-mutant NSCLC[41], highlighting the need for therapies for this lung cancer subtype. *KRAS*-mutant NSCLC is resistant to EGFR TKI treatment[12,43], although there were some responses to combination erlotinib and bexarotene and combination gefitinib and everolimus[44,45]. Recent studies demonstrate a considerable amount of heterogeneity among *KRAS*-mutant lung cancer that may ultimately affect therapeutic responses, and may be part of the reason this genomic subset has been so difficult to target. For example, one study identified subsets of *KRAS*-mutant lung adenocarcinoma with differing co-mutations in *LKB1*, *TP53*, and *CDKN2A/B* that had altered cellular and immune profiles[46]. Recent studies have also revealed unique differences in *KRAS*-mutant NSCLC metabolism, such as enhanced dependency on folate metabolism[47], that suggest other possibilities for therapeutic targeting. Given the prevalence and the current

treatment landscape of *KRAS*-mutated NSCLC outlined above, there is a dire need for improved treatment options.

2.2 Regorafenib and Methotrexate

2.2.1 Regorafenib

Please see Regorafenib Investigational Brochure[48] for further details.

2.2.1.1 Regorafenib Mechanism of Action and Pre-Clinical Studies

Regorafenib is an oral 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. Regorafenib has potent preclinical anti-tumor activity and long-lasting anti-angiogenic activity, as measured by dynamic contrast enhanced (DCE) – magnetic resonance imaging (MRI)[49].

In *in vitro* biochemical or cellular assays, 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. In *in vivo* models, regorafenib demonstrated 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.

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)[49]. 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 (lung)**, 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[49]. These data suggest that regorafenib can target the tumor cell MAPK pathway (tumor cell survival) and tumor vasculature in some but not all tumors.

2.2.1.2 Regorafenib Clinical Trials in Cancer, Including Lung Cancer

FDA-Approved Indications for Regorafenib (3):

1) Colorectal Cancer: Regorafenib is currently FDA-approved at a dose of 160 mg oral once daily for the first 21 days of each 28-day cycle for patients with metastatic colorectal cancer (mCRC) who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if *KRAS*-wild type, an anti-EGFR therapy. This September 2012 approval was supported by two phase III global randomized studies, including the CORRECT study[50] (North America, Europe, Asia, and Australia) and the CONCUR Study[51] (Asia Pacific). The CORRECT study was an international, multicenter, randomized, double-blind, placebo-controlled study of regorafenib that enrolled 760 patients

with mCRC whose disease has progressed after approved standard therapies, with a primary endpoint of overall survival (OS). At a pre-planned second interim analysis, there was a statistically significant survival benefit for regorafenib (OS, median OS was 6.4 months vs. 5.0 months, hazard ratio [HR] = 0.773 [95% confidence interval (CI) 0.635 to 0.941; 1-sided p = 0.0051]). In addition to improved OS, progression-free survival (PFS) and disease control rate (DCR) were superior. Regorafenib demonstrated comparable efficacy benefits across patient subgroups including *KRAS* status.

2) Gastrointestinal Stromal Tumor (GIST): Regorafenib is currently FDA-approved at a dose of 160 mg oral once daily for the first 21 days of each 28-day cycle for patients with locally advanced, unresectable, or metastatic gastrointestinal stromal tumor (GIST) who have been previously treated with imatinib mesylate and sunitinib malate. This 2013 FDA approval was supported by the Phase III GRID randomized study of regorafenib vs. placebo in patients with GISTs who had exhausted all other treatment options, with statistically significant 3.9-month improvement in PFS, compared with placebo (4.8 months vs. 0.9 months; HR = 0.27; $p<.0001$)[52]. OS rate was similar and DCR was also improved.

3) Hepatocellular Carcinoma (HCC): Regorafenib is currently FDA-approved at a dose of 160 mg oral once daily for the first 21 days of each 28-day cycle for patients with hepatocellular carcinoma (HCC) who have been previously treated with sorafenib. This April 2017 approval was supported by the phase III RESORCE randomized trial of regorafenib vs. placebo in patients with HCC who progressed on previous treatment with sorafenib, with a primary endpoint of OS[53]. Regorafenib demonstrated a statistically significant improvement in OS compared with placebo (HR = 0.63; 95% CI 0.50 to 0.79; 1-sided p<0.0001), with a median OS of 10.6 months (95% CI 9.1 to 12.1) for regorafenib compared to 7.8 months (95% CI 6.3 to 8.8) for placebo. PFS and time to progression were also significantly improved with regorafenib.

Clinical Trials in Lung Cancer:

Regorafenib has been studied in lung cancer as a single agent and in combination with cisplatin/pemetrexed chemotherapy[54,55].

The study extension of the phase I study of regorafenib in NSCLC was last presented at the American Society of Clinical Oncology (ASCO) meeting in 2010[54]. Regorafenib was administered orally once daily continuously in repeating cycles of 21 days, with 22 patients receiving 100 mg (recommended phase II dose) and one patient receiving 120 mg. The mean treatment duration was 84.6 days +/-61 days (2-281 days). The majority of patients were heavily pre-treated with a median number of 3 regimens received, including 57% receiving a prior EGFR inhibitor small molecule. There was a high DCR of 83% (N= 15 of 18 patients evaluable had best response of stable disease). However, the median PFS was only 84 days, although one patient had a PFS of 259 days (range 30-259 days). Efficacy was examined by *EGFR* and *KRAS* mutation status when available. Of the 9 patients tested for *EGFR*, 2 had an *EGFR* mutation, subtype unknown, and of the 6 patients tested for *KRAS*, all were negative—none of these patients were part of the 5 patients who had the longest PFS on study. In the waterfall plot, there were 8 patients with some decrease in tumor burden, and only 3 had molecular data available, including one *EGFR/KRAS* negative, one *EGFR* negative/*KRAS* unknown, and one *KRAS* negative/*EGFR* unknown.

Regorafenib was also studied in combination with cisplatin and pemetrexed for the first-line treatment of metastatic NSCLC[55]. Regorafenib 60 mg daily was administered continuously on days 1-21 of each cycle. After completion of 6 cycles of chemotherapy, regorafenib as a single agent or in combination with pemetrexed was dosed until progression. A total of 9 patients were treated on the study before termination. All patients were tested for *EGFR* and *KRAS,* and 3 patients had a *KRAS* mutation, 1 had an exon 20 *EGFR* mutation, and 5 patients were *EGFR/KRAS* wild-type. The objective response rate (ORR) was 55.6%, with 5 partial responses, 3 stable disease, and one progressive disease. Of the 3 *KRAS* codon 12 mutations (2 *KRAS* G12V and 1 *KRAS* G12A), there were 2 partial responses with a 38% and 44% reduction in tumor target lesions. One patient with a *KRAS* mutation had progressive disease in the nontarget lesions but had a 44% decrease in the target lesions. The overall median PFS for the study was 7 months. In the *KRAS* mutated subgroup, the PFS range was from 1.5 months in the patient with progressive disease to 12.4 months. The OS ranged from 13.1 months to 56.7 months. The patient with the second longest PFS and OS in this study had a *KRAS* mutation.

2.2.1.3 Regorafenib Nonclinical and Clinical Toxicology

Nonclinical Toxicology:

Carcinogenesis and Mutagenesis: Studies examining the carcinogenic potential of regorafenib have not been conducted. Regorafenib itself did not demonstrate genotoxicity in *in vitro* or *in vivo* assays; however, a major human active metabolite of regorafenib, (M-2), was positive for clastogenicity, causing chromosome aberration in Chinese hamster V79 cells.

Impairment of Fertility: Dedicated studies to examine the effects of regorafenib on fertility have not been conducted; however, there were histological findings of tubular atrophy and degeneration in the testes, atrophy in the seminal vesicle, and cellular debris and oligospermia in the epididymides in male rats at doses similar to those in human at the clinical recommended dose based on the plasma concentration vs. time curve (AUC). In female rats, there were increased findings of necrotic corpora lutea in the ovaries at the same exposures. There were similar findings in dogs of both sexes in repeat dose studies at exposures approximately 83% of the human exposure at the recommended human dose based on AUC. These findings suggest that regorafenib may adversely affect fertility in humans.

Animal Toxicology and/or Pharmacology: In a chronic 26-week repeat dose study in rats, there was a dose-dependent increase in the finding of thickening of the atrioventricular valve. At a dose that resulted in an exposure of approximately 12% of the human exposure at the recommended dose, this finding was present in half of the examined animals.

Clinical Toxicology:

Toxicity in Gastrointestinal Cancer Studies:

In the CORRECT study of metastatic colorectal cancer, 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%)[50]. Adverse events in CONCUR were consistent with the known safety profile of regorafenib in metastatic colorectal cancer[51]. In the GIST study, the most common grade ≥3 adverse events associated with regorafenib were hand-foot skin reaction (56.1%), hypertension (48.5%), and diarrhea (40.9%)[52]. In the

RESORCE study, the most common grade ≥3 adverse events associated with regorafenib were hypertension (15%), hand-foot skin reaction (13%), fatigue (9%), and diarrhea (3%)[53].

In response to the toxicities observed in the CORRECT and CONCUR trials, the randomized phase II Regorafenib Dose Optimization Study (ReDos) was conducted in 116 patients with refractory metastatic colorectal cancer in which a weekly dose escalation of regorafenib from 80 mg oral daily, 120 mg oral daily, to a target of 160 mg oral daily during cycle one was compared with the standard dose of 160 mg oral daily[56]. In this study, 43% of patients in the dose-escalation arm versus 24% of patients in the standard-dose arm completed two cycles of treatment and intended to initiate cycle three (p=0.0281). The most common grade 3+ adverse events occurring in the dose-escalation arm versus the standard-dose arm included fatigue (13% vs 17.7%), hand-foot skin reaction (14.8% vs 16.1%), abdominal pain (16.7% vs 6.5%), hypertension (7.4% vs 14.5%), increased alkaline phosphatase (5.6% vs 1.6%), and lymphopenia (7.4% vs 0%).

A follow-up dose titration study of regorafenib in metastatic colorectal cancer used 120 mg as a starting dose during cycle one, and found similar efficacy compared to the standard 160 mg dose. In this trial of 60 patients, DCR was 36.7%, with a median PFS of 2.3 months (95% CI 1.8 to 2.8)[57]. About 42% (25/60) of patients needed a dose reduction to 80 mg due to adverse events, and that dose reduction was needed in 10% (6/60) of patients at the first cycle. Overall grade 3-4 adverse events were observed in 55% (33/60) of patients.

Toxicity in Lung Cancer Studies*:*

Regorafenib at 100 mg oral daily dosed continuously had similar side effects in 23 NSCLC patients[54]. Regorafenib was relatively well-tolerated in NSCLC patients, with treatment-emergent study drug-related adverse events including 35% hand-foot skin reaction, 30% pain in extremity, 26% rash/desquamation, 22% hypothyroidism, 17% each of nausea, hypertension, dry skin, neuropathy, fatigue, and 13% each of muscular pain, mucositis, anorexia, voice changes, diarrhea, and 9% each of low platelets, vomiting, hypophosphatemia, mucositis on clinical exam, constipation, elevated amylase, taste alteration. Grade 3 adverse events included 3 hand-foot skin reactions, 1 hypertension, 1 sensory neuropathy, 1 diarrhea, 1 low platelets, and 2 hypophosphatemias. Five out of the 23 patients had dose interruptions, while only 2 out of 23 had dose reductions.

Additional Clinical Safety Information from Package Insert (revision 04/2017) (in addition, please Refer to the Investigator's Brochure):

The frequency of adverse events comes from the large gastrointestinal studies outlined below.

Most Common Adverse Events: In randomized placebo-controlled trials (CORRECT, GRID, RESORCE and CONCUR), the most frequently observed adverse drug reactions (≥20%) in patients receiving regorafenib are pain (including gastrointestinal and abdominal pain), HFSR, asthenia/fatigue, diarrhea, decreased appetite/food intake, hypertension, infection, dysphonia, hyperbilirubinemia, fever, mucositis, weight loss, rash, and nausea.

Most Common Serious Adverse Events: The most serious adverse reactions in patients receiving regorafenib are hepatotoxicity, hemorrhage, and gastrointestinal perforation.

Hepatotoxicity: Severe drug-induced liver injury with fatal outcome occurred in regorafenib-treated patients in clinical trials. In most cases, liver dysfunction occurred within the

first 2 months of therapy and was characterized by a hepatocellular pattern of injury. In the CORRECT study, fatal hepatic failure occurred in 1.6% of patients in the regorafenib arm and in 0.4% of patients in the placebo arm, and all the patients with hepatic failure had metastatic disease in the liver. In the GRID study, fatal hepatic failure occurred in 0.8% of patients in the regorafenib arm. In the RESORCE study, there was no increase in the incidence of fatal hepatic failure as compared to placebo.

Infections: Regorafenib caused an increased risk of infections. The overall incidence of infection (Grades 1-5) was higher (32% vs. 17%) in 1142 regorafenib-treated patients as compared to the control arm in randomized placebo-controlled trials. The incidence of grade 3 or greater infections in regorafenib treated patients was 9%. The most common infections were urinary tract infections (5.7%), nasopharyngitis (4.0%), mucocutaneous and systemic fungal infections (3.3%) and pneumonia (2.6%). Fatal outcomes caused by infection occurred more often in patients treated with regorafenib (1.0%) as compared to patients receiving placebo (0.3%); the most common fatal infections were respiratory (0.6% in regorafenib-treated patients vs 0.2% in patients receiving placebo).

Hemorrhage: Regorafenib caused an increased incidence of hemorrhage in clinical trials. The overall incidence (Grades 1-5) was 18.2% in 1142 patients treated with regorafenib and 9.5% in patients receiving placebo in randomized, placebo-controlled trials. The incidence of grade 3 or greater hemorrhage in patients treated with regorafenib was 3.0%. The incidence of fatal hemorrhagic events was 0.7%, involving the central nervous system or the respiratory, gastrointestinal, or genitourinary tracts.

Dermatologic Toxicity: In randomized, placebo-controlled trials, adverse skin reactions occurred in 71.9% of patients in the regorafenib arm and in 25.5% of patients in the placebo arm, including hand-foot skin reaction (HFSR) also known as palmar-plantar erythrodysesthesia syndrome (PPES), and severe rash requiring dose modification. In the randomized, placebo-controlled trials, the overall incidence of HFSR was higher in 1142 regorafenib-treated patients (53%) than in the placebo-treated patients (8%). Most cases of HFSR in regorafenib-treated patients appeared during the first cycle of treatment. The incidences of Grade 3 HFSR (16% versus <1%), Grade 3 rash (3% versus <1%), serious adverse reactions of erythema multiforme (<0.1% vs. 0%) and Stevens-Johnson Syndrome (<0.1% vs. 0%) were also higher in regorafenib-treated patients. Across all trials, a higher incidence of HFSR was observed in Asian patients treated with regorafenib (all grades: 72%; Grade 3: 18%). Toxic epidermal necrolysis occurred in 0.02% of 4518 regorafenib-treated patients across all clinical trials of regorafenib administered as a single agent.

Hypertension: In randomized, placebo-controlled trials, hypertensive crisis occurred in 0.2% of patients in the regorafenib arms and in none of the patients in the placebo arms. Regorafenib caused an increased incidence of hypertension (30% versus 8% in CORRECT, 59% versus 27% in GRID, and 31% versus 6% in RESORCE). The onset of hypertension occurred during the first cycle of treatment in most patients who developed hypertension (67% in randomized, placebo-controlled trials).

Elevated Lipase: Regorafenib increased lipase levels in 9.2% (46/500) of treated patients in the CORRECT study, 1.8% (9/500) of patients to a Grade 3 level, and 0.4% (2/500) of patients to a Grade 4 level. Regorafenib increased lipase levels in 10.6% (14/132) of treated patients in the GRID study, 0% of patients to a Grade 3 level, and 0.8% (1/132) of patients to a Grade 4 level.

Cardiac Ischemia and Infarction: Regorafenib increased the incidence of myocardial ischemia and infarction (0.9% vs 0.2%) in randomized placebo-controlled trials.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS, also known as posterior reversible encephalopathy syndrome): RPLS occurred in one of 4800 regorafenib-treated patients across all clinical trials.

Gastrointestinal Perforation or Fistula: Gastrointestinal perforation occurred in 0.6% of 4518 patients treated with regorafenib across all clinical trials of regorafenib administered as a single agent; this included eight fatal events. Gastrointestinal fistula occurred in 0.8% of patients treated with regorafenib and 0.2% of patients in placebo arm across randomized, placebo-controlled trials.

Wound Healing: No formal studies of the effect of regorafenib on wound healing have been conducted, although vascular endothelial growth factor receptor (VEGFR) inhibitors such as regorafenib can impair wound healing.

Specific Populations:

Pregnant Women: Based on animal studies and its mechanism of action, regorafenib can cause fetal harm when administered to a pregnant woman. There are no available data on regorafenib use in pregnant women. Regorafenib was embryolethal 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.

Lactation: There are no data on the presence of regorafenib or its metabolites in human milk, the effects of regorafenib on the breastfed infant, or on milk production. In rats, regorafenib and its metabolites are excreted in milk.

Females and Males of Reproductive Potential: There are no data on the effect of regorafenib on human fertility. Results from animal studies indicate that regorafenib can impair male and female fertility.

Geriatric Patients: Of the 1142 regorafenib-treated patients enrolled in randomized, placebo-controlled trials, 40% were 65 years of age and over, while 10% were 75 and over. No overall differences in efficacy were observed between these patients and younger patients. There was an increased incidence of Grade 3 hypertension (18% versus 9%) in the placebo-controlled trials among regorafenib-treated patients 65 years of age and older as compared to younger patients. In addition, one Grade 4 hypertension event has been reported in the 65 years and older age group and none in the younger age group.

Hepatic and Renal Impairment: Regorafenib does not require dose adjustment for mild or moderate hepatic impairment (Child-Pugh Class A or B) or for mild, moderate, or severe renal impairment. However, the investigator should refer to the eligibility (Sections 3.1 and 3.2) and adverse event management section (Section 7) for protocol requirements.

Race: Based on pooled data from three placebo-controlled trials (the CORRECT, CONCUR, and GRID studies), a higher incidence of HFSR and liver function test abnormalities occurred in Asian patients treated with regorafenib as compared with Whites.

2.2.1.4 Regorafenib Pharmacokinetics, Metabolism, Major Route of Elimination, and Potential Drug Interactions

Information in this section was obtained from regorafenib package insert v.4-2017. Please see Investigator's Brochure for more information.

Pharmacokinetics:

Absorption: After a single 160 mg dose of regorafenib in patients with advanced solid tumors, regorafenib reaches a geometric mean peak plasma level (Cmax) of 2.5 μg/mL at a median time of 4 hours and a geometric mean area under the plasma concentration vs. time curve (AUC) of 70.4 μg*h/mL. The AUC of regorafenib at steady-state increases less than dose proportionally at doses greater than 60 mg. At steady-state, regorafenib reaches a geometric mean Cmax of 3.9 μg/mL and a geometric mean AUC of 58.3 μg*h/mL. The coefficient of variation of AUC and Cmax is between 35% and 44%. The mean relative bioavailability of tablets compared to an oral solution is 69% to 83%.

In a food-effect study, 24 healthy men received a single 160 mg dose of regorafenib on three separate occasions: under a fasted state, with a high-fat meal and with a low-fat meal. A high-fat meal (945 calories and 54.6 g fat) increased the mean AUC of regorafenib by 48% and decreased the mean AUC of the M-2 and M-5 metabolites by 20% and 51%, respectively, as compared to the fasted state. A low-fat meal (319 calories and 8.2 g fat) increased the mean AUC of regorafenib, M-2 and M-5 by 36%, 40% and 23%, respectively as compared to fasted conditions. Regorafenib was administered with a low-fat meal in the CORRECT and GRID studies.

Distribution: Regorafenib undergoes enterohepatic circulation with multiple plasma concentration peaks observed across the 24-hour dosing interval. Regorafenib is highly bound (99.5%) to human plasma proteins.

Metabolism: Regorafenib is metabolized by CYP3A4 and UGT1A9. The main circulating metabolites of regorafenib measured at steady-state in human plasma are M-2 (N-oxide) and M-5 (N-oxide and N-desmethyl). Both metabolites have similar *in vitro* pharmacological activity and steady-state concentrations as regorafenib. M-2 and M-5 are highly protein bound (99.8% and 99.95%, respectively).

- *Elimination*: Following a single 160 mg oral dose of regorafenib, the geometric mean (minimum to maximum) elimination half-lives for regorafenib and the M-2 metabolite in plasma are 28 hours (14 to 58 hours) and 25 hours (14 to 32 hours), respectively. M-5 has a longer mean (minimum to maximum) elimination half-life of 51 hours (32 to 70 hours).
- *Excretion*: Approximately 71% of a radiolabeled dose was excreted in feces (47% as parent compound, 24% as metabolites) and 19% of the dose was excreted in urine (17% as glucuronides) within 12 days after administration of a radiolabeled oral solution at a dose of 120 mg.

Drug Interactions:

Extracted directly from package insert v.4-2017. Please refer to Investigator's Brochure for more information. Please refer to section 4.1 for contraindicated medications while on study or medications that should be used with caution.

In vitro studies suggested that regorafenib is an inhibitor of CYP2C8, CYP2C9, CYP2B6, CYP3A4 and CYP2C19; M-2 is an inhibitor of CYP2C9, CYP2C8, CYP3A4 and CYP2D6, and M-5 is an inhibitor of CYP2C8. *In vitro* studies suggested that regorafenib is not an inducer of CYP1A2, CYP2B6, CYP2C19, and CYP3A4 enzyme activity. Relevant drug interactions are listed below.

- Strong CYP3A4 Inducers: Co-administration of a strong CYP3A4 inducer with regorafenib decreased the plasma concentrations of regorafenib, increased the plasma concentrations of the active metabolite M-5, and resulted in no change in the plasma concentrations of the active metabolite M-2, and may lead to decreased efficacy.
- Strong CYP3A4 Inhibitors: Co-administration of a strong CYP3A4 inhibitor with regorafenib increased the plasma concentrations of regorafenib and decreased the plasma concentrations of the active metabolites M-2 and M-5, and may lead to increased toxicity.
- Breast Cancer Resistance Protein (BCRP) Substrates: Co-administration of regorafenib with a BCRP substrate increased the plasma concentrations of the BCRP substrate.

2.2.1.5 Regorafenib Rationale for the Starting Dose and Regimen Chosen

Regorafenib is FDA-approved at a dose of 160 mg (four 40-mg tablets) orally once daily for the first 21 days of each 28-day cycle for metastatic colorectal cancer patients, gastrointestinal stromal tumor patients, and hepatocellular carcinoma patients. The drug has been evaluated previously in patients with NSCLC without known *KRAS* mutations at doses of 100 mg daily continuously, and had expected toxicity with few dose modifications required[54]. Since regorafenib has not been investigated in combination with low-dose oral methotrexate, there is potential for overlapping toxicity (gastrointestinal, liver, and skin). Frequent dose modifications required of single agent regorafenib at the 160 mg dose based on prior studies (i.e., CORRECT and GRID) have resulted in efforts to optimize dosing strategies when initiating regorafenib. Among these, a weekly dose escalation (REDOS) has demonstrated improvements in continuation rates and a trend towards improved overall survival[56]. Based on these data, regorafenib will be administered at an initial dose of 80 mg oral daily 3 weeks on/1 week off of a 28-day cycle. Beginning in Cycle 2, patients may dose escalate to a target dose of 120 mg (same schedule of 3 weeks on/1 week off of 28-day cycle) according to strict criteria, including no evidence of significant drug-related toxicities (SDRT), defined as any event that would require a dose modification of regorafenib (i.e., interruption only, reduction only, or interruption followed by reduction) according to the guidelines/tables in Section 6.2.1 (regorafenib toxicities) and Section 6.2.3 (overlapping regorafenib and methotrexate toxicities).

2.2.2 Methotrexate

Please see Methotrexate Investigational Brochure[58] (Generic, Mylan Pharmaceuticals) for further details.

2.2.2.1 Methotrexate Mechanism of Action and Pre-Clinical Studies in Lung Cancer

Methotrexate (4-amino-10-methyl-folic acid) is an anti-folate that is frequently used in the treatment of head and neck cancers, leukemia, and lymphoma. In smaller doses, it is a common immunomodulatory agent used to treat autoimmune diseases, such as rheumatoid arthritis. Methotrexate acts as a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR), which normally reduces dihydrofolate to tetrahydrofolate that is essential for purine nucleotide

and thymidylate synthesis. As a result, methotrexate disrupts DNA synthesis, causes cell-cycle arrest in the S phase, and ultimately leads to cellular apoptosis.

Rapidly dividing cells are particularly sensitive to the effects of methotrexate. These cells exhibit a higher rate of methotrexate uptake and lower rate of efflux compared to slow-growing and resting-state cells[59]. Intracellularly, methotrexate binds with a much greater affinity to DHFR compared to the endogenous dihydrofolate substrate[60], and is converted to its polyglutamated metabolite by folylpolyglutamyl synthetase (FPGS). Methotrexate polyglutamates (with up to 8 glutamate residues) inhibit DHFR with at least as much potency as the monoglutamate form, and function additionally as potent antagonists of thymidylate synthase, AICAR transformylase, and GAR transformylase to further deplete the supply of reduced folates[61]. Furthermore, pre-clinical studies have shown that methotrexate may interfere with single-carbon transfer reactions, generate reactive oxygen species, and engage in other processes that contribute to its cytotoxicity[60]. One key aspect of methotrexate function that makes it appealing as a chemotherapeutic agent is its selectivity for malignant cells that are actively proliferating. This selectivity may be due, at least in part, to the greater accumulation of methotrexate polyglutamates in tumor cells compared to normal human tissue[62,63].

Pre-clinical studies of methotrexate in lung cancer are few in number, with the majority of the drug's pre-clinical work having been conducted in relation to leukemia and lymphoma. However, a couple of *in vitro* studies in the 1980s examined its properties in small cell lung cancer, which described a general association between methotrexate polyglutamate retention and drug sensitivity[64,65]. In 1991, another *in vitro* study explored the combination effects of methotrexate and 5-fluorouracil in two human NSCLC cell lines (NCI-H23 and NCI-358), revealing an interaction that was highly schedule-dependent in terms of whether it was antagonistic or cytotoxic[66]. Over the past decade, a series of independent studies have evaluated methotrexate action in the A549 cell line (notably a *KRAS*-mutated NSCLC cell line), which together have highlighted its strong anti-proliferative activity and induction of cellular differentiation and apoptosis along specific molecular pathways[67-69]. In 2013, the antagonistic relationship between methotrexate and aspirin was demonstrated in two human lung adenocarcinoma cell lines, CL1-0 and A549[70]. Methotrexate alone reduced cell viability by 75%, whereas combination methotrexate and aspirin treatment resulted in a 46% decrease in cell numbers. The addition of aspirin also reversed the accumulation of CL1-0 cells in the S phase and prevented methotrexate-mediated apoptosis, but did not affect DHFR levels or function.

2.2.2.2 Methotrexate Clinical Trials in Lung Cancer

Although methotrexate is not commonly used for the treatment of lung cancer, it was studied extensively from the 1970s to 1980s with some evidence of activity based on cruder chest x-ray imaging and response criteria.

One of the first studies in lung cancer involved "low" doses of methotrexate (80 to 800 mg) and high doses of methotrexate (3 to 18 g), with 7 out of 8 responses (6/6 patients with squamous cell carcinoma and 1/2 patients with anaplastic carcinoma) seen at the higher dose[71]. In another study of high-dose methotrexate (6 to 10 g/m²) administered in patients with NSCLC, there were 2 responses seen, lasting 13 and 17 weeks[72]. Another study examined the effect of a 30-hour continuous IV infusion of methotrexate at 1.5 g/m² compared to a dose escalation from 1.5 to 12 g/m² of IV methotrexate over 6 hours, both followed by leucovorin rescue, with 3/17 partial responses among the patients treated with 30-hour continuous methotrexate[73]. Another

phase II study of high-dose methotrexate with citrovorum rescue resulted in one patient responding with a partial regression of tumor out of 28 patients with NSCLC, suggesting response rate of less than 20%[74]. Methotrexate has also been studied in small cell lung cancer with variable activity.[75,76]

The following studies at least included doses that more closely approximate the dosing schedule of this protocol (i.e., much lower doses than studies listed above). There was a retrospective study of 195 patients treated with methotrexate using a variety of dosing schedules (0.2 to 0.9 mg/kg *oral* twice a week, 5 to 10 mg/kg IV every 3 weeks)[77]. Of the 29 patients with lung adenocarcinoma, 5 had a response, and a median OS of 8 months. There was a prospective randomized study that compared twice weekly IM (intramuscular) injections of methotrexate at a high dose of 0.6 mg/kg or a low dose 0.2 mg/kg with placebo in 239 lung cancer patients[78]. The investigators found an ORR of 21% in patients with measurable disease receiving the high dose (N = 48) and an ORR of 11% in patients receiving the low dose (N = 37). Patients with a response also had a longer survival. Together these data suggest some but limited efficacy of single agent methotrexate in lung cancer.

2.2.2.3 Methotrexate Clinical Toxicology

In a lung cancer study with similar dose used in our trial of methotrexate IM twice weekly 0.2 mg/kg and 0.6 mg/kg, there was a leukopenia rate (<4500/mm^3) of 26% at the low-dose and 53% at the high-dose, thrombocytopenia (< 100,000/mm^3) rate of 21% at the high-dose and 13% at the low-dose, oral ulcerations of 50% at the high-dose and 14% at the low-dose, and diarrhea and vomiting of 18% at the high-dose and 26% at the low dose[78].

In general, the most common adverse reactions following methotrexate administration include ulcerative stomatitis, leukopenia, nausea, abdominal distress, malaise, fatigue, fever and chills, dizziness, and decreased resistance to infection.

The toxicities below emphasize rheumatologic related toxicity since doses in this study more approximate rheumatologic oral methotrexate dosing.

At rheumatologic dosing of methotrexate (low dose oral (7.5 to 15 mg/week) pulse methotrexate for rheumatoid arthritis, most common adverse events include:

- Incidence Greater Than 10%: Elevated liver function tests 15%, nausea/vomiting 10%.
- Incidence 3% to 10%: Stomatitis, thrombocytopenia, (platelet count less than 100,000/mm).
- Incidence 1% to 3%: Rash/pruritus/dermatitis, diarrhea, alopecia, leukopenia (WBC less than 3000/mm^3), pancytopenia, dizziness.
- Incidence of interstitial pneumonitis 1%

This is similar also for psoriasis studies, which uses slightly higher doses of 25 mg weekly. With the exception of alopecia, photosensitivity, and "burning of skin lesions" (each 3% to 10%), the adverse reaction rates in these reports were very similar to those in the rheumatoid arthritis studies.

Summary of Methotrexate Toxicity by Organ System:

Gastrointestinal: Methotrexate can cause vomiting, diarrhea, or stomatitis occur, which may result in dehydration.

Hematologic: Methotrexate can suppress hematopoiesis and cause anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, and thrombocytopenia. In clinical trials in rheumatoid arthritis (n=128), leukopenia (WBC <3000/mm^3) was seen in 2 patients, thrombocytopenia (platelets <100,000/mm^3) in 6 patients, and pancytopenia in 2 patients.

Hepatic: Methotrexate may cause acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatotoxicity. Chronic toxicity is potentially fatal, and generally has occurred after prolonged use (about 2+ years) and after a total dose of at least 1.5 grams. In studies in psoriatic patients, hepatotoxicity was found to be a function of total cumulative dose, and was enhanced by alcoholism, obesity, diabetes, and advanced age. An accurate incidence rate has not been determined; the rate of progression and reversibility of lesions is not known. Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in this rheumatoid arthritis patients. There is a combined reported experience in 217 rheumatoid arthritis patients with liver biopsies both before and during treatment (after a cumulative dose of at least 1.5 g) and in 714 patients with a biopsy only during treatment. There are 64 (7%) cases of fibrosis and 1 (0.1%) case of cirrhosis. Of the 64 cases of fibrosis, 60 were deemed mild.

Infection or Immunologic Studies: Immunization may be ineffective when given during methotrexate therapy. Hypogammaglobulinemia has been reported rarely. Potentially fatal opportunistic infections, especially *Pneumocystis carinii* pneumonia, may occur with methotrexate therapy.

Pulmonary: Pulmonary symptoms (especially a dry non-productive cough) or a non-specific pneumonitis occurring during methotrexate therapy may develop. Although clinically variable, the typical patient with methotrexate-induced lung disease presents with fever, cough, dyspnea, hypoxemia, and an infiltrate on chest X-ray. This can occur at all dosages.

Renal: Methotrexate may cause renal damage that may lead to acute renal failure. Nephrotoxicity is due primarily to the precipitation of methotrexate and 7-hydroxymethotrexate in the renal tubules.

Skin: Severe, occasionally fatal, dermatologic reactions, including toxic epidermal necrolysis, Stevens-Johnson syndrome, exfoliative dermatitis, skin necrosis, and erythema multiforme, have been reported in children and adults, within days of oral, intramuscular, intravenous, or intrathecal methotrexate administration. Reactions were noted after single or multiple, low, intermediate or high doses of methotrexate in patients with neoplastic and non-neoplastic diseases. Radiation dermatitis and sunburn may be "recalled" by the use of methotrexate.

Specific Populations:

- Pregnant Women: Methotrexate can cause fetal death or teratogenic effects when administered to a pregnant woman.
- Lactation: Methotrexate has been detected in human breast milk, with the highest breast milk to plasma concentration ratio reached at 0.08:1.

2.2.2.4 Methotrexate Pharmacokinetics, Metabolism, Major Route of Elimination, and Potential Drug Interactions

Pharmacokinetics

Absorption: In adults, oral absorption of methotrexate is rapid and dose-dependent, and occurs primarily through the proton-coupled folate transporter in the small intestine. Peak serum levels are typically reached within 1-2 hours. At doses of 30 mg/m^2 or less, methotrexate is well-absorbed, giving a mean bioavailability of about 60%. However, at doses greater than 80 mg/m^2 in adults, and greater than 40 mg/m^2 in children, absorption is significantly less, possibly due to saturation. Administration with food may delay absorption and reduce peak concentrations.

Distribution: About 50% of methotrexate in serum is protein-bound. Laboratory studies have shown that it may be displaced from plasma albumin by sulfonamides, salicylates, tetracyclines, chloramphenicol, and phenytoin. Methotrexate does not penetrate the blood-cerebrospinal fluid barrier in therapeutic amounts when given orally or parenterally.

Half-life: The plasma half-life of high-dose methotrexate varies from 8-15 hours, whereas the half-life for low-dose methotrexate for the treatment of rheumatoid arthritis, psoriasis, or low-dose antineoplastic therapy (less than 30 mg/m^2) ranges from 3-10 hours.

Metabolism: Cellular uptake of methotrexate occurs via active transport mediated by the reduced folate carrier (RFC) family, folate transporter 1 (FOLT, also known as RFC1 encoded by SLC19A1) in particular. The RFC system transports reduced folates and antifolates like methotrexate much more efficiently compared to the transport of folic acid (K_t = 0.7 to 6.0 μmol/L versus K_t = 200 μmol/L)[79,80]. Additional cellular influx is mediated by the α and β isoforms of the folate receptor and the proton-coupled folate transporter. In general, the level of cellular proliferation and activity as well as the extracellular temperature and pH influence the rate of methotrexate influx into the cells.

After absorption, methotrexate undergoes hepatic and intracellular metabolism to polyglutamated forms, which can be converted back to methotrexate by hydrolase enzymes. Methotrexate may bind to and inhibit DHFR or undergo polyglutamation by FPGS. Polyglutamation of folates and antifolates facilitates their intracellular retention, extends the substrate half-life, and occurs as a typical process in most tumors[63,81]. Polyglutamation of methotrexate occurs progressively over several hours. After 24 hours, more than 80% of intracellular methotrexate appears in its polyglutamated form[82]. Methotrexate deglutamation is catalyzed by the lysosomal γ-glutamyl hydrolase. A number of ATP-binding cassette transporters, including ABCC1-ABCC5 and ABCG1/2, and the breast cancer resistance protein (BCRP) mediate methotrexate exportation from cells. However, methotrexate polyglutamates with more than three glutamate residues are not substrates for these transporters, and are not typically exported. As a result, the retention and accumulation of methotrexate polyglutamates provides sustained inhibition of DHFR long after blood levels have decreased[61].

One metabolite of methotrexate, 7-OH-methotrexate, is produced in the liver by the enzyme aldehyde oxidase, and is secreted into bile. 7-OH-methotrexate may accumulate in plasma, reaching peak levels between 24-48 hours following high-dose methotrexate administration, and is readily excreted in urine. Notably, intracellular 7-OH-methotrexate polyglutamates inhibit thymidylate synthase and AICAR transformylase with a similar potency as methotrexate

polyglutamates. 7-OH-methotrexate itself may also bind to DHFR, but acts weakly as an inhibitor compared to methotrexate. DAMPA is another minor metabolite of methotrexate that is produced in the gastrointestinal tract by bacterial carboxypeptidases, and by hydrolysis of methotrexate by the rescue agent carboxypeptidase G_2. DAMPA is considered an inactive metabolite of methotrexate since it does not effectively inhibit DHFR.

Elimination: Methotrexate is excreted primarily through the kidneys, by both glomerular filtration and active tubular secretion. Renal excretion of single daily doses ranges from 55-88% or higher within 24 hours. Biliary excretion is limited, and accounts for less than 10% of the administered dose. Elimination may be non-linear due to saturation of renal tubular reabsorption, and has been observed in patients with psoriasis at doses between 7.5 to 30 mg. Impaired renal function can rapidly increase methotrexate serum levels, which may remain elevated for extended periods. In general, methotrexate clearance rates are widely variable, and usually decrease at higher doses. Delayed drug clearance, either through impaired renal function, a third space effusion, or from other causes, is a major contributor to methotrexate toxicity.

Drug Interactions:

- Nonsteroidal Anti-inflammatory Drugs (NSAIDS): Concomitant administration of some NSAIDs with high dose methotrexate therapy has been reported to elevate and prolong serum methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity. Given potential of toxicity, caution should also be used when NSAIDs and salicylates are administered concomitantly with lower doses of methotrexate such as in this study. These drugs have been reported to reduce the tubular secretion of methotrexate in an animal model and may enhance its toxicity. Despite the potential interactions, studies of methotrexate in patients with rheumatoid arthritis have usually included concurrent use of constant dosage regimens of NSAIDs, without apparent problems. It should be appreciated, however, that the doses used in rheumatoid arthritis (7.5 to 20 mg/week) are somewhat lower than those used in psoriasis and that larger doses such as in this study could lead to unexpected toxicity.
- Drugs Highly Bound to Plasma Proteins: Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, tetracyclines, chloramphenicol, phenylbutazone, phenytoin, and sulfonamides.
- Probenecid: Renal tubular transport is also diminished by probenecid.
- Oral Antibiotics: Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria.
- Penicillins: Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with methotrexate.
- Theophylline: Methotrexate may decrease the clearance of theophylline.

- Trimethoprim/sulfamethoxazole: Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression in patients receiving methotrexate, probably by an additive antifolate effect.

2.2.2.5 Methotrexate Rationale for the Starting Dose and Regimen Chosen

Previous studies in patients with NSCLC have evaluated single-agent methotrexate at doses approximating 12 to 36 mg intramuscularly twice weekly, which produced responses and were well-tolerated. *In vitro* cell line studies, discussed in rationale Section 2.3, suggest our target Cmax should be achieved with the doses of methotrexate used in this study. The dosing frequency of methotrexate twice weekly provides reasonable exposure based on pharmacokinetic (PK) data during regorafenib administration, as methotrexate is hypothesized to enhance the activity of regorafenib. Methotrexate is a known substrate of the BCRP efflux transporter in the intestinal epithelia with the end result of increasing bioavailability. Methotrexate elimination is primarily via the kidneys where it is filtered but is potentially also excreted by the BCRP transporter. Given that regorafenib impairs BCRP activity, methotrexate pharmacokinetics may be affected resulting in a) increased bioavailability and b) decreased renal clearance. To determine the extent of this drug-drug interaction for this novel combination, abbreviated pharmacokinetic studies will be performed as detailed in the study calendar (section 9). In addition, the methotrexate doses used in this study should provide an adequate therapeutic index despite this potential drug interaction given: 1) methotrexate dose is lower than standard doses used as a single agent for cancer therapy and, 2) methotrexate is generally at most 60% bioavailable when administered by mouth. An intrapatient weekly dose escalation of methotrexate will be performed as detailed in the study calendar, Section 9, with a target dose of 20 mg oral twice weekly 3 weeks on/1 week off of a 28-day cycle.

For clinicaltrials.gov compliance

Regorafenib is a FDA approved drug for (1) metastatic colorectal cancer patients who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if KRAS wild type, an anti-EGFR therapy and (2) locally advanced, unresectable or metastatic gastrointestinal stromal tumor (GIST) who have been previously treated with imatinib mesylate and sunitinib malate. Regorafenib is not approved for the indication studied in this protocol. Methotrexate is used as anti-cancer therapies and autoimmune diseases such as rheumatoid arthritis but not for the indication studied in this protocol. Regorafenib and methotrexate as a combination therapy is not FDA approved for any indication.

The protocol director will submit clinical trial information as required using ClinicalTrials.gov to fulfill the requirements of FDAAA 801.

2.3 Rationale

Regorafenib

As detailed in Section 2.2.1, regorafenib[49] has been studied in lung cancer as a single agent and also in combination with cisplatin/pemetrexed chemotherapy[83,84]. In the single agent study, regorafenib achieved a high disease control rate (DCR) of 83% (n=15/18) in heavily pre-treated patients[83]. The median progression free survival (PFS) was 84 days. **This trial studies the combination of regorafenib and methotrexate in a molecularly defined subset of lung**

cancer- *KRAS* mutated NSCLC. Despite the modest activity of regorafenib in an unselected NSCLC population, there were no tumors with known *KRAS* mutations represented. We hypothesize that regorafenib will be more effective in NSCLC patients whose tumors harbor a *KRAS* mutation based on computational modeling (outlined below).

In addition, regorafenib was also studied in combination with cisplatin and pemetrexed for the first line treatment of metastatic NSCLC, with an objective response rate (ORR) of 55.6%, (N=5/9), median PFS of 7 months, and overall survival (OS) of 13.1 months to 56.7 months .[11] Three patients had a *KRAS* mutation, of whom two had a partial response and all had a decrease in target lesions. In addition, the patient with the second longest PFS and OS in this study had a *KRAS* mutation. **There is a suggestion that regorafenib in combination with platinum-pemetrexed chemotherapy may have enhanced activity in *KRAS* mutated NSCLC. Pemetrexed is mechanistically different from methotrexate but it is also an anti-folate chemotherapy. Based on this data, we hypothesize that regorafenib in combination with anti-folate-based methotrexate therapy will result in improved clinical activity in patients with *KRAS* mutated NSCLC.**

The data of regorafenib in NSCLC builds upon the data of sorafenib in NSCLC, particularly in the *KRAS* mutated subgroup. Regorafenib has a different structure from sorafenib by the addition of a fluorine atom in the center phenyl ring and has similar targets as sorafenib but is considerably more potent[85]. Sorafenib has clinical activity in *KRAS* mutated NSCLC[2,86,87]. Sorafenib was the most active agent in the BATTLE trial (Biomarker-Integrated Targeted Therapy Study) and it has been incorporated as one of the treatment arms for BATTLE-2[86,88]. In a phase II trial of sorafenib in heavily pretreated *KRAS* mutated NSCLC patients, the ORR was 10.5%, the DCR was 52.6%, the median PFS was 2.3 months (95% CI 1.6-3.0), and the median OS was 5.3 months (95% CI 3.6-7.0)[2]. In addition, other vascular endothelial growth factor-receptor (VEGFR) oral tyrosine kinase inhibitors (TKI) are useful for the treatment of lung cancer such as nintedanib, which is approved in Europe.[89] PFS was significantly improved in the docetaxel plus nintedanib group compared with the docetaxel plus placebo group (median 3.4 vs 2.7 months; hazard ratio 0.79 [95% CI 0.68-0.92], p=0.0019). **In summary, there is a role for VEGFR inhibition in lung cancer. Sorafenib, a VEGFR inhibitor, has single agent activity in *KRAS* mutated NSCLC. Based on this data, we hypothesize that a more potent version of sorafenib, regorafenib, will have enhanced activity in *KRAS* mutated NSCLC, when combined with methotrexate.**

Methotrexate

As detailed in Section 2.2.2, although methotrexate is not commonly used for the treatment of NSCLC, it was studied extensively from the 1970s to 1980s with some evidence of activity based on cruder chest x-ray imaging and response criteria. There was a retrospective study of 195 patients treated with methotrexate using a variety of dosing schedules, including 0.2 to 0.9 mg/kg *oral* twice a week, for which 5 of 29 patients with lung adenocarcinoma had a response[77]. There was also prospective randomized study comparing twice weekly IM injections of methotrexate at a high dose of 0.6 mg/kg or a low dose 0.2 mg/kg with placebo in 239 lung cancer patients, with an ORR of 21% in patients receiving the high dose and an ORR of 11% in patients receiving the low dose and response was associated longer survival[78]. **Single agent oral methotrexate in an average patient of 60 kg at doses ranging from 12 to 36 mg intramuscular twice weekly produced responses in patients with NSCLC. Molecular status of *KRAS* was not known at this time.**

Repurposing Methotrexate in Combination with Regorafenib for the Treatment of *KRAS* Mutated NSCLC based on Synergy in a Computational Model and Cell Lines

In an experimental proprietary simulation model by Cellworks which models millions of cancer pathway interactions at the genomic, transcriptional, and proteomic level simultaneously, regorafenib and methotrexate were found to be synergistic in *KRAS* mutated lung cancer cell lines. More details on the Cellworks simulation model and the methodology to identify the combination of regorafenib and methotrexate are detailed in Appendix F. The simulation model prediction of synergy of this combination was confirmed *in vitro* in *KRAS* mutated cell lines (i.e., A549, H2122, H358). The synergy at low doses of regorafenib (3.3 uM) and methotrexate (0.25 uM) was differential, with synergy only seen in *KRAS* mutated cell lines (i.e., H2122) but not *KRAS* wild type cell lines (i.e., CALU3) (Figure 2 of Appendix F). With methotrexate at a very low concentration of 0.25 uM, there was a dose response with increasing concentrations of regorafenib from 1-10 uM. Synergy with methotrexate was seen at doses of regorafenib as low as 1 uM. The dose of oral methotrexate required for synergy with regorafenib is significantly *lower* than the dose traditionally used in the treatment of cancer, consistent with the *repurposing* of methotrexate for use in combination with regorafenib in a molecularly defined subset of *KRAS* mutated NSCLC. The target Cmax for methotrexate can be achieved with an oral dose as low as 7.5 mg[90,91]. The Cmax of regorafenib from 1 to 10 uM can be achieved with doses of ≤ 100 mg given non-proportional pharmacokinetic increases[83,92]. Based on preclinical *in vitro* cell line data, our target Cmax is at least 0.25 uM for methotrexate and this should be achieved with oral doses as low as 7.5 mg daily[90]. Our target Cmax for regorafenib is ≥ 1 uM and this is easily achieved at the dose studied in this protocol at 120 mg[83].

The mechanism of the proposed synergy of regorafenib and methotrexate is outlined below and in Figure 1 of Appendix F. Regorafenib inhibits RAF, which is downstream of activated KRAS. Methotrexate inhibits dihydrofolate reductase, which is involved in methionine, purine, and pyrimidine synthesis pathways, affecting the S-phase of the cell cycle. One of the main converging nodes of synergy of this combination is proposed via SP1[93,94] and the transcription factor for dihydrofolate reductase. Methotrexate also causes upregulation of DUSP6 and NF1[95,96], which are inhibitors of the RAF-RAS-ERK pathway. It has been shown that *KRAS* mutated lung cancer cell lines have increased dependency on folate pathways for survival, and therefore, are more sensitive to anti-folate therapies like methotrexate and pemetrexed compared to *KRAS* wild-type lung cancer[47]. Methotrexate has also been shown to decrease KRAS mRNA expression.

The reason methotrexate was found to be more advantageous for combination with regorafenib than alternative anti-folate pemetrexed in the Cellworks proprietary simulation model is outlined below. Although pemetrexed and methotrexate have the potential to target several enzymes in the folate pathway, methotrexate primarily inhibits dihydrofolate reductase while pemetrexed primarily inhibits thimidylate synthase[97-100]. Via dihydrofolate reductase inhibition, methotrexate mainly works via s-adenosylmethionine and DNA methylation machinery while pemetrexed mainly works via pyrimidine synthesis pathway and also via the purine synthesis pathway at a higher concentration. Methotrexate, unlike pemetrexed, also has a unique property of inhibiting isoprenylcysteine carboxyl methyltransferase, which is key regulator of prenylation, an important step for the functionality of RAS isoforms[101,102]. Therefore, its effect will be specific for RAS mutant tumors.

Although not specifically examined in the Cellworks model, methotrexate may also have beneficial immunomodulatory effects. Methotrexate effects may be prolonged more than the half-life (~ 3 hours) suggests due to accumulation of polyglutamate metabolites in tissues, which can take weeks to months to achieve steady state (6.2 weeks-139.8 weeks)[103]. These may have lasting immunomodulatory effects that are beneficial. For example, methotrexate inhibits polyamines (small organic cations) that are required for tumor growth[104-106]; methotrexate increases adenosine, which has pleiotropic effects and in some studies when interacting through A3 receptors, inhibits proliferation and promotes apoptosis in tumors[106-108]; methotrexate has been shown to decrease levels of interleukin-1 and tumor necrosis factor, which are involved in cancer progression[106,109,110]. In addition, this more attractive *oral* combination of methotrexate and regorafenib is positioned for the 2nd/3rd line setting and beyond, and majority of patients will have already been treated with pemetrexed. There is no published data that failure to pemetrexed would result in failure to methotrexate. The purpose of methotrexate in this trial is to at low doses synergistically enhance the activity of regorafenib.

In summary, the significant crosstalk of pathways explains the synergy of regorafenib and methotrexate in *KRAS* mutated NSCLC. Methotrexate is also the ideal anti-folate given ability of oral administration, direct impact on RAS via prenylation, and potential beneficial immunomodulatory effects.

2.4 Study Design

This is a prospective open-label non-randomized single-arm treatment trial of regorafenib in combination with oral methotrexate for metastatic KRAS mutated non-squamous NSCLC patients who have received at least 1 systemic therapy, with a primary endpoint of progression free survival.

2.5 Correlative Studies Background

2.5.1 Detecting Circulating Tumor DNA (ctDNA) and Tumor Mutations using CAPP-Seq

Peripheral blood monitoring of cancer while on treatment is attractive. CAncer Personalized Profiling by Deep Sequencing (CAPP-Seq) is a method to measure ctDNA using a selector of biotinylated DNA oligonucleotides that detect recurrent mutations in cancers[111]. The initial design of the CAPP-Seq selector was created for NSCLC. The initial selector targeted 521 exons and 13 introns from a total of 139 mutated genes, and could identify 96% of patients with NSCLC. Using CAPP-Seq for noninvasive monitoring of cancer in the initial publication, had an impressive receiving operating characteristic (AUC 0.95), with a sensitivity of 85% and specificity of 96%. The sensitivity was higher in tumors of more advanced stage, reaching 100% in stage II-IV tumors. Newman et al (Diehn/Alizadeh labs, Stanford University, Stanford, CA) were able to correlate ctDNA levels with tumor volumes determined radiographically by PET and/or CT ($R^2 = 0.89$, $P = 0.0002$). ctDNA levels were also shown to fall dramatically in patients responding to chemotherapy and targeted therapeutics. ctDNA was also helpful in determining if a patient had progressed after receiving definitive radiotherapy as CT imaging

after radiotherapy is often difficult to interpret. ctDNA was also useful in surveillance monitoring after curative intent therapy for early stage NSCLC. In addition, ctDNA by CAPP-Seq has the ability to detect resistance mechanisms to oral targeted therapeutics. For example, in 43 NSCLC patients treated with a 3rd generation EGFR inhibitor rociletinib, there were multiple resistance mechanisms detected with CAPP-Seq in almost half of the patients including MET, EGFR, PIK3CA, ERBB2, KRAS, and RB1 along with novel EGFR mutations (L798I and C797S)[112]. Therefore, CAPP-Seq is also a robust platform to study resistance mechanisms.

ctDNA as measured by CAPP-Seq technology may serve as a useful surrogate biomarker of clinical activity to regorafenib and methotrexate using baseline levels of ctDNA or change in levels of ctDNA longitudinally. In this trial, we will measure ctDNA levels as surrogate biomarkers of response. ctDNA may be able to distinguish at early timepoints which patients will benefit from the combination of regorafenib and methotrexate. CAPP-Seq may also identify resistance mutations to this oral combination targeted therapy.

2.5.2 Computational Simulation Model

The Cellworks computational model is described in Section 2.3. We will send available de-identified genomic data to Cellworks via secure encrypted communication. Sources of genomic data may have been collected from blood, tissue, urine, or other body fluid (i.e., pleural fluid). Genomic data may include copy number variation, targeted gene sequencing, whole exome sequencing, are whole genome sequencing. The genomic data may have been generated previously for standard of care management of the patient. In addition, de-identified genomic data from circulating tumor DNA may be shared for modeling. Therefore, prediction of sensitivity to this therapy based on the patient's available genomic data, which may go beyond KRAS, can then potentially be correlated with clinical outcomes of the patient on study.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

In order to be eligible for participation in this trial, the patient must meet *ALL* of the following criteria (i.e., mark "yes" or "N/A" to all criteria):

1. Histologic or cytologic confirmed diagnosis of non-squamous non-small cell lung cancer that is recurrent or metastatic. Adenosquamous is allowed provided the patient has confirmed adenocarcinoma component.
2. Documentation of pathogenic KRAS mutation
3. Previous receipt of at least one systemic therapy for recurrent or metastatic disease **OR** previous receipt of adjuvant systemic therapy within 6 months of enrollment. There is no limit on number of prior therapies allowed.
4. Prior systemic therapy must be completed at least 2 weeks prior to study treatment, with either improvement of clinically significant treatment-related toxicities to grade 0-1 **OR** stabilized to a new baseline.

5. Previously treated **OR** asymptomatic non-progressing < 1 cm untreated brain metastases are allowed
6. Measurable disease based on RECIST version 1.1 criteria (Appendix B)
7. Ability to understand and the willingness to sign a written informed consent document
8. Age ≥ 18 years-old
9. ECOG performance status of 0 or 1 (Appendix A)
10. Adequate bone marrow, liver and renal function as assessed by the following laboratory requirements:
    a. Absolute neutrophil count (ANC) ≥ 1500/mm^3
    b. Platelet count ≥ 100,000 /mm^3
    c. Hemoglobin (Hb) ≥ 9 g/dL
    d. Serum creatinine ≤ 1.5x upper limit of normal (ULN) **OR** calculated (Cockcroft-Gault formula) *or* measured creatinine clearance ≥ 50 mL/min for patients with creatinine levels > 1.5x ULN
    e. Total bilirubin ≤ 1.5x ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5x ULN
    f. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3x ULN (≤ 5x ULN for patients with liver involvement of their cancer)
11. Must be able to swallow and retain oral medication.
12. Women patients of childbearing potential and men patients with women partners of childbearing potential must agree to use adequate contraception or agree to abstain from heterosexual activity beginning at the time of signing informed consent until at least 3 months after the last dose of study treatment. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not considered childbearing.

3.2 Exclusion Criteria

In order to be eligible for participation in this trial, the patient must *NOT* meet the following criteria (i.e., mark "no" or "N/A" to all criteria):

1. Previously treated with regorafenib
2. Known allergy to regorafenib or methotrexate
3. Currently receiving another systemic standard or investigational anti-cancer therapy. Prior investigational therapy must be completed within 4 half-lives (if known) or 2 weeks, whichever is longer. The maximal washout of investigational therapy will not exceed 4 weeks prior to study treatment. Bone medications such as bisphosphonates and RANK ligand inhibitors permitted.
4. Leptomeningeal disease as documented by CSF cytology.
5. Clinically significant cardiovascular-related disease including:

a. Uncontrolled hypertension (systolic pressure >150 mm Hg or diastolic pressure > 90 mm Hg on *repeated* measurements that does not resolve prior to study treatment on C1D1 despite optimal medical management
b. Congestive heart failure – New York Heart Association (NYHA) Class III or greater
c. Active coronary artery disease (i.e., unstable or new onset angina within 3 months of study treatment; myocardial infarction within 6 months of study treatment)
d. Clinically significant cardiac arrhythmias other than atrial flutter/fibrillation
e. Stroke, including transient ischemic attacks, within 6 months of study treatment
f. Other clinically significant arterial events, *except* for controlled asymptomatic pulmonary embolism, within 6 months of study treatment

6. Clinically significant hemorrhage or bleeding event within 1 month of study treatment
7. Uncontrolled symptomatic pleural effusion or ascites
8. Known active additional malignancy that is undergoing or expected to undergo systemic treatment during duration of study participation.
9. Known history of human immunodeficiency virus (HIV) infection or known current active hepatitis B (i.e., Hep B DNA positive in prior 3 months) or hepatitis C infection (i.e., Hep C RNA positive in prior 3 months, with the exception of patients who have completed curative therapy and are Hep C RNA negative on retest).
10. Major surgical procedure (e.g., involving the opening of a major body cavity) within 4 weeks of study treatment. This does *not* apply to low-risk procedures (i.e., thoracentesis; paracentesis; chest tube/PleurX catheter placement; line placement; needle biopsy of tumor; and bronchoscopy).
11. Presence of a clinically significant non-healing wound or non-healing ulcer
12. Concomitant therapy required at time of first dose of study treatment, including:
    a. Strong CYP3A4 inhibitors and CYP3A4 inducers (Appendix C)
    b. Regular use of NSAIDs, proton pump inhibitors, and probenecid
13. Women who are pregnant or breast-feeding.
14. Any condition which, in the investigator's opinion, including substance abuse, medical, psychological or social conditions that makes the patient unsuitable for trial participation or may interfere with the patient's participation in the study.

See Participant Eligibility Checklist in Appendix E.

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

Special Informed Consent Circumstances:

1. If the patient is not physically capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.
2. For adults under legal protection, consent shall be given by the legal guardian(s). The consent of an adult under legal protection shall also be requested where such a person is able to express his/her own will. His/her refusal or the withdrawal of his/her consent may not be disregarded.
3. In emergency situations, when prior consent of the patient is not possible, the consent of the patient's legal representative(s) or proxy consenter, if present, should be requested. The patient should be informed about the study as soon as possible and his/her consent to continue the study should be requested.

The informed consent form provided to patients/legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the patient's consent, or if there is an amendment to the protocol that necessitates a change to the content of the patient information and/or the written informed consent form. The investigator will inform the patient/legal representative or proxy consenter of changes in a timely manner and will ask the patient to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form must receive the IRB`s approval in advance of use.

3.4 Randomization Procedures

There is no randomization for this study.

3.5 Study Timeline

3.5.1 Primary Completion

Assuming no dose limiting criteria are met, with a sample size of 18 patients, with at least 15 evaluable for PFS, and an accrual rate of 2 patients per month, the time for the study to reach primary completion for the primary endpoint of progression-free survival will take 12-15 months (from the time study opens to accrual).

3.5.2 Study Completion

The study will reach study completion 24 months from the time the study opens to accrual.

4. TREATMENT PLAN

- Screening, study visits, and end of treatment visit will be conducted as outlined in the Study Calendar (Section 9).

- Study treatment will be administered in 28-day cycles. Refer to Section 5.1 for dose and schedule.

4.1 General Concomitant Medication and Supportive Care Guidelines

4.1.1 Prohibited Therapies

- Other concurrent systemic standard or investigational anti-cancer therapy, with the exception of bone strengthening medications such as RANK ligand inhibitors or bisphosphonates
- Concurrent radiation therapy, with the exception of:
    - 1) Central nervous system radiation, either stereotactic radiotherapy or whole brain radiation, as long as there is evidence of systemic disease control per investigator-assessment
    - 2) Palliative radiation therapy, as long as (1) the target lesion(s) are not included within the radiation field (or can be replaced with an alternative target lesion), and (2) that the radiation is not required due to progression per investigator-assessment.
    - **Of note, study treatment (both regorafenib and methotrexate) should be held on the days of radiation.**

4.1.2 Therapies to Avoid (To the Extent Possible)

The following therapies should be avoided to the extent possible, particularly during Cycle 1.

- Regorafenib-related
    - Strong CYP3A4 inducers (decrease exposure of regorafenib). Examples include rifampin, phenytoin, carbamazepine, phenobarbital, and St. John's Wort. (Appendix C)
    - Strong CYP3A4 inhibitors (increase exposure of regorafenib). Examples include clarithromycin, grapefruit juice, itraconazole, ketoconazole, posaconazole, nefazodone, telithromycin, and voriconazole. (Appendix C)
- Methotrexate-related
    - Regular use of non-steroidal anti-inflammatories (NSAIDs)
    - Regular use of proton pump inhibitors (PPIs)
    - Regular use of Probenecid
- Regorafenib and methotrexate-related
    - Regular or excessive use of alcohol

4.1.3 Therapies that May Require Additional Monitoring

The following therapies may require additional monitoring per institutional guidelines.

- Regorafenib-related
    - Medication that is metabolized by the cytochrome enzymes CYP2C8, CYP2B6, and CYP2C9 (Appendix D)

- Therapeutic anticoagulation with Vitamin-K antagonists (i.e., warfarin) or with other anticoagulants (i.e., heparins, low molecular weight heparin, oral direct anti-XA inhibitors)
    - Of note, warfarin is metabolized by the cytochrome enzyme CYP2C9 and its levels may be affected by regorafenib.
- BCRP substrates such as statins, prazosin, glyburide, nitrofurantoin, and cimetidine.
    - *Regorafenib is an inhibitor of BCRP transporters and the investigator should be aware of the potential increased serum concentrations of BCRP substrates. Of note, methotrexate (investigational compound in this study) is a BCRP substrate, and dose limiting toxicity definitions, drug levels, and safety monitoring have been incorporated in this study. However, the target dose for this study is relatively low at 20 mg oral methotrexate twice weekly. This dose is lower dose than the dose used in other cancers such as head and neck at 40 mg/m^2 iv weekly, which is well-tolerated.[113,114] Not only is the target dose of methotrexate for this study lower, there will also be decreased absorption due to route of administration, oral, compared to intravenous, subcutaneous, or intramuscular.*

- Methotrexate-related. Drugs that can affect methotrexate levels.
  These should be avoided in Cycle 1 to the extent possible.
    - Can increase methotrexate levels
        - Penicillins (can reduce renal clearance of methotrexate)
        - Drugs highly bound to plasma protein such as such as salicylates, phenylbutazone, phenytoin, and sulfonamides (can decrease amount of methotrexate bound to serum albumin)
    - Can decrease methotrexate levels
        - Oral antibiotics such as tetracycline and chloramphenicol (can decrease intestinal absorption or interfere with metabolism by inhibiting bowel flora)
- Methotrexate-related (other)
    - Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression

4.1.4 Permitted Medications

- All medications and therapies that are considered necessary for the patient's welfare (i.e., concurrent medical conditions, supportive care), and which are not expected to interfere with the study treatment, may be given at the discretion of the investigator.
- Aspirin use up to 325 mg daily is permitted. Daily aspirin doses above 325 mg may be permitted under investigator discretion. Previous studies of rheumatoid arthritis report no increases in methotrexate-induced pulmonary disease or major toxic reactions in those

receiving aspirin[115-119]. Another study found no increase in adverse events or effect on laboratory parameters in those on 975 mg aspirin four times per day for one week[120]. Subjects who regularly use aspirin will be monitored closely in this trial.

- 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.
- Chronic erythropoietin
- Bisphosphonates or RANK-ligand inhibitors

4.2 Criteria for Removal from Study

4.2.1 Withdrawal of Consent

Patient (or his/her legally acceptable representative) may choose to discontinue the trial at any time, for any reason, and without prejudice to further treatment and care. A patient who withdraws consent from study participation will be asked to state the level of withdrawal:

- 1) Withdrawal from study treatment only; in this case, follow up data will continue as planned.
- 2) Withdrawal from study, including study treatment and post-treatment follow-up.

In the event of withdrawal of consent, the study staff and/or investigator must make every effort to ascertain the level of consent withdrawn. The type of consent withdrawal must be noted in source documents.

4.2.2 Discontinuing Study Treatment

Patients will <u>stop study treatment</u> for any of the following reasons (bullets below). These patients will be followed for outlined outcomes until the end of the study. To the extent possible, they should be followed for safety until improvement to grade 0-1 or stabilization to new baseline (i.e., potentially permanent sequelae) of toxicities attributable to the study treatment.

- Withdraws consent for study treatment
  - *If a patient withdraws from study treatment, the investigator should promptly notify the Stanford Medical Monitor and will make every effort to complete the End-of-Treatment visit. Every effort must be made to ascertain if patients will permit follow-up.*
- Unacceptable toxicity
- Pregnancy
- Investigator decision
- Lost to follow-up
  - *Before a patient is considered "lost to follow-up", study personnel should contact the patient at least twice by phone and once by mail and document these attempts. Study*

personnel may use public records to check for mortality for any patients considered "lost to follow-up," as permitted by applicable laws or regulations.

- Disease progression (Refer to Section 4.2.4 for further details)
- Death
- Consistent and significant non-compliance with study treatment, protocol-mandated procedures, or both
- The study is terminated by the investigator or Bayer. (Refer to Section 4.2.3)

4.2.3 Premature Termination of the Study

The premature termination of the study may occur (but is not limited to) the following:

-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

-If the study conduct (e.g., recruitment rate; drop-out rate) 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., IRB(s); competent authority(ies); study center; head of study center) should be informed as applicable according to local law.
- In case of a partial study closure, ongoing patients, including those in post study follow-up, should be taken care of in an ethical manner.

4.2.4 Special Situations Requiring PI Permission

Patients may continue on study therapy with permission of the overall study PI in the following situations:

- Patients with progression of disease as determined by the investigator should generally discontinue study therapy. However, if the patient is having clinical benefit (for example, response or stable disease systemically, with mild progression in central nervous system), patients may be considered to continue on therapy post-progression. The patient should have permission from the overall study PI to receive post-progression therapy before the second week of post-progression study treatment is given (i.e., patient can receive 1 week of study treatment post-progression without prospective permission).
- For patients who require > 28 days delay of regorafenib secondary to a treatment-related adverse event or for another reason (starting from the time the study treatment was supposed to be administered), they will proceed with the End-of-Treatment visit, unless, in the investigator's opinion, continuing on study treatment is of benefit for the patient.

In this case, the reason of continuation should be documented and there should be permission from the overall study PI before resuming treatment.

- *If there is a methotrexate-related toxicity that requires discontinuation of methotrexate but the patient is tolerating regorafenib, regorafenib may be continued without methotrexate (permission not required by study PI).*

4.3 Alternatives

All patients will sign informed consent. To minimize potential risks related to potential overlapping toxicities from regorafenib and methotrexate, patients will undergo an intra-patient weekly dose escalation of methotrexate and be followed very closely in the first two cycles for safety. Peripheral blood research studies will be drawn with every effort to coincide with scheduled safety blood draws. Patients may withdraw from the study at any time without prejudice to their further care. Patients with progressing cancer may be offered a variety of alternate treatment options. They may also be offered palliative and supportive care services.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

Please refer to Section 2 and investigator's brochure (IB) for regorafenib, and Section 2 and package insert for methotrexate for details regarding mechanism of action, summaries of animal and clinical studies, non-clinical and clinical pharmacokinetics, major route of elimination, and safety profile.

5.1 Investigational Agent/Device/Procedure

5.1.1 Regorafenib

Regorafenib will begin on Cycle 1 Day 1 and will be self-administered at 80 mg oral daily for 3 weeks on /1 week off of a 28-day cycle. Regorafenib is provided as 40 mg tablets. Regorafenib will NOT be dose escalated during Cycle 1.

Regorafenib can be dose escalated to 120 mg oral daily (same schedule of 3 weeks on/1 week off of the 28-day cycle) according to strict criteria beginning on Cycle 2 Day 1. The strict criteria for escalation include no evidence of significant drug-related toxicities (SDRT), defined as any event that would require a dose modification of regorafenib (i.e., interruption only, reduction only, or interruption followed by reduction) according to the toxicity guidelines/tables in Section 6.2.1 (regorafenib toxicities) and Section 6.2.3 (overlapping regorafenib and methotrexate toxicities). If after the first two cycles a patient has not yet escalated or is considering re-escalation to regorafenib 120 mg, this is permitted as long as all treatment-related toxicities have resolved to grade 0-1 or to the patient's baseline and at the discretion of the investigator. **However, a patient is not required to dose escalate regorafenib to 120 mg during the course of study treatment and is permitted to remain at the 80 mg dose if it is better tolerated and per investigator discretion.**

Regorafenib should be taken ideally at a similar time each day and with approximately 8 fluid ounces (240 mL) of water after a low-fat (<30% fat) meal. Some examples of low-fat meals 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, 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.

5.1.2 Methotrexate

Methotrexate will be self-administered during the study twice weekly during the same weeks as regorafenib (i.e., a schedule of 3 weeks on/1 week off of a 28-day cycle). Methotrexate is provided as 2.5 mg tablets. The initial starting dose during Cycle 1 Week 1 is 10 mg oral twice weekly. Doses of methotrexate should be separated by at least 2 days each week. However, it is preferred that methotrexate doses are separated by 3 days. During Cycle 1, methotrexate and regorafenib will be taken at a similar time of day to allow for ease of pharmacokinetic sampling. During Cycle 1, methotrexate will be taken within 30 minutes of regorafenib, and therefore, will be taken with a low-fat meal.

Methotrexate will be dose escalated each week during Cycle 1 as tolerated to a maximum dose of 20 mg oral twice weekly:

- Cycle 1 D1 ($\pm$ 1 day): 10 mg (i.e., 4 tablets) oral twice weekly
- Cycle 1 D8 ($\pm$ 1 day): 15 mg (i.e., 6 tablets) oral twice weekly
- Cycle 1 D15 ($\pm$ 1 day): 20 mg (i.e., 8 tablets) oral twice weekly
- Cycle 1 D22 ($\pm$ 1 day): OFF

Beginning in Cycle 2, methotrexate will be self-administered at doses ranging from 10 to 20 mg oral twice weekly, depending on tolerability, with 2-3 days between doses and on the same weeks as regorafenib (3 weeks on/1 week off of a 28 day cycle). Dose escalation of methotrexate can continue after Cycle 1 depending on the patient's tolerability, if the patient has not dose escalated to 20 mg by the end of Cycle 1. Beginning in Cycle 2, methotrexate may be taken at a different time of day than regorafenib per patient preference. Methotrexate may be taken with or without food per patient preference. Although methotrexate administration with food may delay absorption and reduce peak concentrations of methotrexate, it may improve tolerability and is therefore encouraged.

5.2 Availability

BAYER U.S. LLC will provide regorafenib.

Methotrexate (generic) will be purchased through the Stanford Research Pharmacy. Bayer will supply funding for purchase of methotrexate.

5.3 Agent Ordering

5.3.1 Regorafenib

Supply of regorafenib will occur through direct contact with the supplier, BAYER U.S. LLC.

BAYER U.S. LLC

[redacted]

Phone: [redacted]

5.3.2 Methotrexate

An Interdepartmental Request Form, which can be obtained from the Standard Register, will be filled out and submitted to the Stanford Research Pharmacy for supply of methotrexate.

Stanford Hospital & Clinics

Department of Pharmacy

[redacted]

Stanford, CA [redacted]

Phone: [redacted]

Fax: [redacted]

5.4 Agent Accountability

All study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and the instructions given by the clinical supplies department of the Institution and will be inaccessible to unauthorized personnel.

5.4.1 Regorafenib

Regorafenib tablets will be packaged in high density polyethylene bottles with a white child resistant closure and induction seal. Each bottle contains 28 tablets and a 3-gram desiccant. The bottles will have a label affixed containing study identification, product identification, and quantity of tablets. Once the drug has been received it should be kept in a secure, dry location. Study drug should be stored in its original bottle at a temperature not above 25°C (77°F). The study drug must be exclusively used for the investigation specified in this protocol and it will only be accessible to authorized staff.

The investigator, or a responsible party designated by the investigator (i.e., site pharmacist), will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record or another comparable drug accountability form.

At the end of the study, unused supplies of regorafenib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form. The certificate of destruction for regorafenib should be sent to Bayer.

A completed "Unused Study Drug Disposition Form Destruction or Return Confirmation" should be sent to Bayer at the following address:

E-mail: [redacted]

OR

Mail: (VP of Medical Affairs named in contract) at

BAYER U.S. LLC

[redacted]

5.4.2 Methotrexate

Methotrexate tablets (2.5 mg each) will be packaged in a tight, light-resistant container using a child-resistant closure. The methotrexate packages will have a label affixed containing study identification, product identification, and quantity of tablets. Once methotrexate has been received, it will be kept in a secure, dry location. Methotrexate will be stored in its original package at a temperature not above 25°C (77°F). Methotrexate will be exclusively used for the investigation specified in this protocol, and it will only be accessible to authorized staff.

At the end of the study, unused supplies of methotrexate will be destroyed according to institutional policies.

6. DOSE MODIFICATIONS

6.1 Dose Limiting Toxicity Definitions (First 28-Day Period)

Dose Limiting Toxicities (DLT) will be monitored during Cycle 1 (first 28 days). During Cycle 1, patients will be on regorafenib 80 mg oral daily 3 weeks on/1 week off and methotrexate with doses ranging three dose levels between 10-20 mg oral twice weekly 3 weeks on/1 week off. A patient will be eligible for DLT assessments if he/she has received at least 80% of the planned doses of study treatment, which will be calculated by dosing days of regorafenib in case a patient is off treatment during Cycle 1 for a reason other than toxicity.
A DLT will be generally defined as any Grade 3 or 4 toxicity occurring during Cycle 1 (first 28 days), which is regarded as clinically significant and related to study treatment with clarifications and exceptions for adverse event terms noted below. The NCI-CTCAE version 4.03 will be used to assess toxicities.

<u>Only the following hematologic toxicities will be considered a DLT</u>:

- Absolute Neutrophil Count (ANC) < 500/mm^3 for > 7 days despite dose interruption
- Febrile neutropenia [ANC <1000/mm^3 with a single temperature of > 38.3 degrees C (101 degrees F) or a sustained temperature of ≥ 38 degrees C (100.4 degrees F) for more than one hour]
- Platelets < 25,000/mm^3 or ≥ Grade 3 thrombocytopenia associated with clinically significant bleeding

Non-hematologic Grade 3 or Grade 4 toxicity considered clinically significant and study treatment-related is a DLT, except for the following, *permitting protocol therapy can resume according to the toxicity guidelines in Section 6.2. (Note: If patient declines to resume protocol therapy despite being able to, this will not be considered a DLT).*

- Grade 3 nausea, vomiting, or diarrhea that improves to grade 2 or less with dose interruption and appropriate supportive care within 5 days
- Grade 3 mucositis or stomatitis that improves to grade 0-1 with dose interruption and appropriate supportive care within 28 days
- Grade 3 hypertension that improves to grade 2 or less with dose interruption and appropriate supportive care within 7 days
- Grade 3-4 electrolyte abnormalities that are asymptomatic and can be corrected with supplementation and/or appropriate supportive care (e.g., hypomagnesemia, hypokalemia, hypocalcemia, hyponatremia, hypophosphatemia) or any grade of electrolyte abnormality that is related to another adverse event such as diarrhea, nausea, or vomiting
- Grade 3 asymptomatic maculo-papular rash that improves to grade 2 or less with dose interruption and appropriate supportive care within 28 days

The following will not be considered a DLT:

- ≥ Grade 3 lipase and amylase that is asymptomatic
- Grade 3 unconjugated/indirect bilirubin secondary to Gilbert's disease or equivalent

Miscellaneous: In case an unexpected drug-related toxicity (even at a lower grade) is seen more frequently than expected or beyond the DLT time period, this toxicity may be declared as a DLT for the remainder of the study after consultation with the PI.

6.2 Dose Modifications

The guidelines in this section (applies to all text and tables listed under the Section 6.2) are STRICT for adverse event management for the FIRST TWO CYCLES of therapy. After the first two cycles of therapy, the final adverse event management decision is per investigator discretion, although these guidelines should be strongly considered

Separate guidelines are given for (1) regorafenib-related adverse event management, (2) methotrexate-related adverse event management, and (3) potential overlapping toxicities of the two investigational agents including dermatologic (rash), gastrointestinal (diarrhea, nausea, vomiting, stomatitis), and hepatitis.

In general, if regorafenib dose is interrupted, methotrexate dose should be interrupted. However, if methotrexate dose is interrupted, regorafenib dose does not necessarily need to be interrupted if the toxicity is thought to be unrelated to regorafenib (i.e., cytopenias). In general, for

overlapping toxicities of both study drugs that necessitate dose reduction, both regorafenib and methotrexate should be dose reduced, although some toxicities may allow for stepwise reduction upon resuming treatment (i.e., dose reducing one drug first and keeping the other study drug at the same dose). Re-escalation of one or both drugs in certain circumstances can be considered per guidelines below. Monitoring should be performed following dose interruption or modification of regorafenib and/or methotrexate, in a manner consistent with the local clinical standard of care.

6.2.1 Regorafenib

6.2.1.1 Regorafenib Dose Levels

The starting dose of regorafenib is 80 mg oral once daily on a 3 weeks on/1week off schedule of a 28-day cycle with a low-fat meal during Cycle 1. Regorafenib will NOT be dose escalated during Cycle 1. Regorafenib can be dose escalated to 120 mg starting at Cycle 2 based on absence of significant-drug related toxicities (SDRT), defined as any event that would require a dose modification of regorafenib (i.e., interruption only, reduction only, or interruption followed by reduction) according to the guidelines/tables in this Section 6.2.1 (regorafenib toxicities) and Section 6.2.3 (overlapping toxicities with methotrexate).

Doses will be delayed or reduced for clinically significant hematologic and non-hematologic toxicities that are related to study treatment according to the guidelines shown in the Dose Delays/Dose Modifications tables that follow. Dose modifications will follow predefined dose levels.

The modifications of regorafenib will follow the following predefined dose levels:		
Dose level 0 (starting dose)	80 mg oral daily	Two 40-mg tablets of regorafenib
Dose level 1	120 mg oral daily	Three 40-mg tablets of regorafenib

If after the first two cycles, a patient has not yet escalated or is considering re-escalation to regorafenib 120 mg oral daily, this is permitted as long as all treatment-related toxicities have resolved to grade 0-1 or to the patient's baseline and at the discretion of the investigator.

If a patient requires a dose reduction below 80 mg daily due to a treatment-related toxicity, the patient should discontinue study treatment. If a patient requires >4 week delay for treatment-related toxicity, it is encouraged that the patient discontinue study treatment, unless the patient may derive ongoing benefit by resuming treatment as per investigator discretion and after discussion with the overall study PI.

6.2.1.2 Regorafenib-Related Toxicities (General)

The following table outlines dose adjustments for clinically significant toxicities (hematologic and non-hematologic) related to regorafenib except hand-foot skin reaction (HFSR), hypertension, mucositis/stomatitis, and liver function test abnormalities.

In addition to these recommended dose modifications, subjects who develop diarrhea, mucositis or stomatitis, anorexia or other events predisposing to fluid loss or inadequate fluid intake should be carefully monitored and rehydrated as clinically necessary. Dose reductions when recommended are only permitted for those patients who have already escalated to the 120 mg oral daily dose. No dose reductions below 80 mg are permitted and if this is required, patients should go off protocol treatment.

Patients do not necessarily need to discontinue protocol therapy if intolerable or severe toxicity occurs at the regorafenib 80 mg dose if it is possible for (i) a treatment-related toxicity to improve to a lower grade with dose interruption alone +/- maximal supportive care and the patient is able to resume regorafenib at a dose of 80 mg oral daily or (ii) if the toxicity is thought to be at least contributed to methotrexate whereby a dose reduction of only methotrexate may improve the toxicity per investigator discretion. These scenarios are further described in tables below.

Recommended dose modification for Regorafenib treatment-related toxicities except hand-foot-skin reaction, hypertension and ALT/AST/bilirubin			
NCI-CTCAE v4.03[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 patient on 120 mg dose)[d]	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 (if patient on 120 mg dose)[d] Permanent discontinuation can be considered at treating investigator's discretion.	Dose escalation not recommended.

a. NCI-CTCAE = National Cancer Institute - Common Terminology Criteria for Adverse Events, version 4.03
b. Excludes (1) alopecia; (2) non-refractory nausea/vomiting, diarrhea responsive to supportive care; (3) non-refractory hypersensitivity reaction responsive to supportive care; (4) laboratory abnormalities except those specifically noted in Section 6.2. See separate toxicity table for mucositis/stomatitis.
c. If no recovery after a 28-day delay, treatment should be permanently discontinued unless patient is deriving clinical benefit per-investigator assessment and after discussion with overall study PI. (Refer to Section 4.2.4)
d. Patients requiring dose reduction of regorafenib below 80 mg daily should go off protocol therapy. The maximum daily dose of regorafenib is 120 mg.

6.2.1.3 Regorafenib-Related Hand-Foot Skin Reaction (HFSR)

Grading for Hand-Foot-Skin-Reaction			
	Grade 1	**Grade 2**	**Grade 3**
NCI-CTCAE v4.03 Palmar-plantar erythrodysesthesia syndrome[a]	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.			

Recommended dose modification for Regorafenib-related hand-foot-skin reaction (HFSR) [a,e]		
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	Immediately institute supportive measures and consider decreasing dose by one dose level (i.e., if patient is on 120 mg dose)[b,d]. If no improvement or dose level maintained (i.e., particularly if patient is only on 80 mg dose), interrupt therapy for a minimum of 7 days, until toxicity resolves to Grade 0 to 1.
	No improvement within 7 days or 2nd occurrence	Interrupt therapy until toxicity resolves to Grade 0 to 1. When resuming treatment, treat at reduced dose level [b,d]
	3rd occurrence	Interrupt therapy until toxicity resolves to Grade 0 to 1. When resuming treatment, can consider continuing treatment at reduced dose level.[b, d] **However, discontinuing therapy strongly encouraged**.
	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 to 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 to 1.[c] When resuming treatment, can consider continuing treatment at reduced dose level.[b, d] **However, discontinuing therapy strongly encouraged.**
	3rd occurrence	Discontinue treatment

a. More conservative management is allowed if judged medically appropriate by the investigator.
b. If toxicity returns to Grade 0 to 1 after dose reduction, dose re-escalation is strongly discouraged. However, it is permitted at the discretion of the investigator after the patient has completed one cycle at reduced dose without recurrence of event.
c. If no recovery after a 28 day delay, treatment should be permanently discontinued unless patient is deriving clinical benefit per-investigator assessment and after discussion with overall study PI. (Refer to Section 4.2.4)
d. Patients requiring dose reduction of regorafenib below 80 mg daily should go off protocol therapy. The maximum daily dose of regorafenib is 120 mg.
e. It should be noted that oral methotrexate can cause dermatologic toxicity including alopecia, dermatitis, erythema multiforme, erythroderma, photosensitivity, pruritus, rash, sensation of burning skin, skin necrosis, skin plaque erosion, Steven-Johnson syndrome, tissue necrosis, and toxic epidermal necrolysis. However, methotrexate does not generally cause HFSR. In general, when adverse management guidelines suggest interrupting therapy with regorafenib, methotrexate therapy should also be interrupted. **If the investigator cannot rule out relatedness to methotrexate, if it is recommended to reduce regorafenib by one dose level, methotrexate can also be reduced by one dose level** (Refer to Section 6.2.2.1 for methotrexate dose levels).

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. Early referral to supportive dermatology is strongly encouraged for all patients given the relatively high incidence of this toxicity with regorafenib.

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, when indicated.

During regorafenib treatment, patient should:

- 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. It is preferable to 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

6.2.1.4 Regorafenib-Related Hypertension

Hypertension is a known adverse event associated with regorafenib treatment. Patients will have their blood pressure measured per Study Calendar (Section 9). If additional blood pressure measurements are done outside the study site, and the blood pressure is > 150 mm Hg systolic or > 90 mm Hg diastolic (NCI CTCAE v4.03), then the patient should contact study personnel for guidance. 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, the following table outlines suggested dose reductions.

Recommended Management of Regorafenib Related Treatment-Emergent Hypertension		
Grade (CTCAE v4.03)	**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 Grade 3 hypertension recurs, reduce by 1 dose level.[b,c] • If BP is not controlled with the addition of new or more intensive therapy, reduce by 1 dose level.[b,c] • If Grade 3 hypertension recurs despite dose reduction and appropriate antihypertensive therapy, discontinue therapy.
4 Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis)	Per institutional guidelines	Discontinue therapy
a. If no recovery after a 28-day delay, treatment should be permanently discontinued unless patient is deriving clinical benefit per-investigator assessment and after discussion with overall study PI. (Refer to Section 4.2.4) b. After the patient's BP remains controlled for at least one cycle, dose re-escalation permitted per investigator's discretion. c. Patients requiring dose reduction of regorafenib below 80 mg daily should go off protocol therapy.		

6.2.2 Methotrexate

6.2.2.1 Methotrexate Dose Levels

Methotrexate is given at low doses, no more than 20 mg oral twice weekly, separated by at least 2 days (preferably 3 days), 3 weeks on/1 week off of a 28-day cycle.

The modifications of methotrexate will follow the predefined dose levels[a]:		
Dose level 0	20 mg oral twice weekly	Eight, 2.5-mg tablets
Dose level -1	15 mg oral twice weekly	Six, 2.5-mg tablets
Dose level -2	10 mg oral twice weekly	Four, 2.5-mg tables

[a] Of note, methotrexate will be dose escalated weekly from dose level -2 to dose level 0 during Cycle 1 to each individual patient's maximum tolerated dose.

In general, if regorafenib dose is interrupted, methotrexate dose should be interrupted. However, if methotrexate dose is interrupted, regorafenib dose does not necessarily need to be interrupted if the toxicity is thought to be unrelated to regorafenib (i.e., cytopenias). In general, for overlapping toxicities of both study drugs that necessitate dose reduction, both regorafenib and methotrexate should be dose reduced, although some toxicities may allow for stepwise reduction upon resuming treatment (i.e., dose reducing one drug first and keeping the other study drug at the same dose). Dose reduction for methotrexate is usually decreased by one dose level but can be decreased by two dose levels per investigator discretion. Re-escalation of one or both drugs can be considered according to toxicity tables and where noted, investigator discretion.

Methotrexate may cause cytopenias, with guidelines for methotrexate dose management in Section 6.2.2.2. Regorafenib does not necessarily need to be dose interrupted or reduced for cytopenias. **For severe cytopenias, the investigator should check a methotrexate level** (Refer to Section 6.2.2.3 for Methotrexate Overdose). **If a patient requires discontinuation of methotrexate due to cytopenias, regorafenib may be continued alone at the discretion of the investigator.**

6.2.2.2 Methotrexate-Related Cytopenias

Neutrophil Count Decreased

CTCAE v. 4.03	Definition	Methotrexate Dosing
Grade 1	<LLN-1500/mm^3; <LLN -1.x 10e9/L	Continue methotrexate
Grade 2	<1500-1000/mm^3; <1.5-1.0x10e9/L	Continue methotrexate and follow blood counts
Grade 3	<1000-500/mm^3; <1.0-0.5x 10e9/L	Interrupt methotrexate until ≤ Grade 2 and resume at one lower dose level[a,b]; check methotrexate level
Grade 4	<500/mm^3; <0.5 x 10e9/L	Interrupt methotrexate until ≤ Grade 2 and resume at one lower dose level[a,b]; check methotrexate level
a: If there is an interruption of methotrexate recommended to manage cytopenias, it is recommended to resume at one lower dose level (i.e., dose reduction from 20 mg → 15 mg). However, it is permitted to resume at two lower dose levels per investigator discretion (i.e., 20 mg → 10 mg). If a dose reduction is made for cytopenia, the dose of methotrexate should not be escalated, unless a patient has decreased by more than one dose level. b: If patient is already on the lowest dose of methotrexate (10 mg), methotrexate should be discontinued and regorafenib may be continued per investigator discretion.		

Febrile Neutropenia

CTCAE v. 4.03	Definition	Methotrexate Dosing
Grade 3	ANC < 1000/mm^3 with single temperature of >38.3 degrees C (101 degrees F) or sustained temperature of >= 38 degrees C (100.4 degrees F) for more than one hour	Interrupt methotrexate until neutrophil count recovers to ≤ Grade 2, fevers resolve, and resume at one lower dose level[a,b]; check methotrexate level
Grade 4	Life-threatening consequences; urgent intervention indicated	Interrupt methotrexate until neutrophil count recovers to ≤ Grade 2, fevers resolve, and resume at one lower dose level[a,b]; check methotrexate level

a: If there is an interruption of methotrexate recommended, it is recommended to resume at one lower dose level (i.e., dose reduction from 20 mg → 15 mg). However, it is permitted to resume at two lower dose levels per investigator discretion (i.e., 20 mg → 10 mg). If a dose reduction is made, the dose of methotrexate should not be escalated, unless a patient has decreased by more than one dose level.

b: If patient is already on the lowest dose of methotrexate (10 mg), methotrexate should be discontinued and regorafenib may be continued per investigator discretion.

Anemia

CTCAE v. 4.03	**Definition**	**Methotrexate Dosing**
Grade 1	Hemoglobin (hb) <LLN-10.0 g/dl	Continue methotrexate
Grade 2	Hemoglobin (hb) <10.0-8.0 g/dl	Continue methotrexate and follow blood counts
Grade 3	Hemoglobin (hb) <8.0 g/dl; transfusion indicated	Interrupt methotrexate until Hb resolves to ≤ Grade 2, consider red blood cell transfusion, and resume at one lower dose level[a,b]
Grade 4	Life-threatening consequences; urgent intervention indicated	Interrupt methotrexate until Hb resolves to ≤ Grade 2, transfuse, and resume at one lower dose level[a,b]; check methotrexate level

a: If there is an interruption of methotrexate recommended to manage cytopenias, it is recommended to resume at one lower dose level (i.e., dose reduction from 20 mg → 15 mg). However, it is permitted to resume at two lower dose levels per investigator discretion (i.e., 20 mg → 10 mg). If a dose reduction is made for cytopenia, the dose of methotrexate should not be escalated, unless a patient has decreased more than one dose level.

b: If patient is already on the lowest dose of methotrexate (10 mg), methotrexate should be discontinued and regorafenib may be continued per investigator discretion.

Platelet count decreased (Thrombocytopenia)

CTCAE v. 4.03	Definition	Methotrexate Dosing
Grade 1	<LLN-75,000/mm^3	Continue methotrexate
Grade 2	<75,000-50,000/mm^3	Interrupt methotrexate until platelet count recovers to ≤ Grade 1 and resume at one lower dose level[a,b]
Grade 3	<50,000-25,000/mm^3	Interrupt methotrexate until platelet count recovers to ≤ Grade 1 and resume at one lower dose level[a,b]; check methotrexate level
Grade 4	<25,000/mm^3	Interrupt methotrexate until platelet count recovers to ≤ Grade 1 and resume at one lower dose level[a,b]; consider transfusing platelets for clinically significant bleeding; check methotrexate level

a: If there is an interruption of methotrexate recommended to manage cytopenias, it is recommended to resume at one lower dose level (i.e., dose reduction from 20 mg → 15 mg). However, it is permitted to resume at two lower dose levels per investigator discretion (i.e., 20 mg → 10 mg). If a dose reduction is made for cytopenia, the dose of methotrexate should not be escalated, unless a patient has decreased more than one dose level.

b: If patient is already on the lowest dose of methotrexate (10 mg), methotrexate should be discontinued and regorafenib may be continued per investigator discretion.

6.2.2.3 Methotrexate Overdose Management

Given low doses of oral methotrexate used in this protocol, leucovorin is very unlikely to be necessary. However, if there is a suspected methotrexate overdose, check methotrexate level promptly. Leucovorin is indicated to diminish the toxicity and counteract the effect of inadvertently administered overdosages of methotrexate per institutional guidelines, with leucovorin administration beginning as promptly as possible. As the time interval between methotrexate administration and leucovorin initiation increases, the effectiveness of leucovorin in counteracting toxicity decreases. Monitoring of the serum methotrexate concentration is essential in determining the optimal dose and duration of treatment with leucovorin.

In cases of massive overdosage, hydration and urinary alkalinization may be necessary to prevent the precipitation of methotrexate and/or its metabolites in the renal tubules. Generally speaking, neither hemodialysis nor peritoneal dialysis have been shown to improve methotrexate elimination. However, effective clearance of methotrexate has been reported with acute, intermittent hemodialysis using a high-flux dialyzer and consultation with nephrology may be

useful. Another option for toxic plasma methotrexate concentrations (>1 micromole per liter) is glucarpidase in patients with delayed methotrexate clearance due to impaired renal function. Leucovorin should not be administered within 2 hours before or after dose of glucarpidase. For 48 hours after glucarpidase administration, leucovorin dose is based on pre-glucarpidase concentrations.

6.2.3 Overlapping Toxicities Between Regorafenib and Methotrexate

Potential overlapping toxicities of methotrexate and regorafenib include dermatologic (rash), gastrointestinal (diarrhea, nausea, vomiting, stomatitis), and hepatitis.

6.2.3.1 Liver Function Abnormalities

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

Methotrexate has been described to cause cirrhosis of liver (0.1%), hepatic fibrosis (7%), acute hepatitis, other hepatotoxicity, liver failure, abnormal liver function tests (15%). Manifestations of hepatotoxicity or abnormal liver function tests may include elevations in AST/ALT, alkaline phosphatase, or bilirubin. Regorafenib has resulted in severe drug induced liver injury in 0.3% across clinical trials. Regorafenib is a UGT1A1 inhibitor. Mild, indirect (unconjugated) hyperbilirubinemia may occur in patients with Gilbert's syndrome.

Recommended Dose Modification/interruption for alanine aminotransferase and/or aspartate aminotransferase increases related to study treatment (regorafenib and/or methotrexate)[a]			
Increases in ASL/ALT (per NCI-CTCAE v 4.03)	**1st Occurrence**	**Restart**	**Recurrence**
AST and/or ALT ≤ 5x ULN (<Grade 3) - Baseline Grade 0→ Grade 1 (>ULN-3x ULN) - Baseline Grade 1 (>ULN-3x ULN)→ Grade 2 (>3-5x ULN)	Continue dosing of regorafenib and methotrexate, with weekly monitoring of liver function until transaminases return to ≤ 3x ULN (≤ Grade 1) or baseline.		
AST and/or ALT ≤ 5x ULN (<Grade 3) - Baseline Grade 0→ Grade 2 (>3.0-5.0 x ULN)	Interrupt dosing of regorafenib and methotrexate, with at least weekly monitoring of liver function until transaminases return to ≤ 3x ULN (≤ Grade 1).	Consider dose reducing regorafenib and methotrexate by one dose level, with at least weekly monitoring of liver function.[b]	
ALT and/or AST > 5 x ULN (≥ Grade 3)[a]	Interrupt dosing of regorafenib and methotrexate, with weekly monitoring until transaminases return to ≤ 3x ULN (≤ Grade 1) or baseline.	If the potential benefit of reinitiating regorafenib and methotrexate is considered to outweigh the risk of hepatotoxicity: reduce 1	Discontinue regorafenib and methotrexate

		dose level of both drugs and measure serum transaminases weekly for at least 4 weeks.[a] If methotrexate at lowest dose of 10 mg, it is permitted to only dose reduce regorafenib one dose level when considering resuming treatment.	
ALT and/or AST > 20 x ULN (≥ Grade 4)	Discontinue regorafenib and methotrexate and follow serum transaminases closely until resolution.		
ALT or AST >3x ULN (≥ Grade 2), with concomitant rise in bilirubin (>2x ULN), primarily direct bilirubin, if grade 0-1 baseline AST and/or ALT (≤ 3 x ULN) **ALT or AST >8x ULN, with concomitant rise in bilirubin (>2x ULN), primarily direct bilirubin, if grade 2 baseline AST and/or ALT (> 3- 5x ULN)**	Discontinue regorafenib and methotrexate and follow serum transaminases closely until resolution (at least weekly). Exception: subjects with Gilbert's syndrome who develop elevated transaminases should be managed as per the recommendations outlined above for ALT/AST elevations.		
a: It will not always clear whether regorafenib or methotrexate or both caused the hepatotoxicity. Regorafenib is more likely to contribute to severe hepatotoxicity than low dose methotrexate. After an adverse event of grade 3 hepatotoxicity, regorafenib should generally not be dose escalated. However, after one cycle of both regorafenib and methotrexate at reduced dose level, methotrexate can be escalated with weekly monitoring of liver function tests. b: Patients requiring dose reduction below regorafenib 80 mg should go off protocol therapy. The maximum daily dose of regorafenib is 120 mg.			

6.2.3.2 Prevention/Management Strategies for Diarrhea, Nausea, Vomiting, Mucositis/Stomatitis, and Maculo-Papular Rash

Both regorafenib and methotrexate have overlapping gastrointestinal toxicities. Many of these toxicities can be managed with appropriate supportive care.

NAUSEA, VOMITING, DIARRHEA DOSE MANAGEMENT:

- If the toxicity (diarrhea, nausea, vomiting) is severe (CTCAE v. 4.03 grade 3-4), interrupt regorafenib and methotrexate.
    - Grade 3: Dose reduction is not always necessary, although the dose should generally be reduced by one dose level for each study drug if it is refractory to maximum supportive care after 5 days. Upon improvement of toxicity to ≤ grade

2, can consider stepwise dose reduction when resuming treatment (i.e., reduce methotrexate by one dose level and continue regorafenib at same dose level or vice versa). In general, if dose reduction is performed, dose should not be re-escalated until the patient has completed one cycle at reduced dose without recurrence of the event at the discretion of the investigator.

- Grade 4: Upon improvement of toxicity to ≤ grade 2, dose reduction by one dose level of both drugs (or at least regorafenib if methotrexate at lowest 10 mg dose) is recommended and consideration should be given to discontinuing protocol treatment. The dose should not be re-escalated.

DIARRHEA: Diarrhea can be a common side effect of regorafenib (43-47%). Diarrhea can also occur with oral methotrexate (1-3%). 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 dose of loperamide is two tablets (4 mg) initially, then 2 mg after each unformed stool, not to exceed 16 mg/day for ≤2 days. Diphenoxylate (Lomotil) or tincture of opium are additional agents.

NAUSEA/VOMITING: Nausea and vomiting can occur with both regorafenib (17-20%) and methotrexate (>20%). Anti-emetics per institutional standards including but not limited to ondansetron, metoclopramide, prochlorperazine. The patient may also be pre-medicated with anti-emetics prior to dosing regorafenib and/or methotrexate.

MUCOSITIS/STOMATITIS: Mucositis/stomatitis can occur with both regorafenib (33-40%) and methotrexate (2-10%).

Recommended dose modification for mucositis or stomatitis[a]		
Grade of event (NCI-CTCAE v4.0)	**Occurrence**	**Suggested Dose Modification**
Grade 1	Any	Maintain dose level of regorafenib and methotrexate and immediately institute supportive measures for symptomatic relief
Grade 2	1st occurrence	Immediately institute supportive measures and consider decreasing dose by one dose level of regorafenib (if patient on 120 mg dose level) and methotrexate (if patient on >10 mg dose level) [b,d]. If no improvement or dose level maintained (i.e., particularly if patient is only on a 80 mg dose of regorafenib or 10 mg dose of methotrexate), interrupt regorafenib and methotrexate for a minimum of 7 days, until toxicity resolves to Grade 0 to 1
	No improvement within 7 days or 2nd occurrence	Interrupt regorafenib and methotrexate until toxicity resolves to Grade 0 to 1. When resuming treatment, treat at reduced dose level of regorafenib and methotrexate[b,d]. Can also consider stepwise approach when resuming treatment of first decreasing dose level of methotrexate and only decreasing dose level of regorafenib if required (or vice versa).
	3rd occurrence	Interrupt regorafenib and methotrexate until toxicity resolves to Grade 0 to 1. When resuming treatment, decrease dose by one dose level for regorafenib and methotrexate OR can consider continuing treatment at the lowest dose levels regorafenib (i.e., 80 mg) and methotrexate (i.e., 10 mg) if further dose reduction not possible.[b,d] **However, discontinuing therapy strongly encouraged.**
	4th occurrence	Discontinue therapy
Grade 3	1st occurrence	Institute supportive measures. Interrupt regorafenib and methotrexate for a minimum of 7 days until toxicity resolves to Grade 0 to 1.[c] When resuming regorafenib and methotrexate, decrease dose by one dose level for both regorafenib and methotrexate.[b,d] Can also carefully consider stepwise approach when resuming treatment of first decreasing dose level of methotrexate and only decreasing dose level of regorafenib if required (or vice versa).
	2nd occurrence	Interrupt regorafenib and methotrexate for a minimum of 7 days until toxicity resolves to Grade 0 to 1.[c] Can consider continuing treatment at the lowest dose levels of regorafenib (i.e., 80 mg) and methotrexate (i.e., 10 mg) if further dose reduction not possible.[b,d] **However, discontinuing therapy strongly encouraged.**
	3rd occurrence	Discontinue treatment.
Grade 4	Any occurrence	Discontinue treatment.

a. More conservative management is allowed if judged medically appropriate by the investigator.
b. If toxicity returns to Grade 0 to 1 after dose reduction, dose re-escalation is strongly discouraged. However, it is permitted at the discretion of the investigator after the patient has completed one cycle at reduced dose without recurrence of event.
c. If no recovery after a 28-day delay, treatment should be permanently discontinued unless patient is deriving clinical benefit per-investigator assessment and after discussion with overall study PI. (Refer to Section 4.2.4)
d. Patients requiring dose reduction below regorafenib 80 mg should go off protocol therapy. The maximum daily dose of regorafenib is 120 mg.

Treatment is per institutional standards and may include:

- Routine mouth care, including:
    - removal of dentures
    - oral rinses with weak solution of salt and baking soda (i.e., one-half teaspoon of salt and one teaspoon of baking soda in a quart of water) every four hours
    - avoidance of acidic, salty, dry foods
- Topical lidocaine solutions including "Magic Mouthwash", with variety of ingredients (examples listed below), swish and spit (or swallow depending on extent) four –six times daily
    - One consists of viscous lidocaine (50 mL of a 2 percent solution), sodium bicarbonate (100 mL of a 1 mEq/mL solution), and diphenhydramine (50 mL of a 12.5 mg/5 mL solution) in 500 mL normal saline (resultant fluid volume 700 mL) with instructions to swish and expectorate 10 to 15 mL four to six times per day.
    - Another type consists of a mixture of equal parts of viscous lidocaine, diphenhydramine, and magnesium aluminum hydroxide (Maalox).
- Steroid solution rinses (i.e., dexamethasone elixirs)
- Systemic opiates including morphine (oral route preferred)

MACULO-PAPULAR RASH

- Grade 3 AND symptomatic: Interrupt regorafenib and methotrexate and institute supportive measures. Dose reduction is not always necessary, although the dose should generally be reduced by one dose level for each study drug if it is refractory to supportive care after 7 days. Upon improvement of toxicity to ≤ grade 2, can consider stepwise dose reduction when resuming treatment (i.e., reduce methotrexate by one dose level and continue regorafenib at same dose level or vice versa). In general, if dose reduction is performed, dose should not be re-escalated until the patient has completed one cycle at reduced dose without recurrence of the event at the discretion of the investigator.
- Grade 3 AND asymptomatic: Continue regorafenib and methotrexate at current dose level and institute supportive measures.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Refer to Section 2.2 for detailed adverse events of regorafenib and methotrexate. Refer also to investigator's brochure for Regorafenib and generic package insert for Methotrexate.

7.2 Adverse Events

7.2.1 Definition of Adverse Event

Adverse events are defined as clinically significant untoward medical event that occurs during the course of the use of a study treatment, both laboratory and non-laboratory, irrespective of relatedness to study treatment.

Non-clinically significant lab values as determined by the treating investigator or designee are not considered adverse events. In more detail, elective surgical or medical procedures are not considered adverse events. Conditions that started before initiation of study therapy are considered baseline medical history and not adverse events. However, baseline conditions that worsen in frequency or severity after initiation of study therapy will be documented as adverse events.

7.2.2 Grading, Relationship, Expectedness of Adverse Events

Adverse events will be graded according to NCI-CTCAE v4.03. In general, it should be noted if the adverse event is a serious adverse event (Refer to Section 7.2.5). For all events, the relationship to treatment and the grade of the event should be determined by the investigator and as much as possible, the causality assessment should be done separately for each study treatment. For example, distinction should be made between relatedness to regorafenib, methotrexate, or both. In order to determine relatedness, the following factors may be useful: 1) temporal sequence from drug administration (i.e., the event occurs after study treatment is given); 2) recovery on drug discontinuation OR, recurrence on study treatment re-introduction (re-challenge): 3) underlying, concomitant, intercurrent diseases or concomitant medication or treatment; 4) the pharmacology and pharmacokinetics of the study treatment (i.e., absorption, distribution, metabolism and excretion). Usually, if an adverse event is not deemed related is because there is the existence of an alternative explanation.

Investigators should refer to the Safety Information section (2.2) along with the current IB for regorafenib, including the DCSI (development core safety information for the expected side effects of regorafenib. Overview listings of frequent events that have occurred so far in the clinical development are shown in the current IB. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions. Given current knowledge of regorafenib toxicities, adverse events of special interest may include: acute renal failure (NCI-CTCAE version 4.03 ≥ grade 3) or severe proteinuria (NCI-CTCAE version 4.03 ≥ grade 3); interstitial lung disease; acute cardiac failure; clinically significant bleeding (NCI-CTCAE version 4.03 ≥ grade 3); Stevens-Johnson Syndrome and erythema multiforme;

hepatic failure; reversible posterior leukoencephalopathy syndrome; and gastrointestinal perforation or fistula For methotrexate, investigators should refer to the Safety Information (Section 2.2) of the protocol. The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation.

CTCAE v4.03 (Refer to official guide for more details per each adverse event term):

- **Grade 1**: mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
- **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.).
- **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).
- **Grade 4**: life-threatening consequences; urgent intervention is indicated.
- **Grade 5**: death due to an AE.

7.2.3 Recording Adverse Events

Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). In general, in the source documentation, adverse events should include adverse event term (i.e., diagnosis), start/stop dates, action taken [i.e., study treatment (regorafenib or methotrexate or both) withdrawn, interrupted; dose reduced, not changed, increased or both)] and outcome (i.e., ongoing, resolving, resolved). All AEs regardless of seriousness or relatedness to either study drug will be documented from the start of therapy (C1D1) until the end of treatment visit. SAEs will also be documented from the time of informed consent until 30 days after the last dose of either study drug regardless of relatedness.

7.2.4 Serious Adverse Events (SAE)

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

- Fatal: Adverse event (AE) results in death.
- Life-threatening: AE placed patient at immediate risk of death. This classification does not refer to an event, which hypothetically might have caused death if it were more severe.
- Hospitalization: AE that required or prolonged inpatient hospitalization. Hospitalizations for elective medical treatments or surgical procedures are not considered SAEs.

Treatments planned before enrolment in study or routine check-ups are not considered SAEs. Admission to palliative unit or hospice facility is not considered hospitalization.

- If progressive disease leads to signs and symptoms that meet the criteria for an SAE (i.e., hospitalization, disability, death, or important medical event), the signs and symptoms should be reported as an SAE and not the underlying progressive disease.

- Disabling / incapacitating: AE that resulted in substantial and permanent disruption of a person's ability to conduct normal life's functions.
- Congenital anomaly / birth defect: AE outcome in a child or fetus of a patient exposed to the treatment regimen before conception or during pregnancy.
- Medically Significant: The AE did not meet any of the above criteria, but could have jeopardized the patient and might have required medical or surgical intervention to prevent one of the outcomes listed above as judged by the investigator.

All Serious Adverse Events (SAEs) will be tracked until resolution, or until 30 after the last dose of the study treatment, whichever is earlier.

7.2.5 Adverse Event Reporting

Throughout the course of the study, the Principal Investigator and study personnel agree to comply with the obligations of adverse event reporting as set forth below and with FDA regulations.

7.2.5.1 Serious Adverse Events, Stanford Reporting

SAEs CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences. (DSMC plan detailed in Section 11.2).

7.2.5.2 Bayer Reporting

Additionally, the Stanford University shall within 24 hours from the time of awareness of the event report to BAYER by fax or and/or email.

1) All Serious Adverse Events occurring after start of administration of BAYER product, independent of their causal relationship to the study treatment (Details Below)

   -- if linked to a serious adverse event, reports of misuse, abuse, overdose, medication error and other uses outside what is foreseen in the protocol, with respect to the study treatment

2) Reports of drug exposure via mother / father with and without adverse events, such as exposure during conception, pregnancy, childbirth and breastfeeding, including their outcome (if it is patient's partner, outcome is subject to partner's consent).

3) Communication concerning safety related information to regulatory authorities or ethics committees including but not limited to (*This is exempt from 24 hour reporting from time of awareness but should be reported in an expedited manner)*:

- Development Safety Update Reports / relevant parts of IND reports for the study;
- Other safety related reports, issues and queries that are either raised by or communicated to regulatory authorities or ethics committees (e.g., reportable non-serious cases)

4) If a patient dies during the trial or within 30 days of the end-of-treatment visit, the investigator will inform Bayer and record the cause of death if available (using the SAE Form).

Details for Reporting of Serious Adverse Events:

All SAEs should be reported to Bayer within 24 hours of the Principal Investigator's awareness and should include the following minimum information:

1. The name and contact information of the reporter
2. The name of the study drug(s)
3. A description of the reported SAE
4. A patient identified by one or more of the following:
    a. Patient initials
    b. Patient number
    c. Knowledge that a patient who experienced the adverse event exists
    d. Age
    e. Sex

5. An investigator assessment of study drug causality. A separate causality assessment should be provided for each study drug (i.e., regorafenib, methotrexate).

The Investigator/Sponsor may report SAEs using:

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

All reports shall be sent electronically to one of the following methods of contact:

Electronic Mailbox: [redacted]

Facsimile: [redacted]

Address: Global Pharmacovigilance - USA

Mail only BAYER U.S. LLC

[redacted]

[redacted]

Address: [redacted]

FDX or UPS only [redacted]

Reports for all Bayer products can also be phoned in via our Medical Communications Department

Phone: [redacted]

8. Planned Research Studies

Additional/alternative correlative exploratory research may be performed pending development of new technology and scientific advances. Archival tissue may be retrieved for additional correlative exploratory research. This may require the cooperation of other laboratories.

8.1 Blood Samples for Correlative Research Studies

8.1.1 CAPP-Seq Circulating Tumor DNA[1]

Blood will be drawn (goal of 20 to 50 mL whole blood at each timepoint) at the timepoints listed in Section 9 Study Calendar. The blood will be brought to the Diehn/Alizadeh labs as soon as possible, but always within 4 hours of collection, for further processing.

Blood samples will be hand-transported to the Diehn/Alizadeh lab within 4 hours of draw.

We do not anticipate shipping of specimens.

CAPP-Seq will be performed by Diehn/Alizadeh labs (Stanford Campus). CAPP-Seq result will be reported as circulating tumor DNA (ctDNA) either as a percentage of total circulating free DNA and/or ctDNA titer (a continuous variable in units pg/mL). The assay can identify less than 0.005% ctDNA, though many advanced NSCLC tumors have greater than 1% ctDNA, resulting in a wide dynamic range for analysis.

All specimens will be de-identified with a unique code identifier.

Tissue and blood samples will be retained and used for future research. For example, CAPP-Seq can also be performed on archival or fresh tissue.

8.1.2 Computational Simulation Model (Refer to Section 2.5.2)

We will send available de-identified genomic data to Cellworks via secure encrypted communication. In addition, de-identified genomic data from circulating tumor DNA may be shared for modeling.

9. STUDY CALENDAR*

	Screening[a] (Day -30 to Day 1)	Cycle 1				Cycle 2				Cycle 3*				Off Study[m]
		D1	D8	D15	D22	D1	D8	D15	D22	D1	D8	D15	D22	
Regorafenib[b]		X	X	X		X	X	X		X	X	X		
Methotrexate[c]		X	X	X		X	X	X		X	X	X		
Informed Consent	X													
Demographics	X													
Medical History	X													
Beta-hCG[d]	X													
EKG (as indicated)	X													
Concurrent Medications	X	X----------											----X	X
Physical Exam	X	X	X	X	X	X		X		X				X
Vital Signs[e]	X	X	X	X	X	X	X[e]	X		X				X
Height	X													
Weight	X	X				X				X				X
ECOG Performance Status	X	X				X				X				X
Pill Count		X	X	X	X	X				X				
Complete Blood Count with Differential	X	X	X	X	X	X		X		X				X
Serum Chemistry[f]	X	X	X	X	X	X		X		X				X
Random Urine Protein:Creatinine Ratio[g]	X													
Serum Amylase/Lipase and TSH/Free T4		X				X				X				
Pharmacokinetic Sample: Methotrexate[h]		X	X	X	X									
Circulating Tumor DNA (ctDNA)[i]		X		X		X				X				X
Radiologic Evaluation (CT)[j]	X	Radiologic measurements will be performed every 8 weeks ± 1 week for the first 1 year and then after 1 year, every 12 weeks ± 1 week												X
Brain MRI (or CT head)[k]	X	Brain MRI every 8 weeks ± 1 week if untreated brain metastases at baseline for the first 1 year and then after 1 year, every 12 weeks ± 1 week												X
Chest XRAY 2-View[l]		X				X				X				
Archival Tumor Tissue (if available)	X													
Adverse Event Evaluation		X ----------											----X	X

*Study treatment is given 3 weeks ON/ 1 week OFF of a 28-day cycle, ongoing until disease progression or intolerable toxicity.

1) WINDOWS:

- Unless otherwise specified, each cycle with its associated study-specific procedures has the following window: (-3 days/+7 days).
- Specific to Cycle 1, the required weekly visits have a ± 1 day window.
- If there is a clinic holiday or other unforeseen circumstance that prevents the performance of study specific procedures in the specified timeframe, a longer window should be approved by PI *prior* to scheduled visit with a clear reason stated.

2) DOSE INTERRUPTIONS:

- For dose interruptions, patients will continue on cycles as scheduled (e.g., patient will start Cycle 2 28 days after Cycle 1 regardless of any dose interruptions).
- If patient has dose interruption, protocol-specified procedures should be followed as feasible and applicable.
- If patient has a dose interruption during Cycle 1 but still requires *first* time intrapatient dose escalation of methotrexate, the weekly protocol-specified procedures specified during Cycle 1 should be followed *until the dose is maximally escalated* (i.e., weekly CBC+diff, CMP, vital signs, physical exam, and PK sampling).

a: Screening studies must be performed within 30 days of Cycle 1 D1. Exceptions include brain MRI, which can be performed within 45 days of Cycle 1 D1. Screening studies may be completed on Cycle 1 D1 as long as they are completed *prior* to the first dose of study treatment.

b: Regorafenib will begin on Cycle 1 D1 and be self-administered at 80 mg oral daily 3 weeks on/1 week off of a 28-day cycle. Regorafenib may be dose escalated to 120 mg oral daily (3 weeks on/1 week off of a 28-day cycle) beginning on Cycle 2 D1 according to strict criteria, including no evidence of significant drug-related toxicities (SDRT), defined as any event that would require a dose modification of regorafenib (i.e., interruption only, reduction only, or interruption followed by reduction) according to the toxicity guidelines/tables in Section 6.2.1 (regorafenib toxicities) and Section 6.2.3 (overlapping regorafenib and methotrexate toxicities). Refer to Section 5.1.1 for more details on self-administration of regorafenib and Section 6.2.1 for regorafenib dose modifications.

c: Methotrexate will begin on Cycle 1 D1 and is self-administered during the same weeks as regorafenib (i.e., 3 weeks on /1 week off). The initial starting dose during Cycle 1 Week 1 of methotrexate is 10 mg oral twice weekly. Methotrexate doses should be separated by at least 2 days. However, it is preferred that methotrexate dosing is separated by 3 days. Methotrexate will be dose escalated in the following manner as tolerated weekly: Cycle 1 Week 1: 10 mg oral twice weekly, Cycle 1 Week 2: 15 mg oral twice weekly, Cycle 1 Week 3: 20 mg oral twice weekly. Beginning in Cycle 2, methotrexate will be self-administered at doses ranging from 10 to 20 mg oral twice weekly (depending on tolerability), with at 2-3 days between doses. Refer to Section 5.1.2 for more details on self-administration of methotrexate and Section 6.2.2 for methotrexate dose modifications. When methotrexate is scheduled, it should be taken together with regorafenib (within 30 minutes, if feasible) during Cycle 1 for ease of pharmacokinetic studies. Beginning in Cycle 2, patients may take regorafenib and methotrexate at different times of day.

d: Urine or serum pregnancy test (women of childbearing potential) must be performed and confirmed negative prior to self-administration of study treatment on Cycle 1 D1.

e: Patient needs to document blood pressure locally anytime during the week of Cycle 2 D8 (i.e., week 2 cycle 2) and only report to investigator team if blood pressure is >140/90.

f: Serum chemistry includes electrolytes (bicarbonate, chloride, potassium, sodium, calcium) along with phosphorus; liver function including albumin, alkaline phosphatase, total bilirubin, total protein, SGOT[AST], and SGPT[ALT]; renal function including BUN and creatinine; and glucose.

g: We will check urine protein:creatinine ratio at screening as a baseline measure for comparison in the event of subsequent development of proteinuria, but not for eligibility. We will periodically assess ratio throughout the study depending on patient's symptoms.

h: Methotrexate: PK blood samples will be measured during Cycle 1 *prior* to self-administration of study treatment (i.e., baseline for Cycle 1 D1, trough) and 1 hour *post*-self administration (±15 minutes) (i.e., Cmax) of methotrexate during Weeks 1, 2, and 3 of cycle 1. Cycle 1 Week 4 will only include a single methotrexate level. Methotrexate will be taken within 30 minutes of regorafenib on days of PK sampling.

i: Blood samples for ctDNA will be collected at the specified timepoints in study calendar and then only at a timepoint that is closest to time of radiologic imaging. Refer to Section 8.1 for further details on procedures for sample collection.

j. Computed tomography (CT) chest ± abdomen/pelvis with contrast (unless contraindicated) to image all known baseline sites of disease. To the extent possible, the same imaging modality should be used for follow-up studies. If CT is not best suited for following the patient's cancer, any study that conforms to RECIST 1.1 criteria is allowed. **Imaging will be performed every 8 weeks ± 1 week irrespective of dose interruptions.** If the patient is on study for > 1 year, imaging frequency can be decreased to as infrequent as every 12 weeks (± 1 week). For patients who come off study for toxicity or any other reason, obtaining interval imaging (i.e., unscheduled scan) will be strongly encouraged to monitor response.

k: Brain MRI (preferred over CT head) will be performed every 8 week ± 1 week if patient has untreated brain metastases at baseline; **imaging will be performed every 8 weeks ± 1 week irrespective of dose interruptions.** If the patient is on study for > 1 year, imaging frequency can be decreased to as infrequent as every 12 weeks (± 1 week). If patient has history of treated brain metastases or no history of brain metastases, frequency of brain imaging follow-up is per the investigator discretion, although it is encouraged

	to perform the imaging every 8-16 weeks (± 1 week). Imaging the brain as well as systemic sites of disease at the same time is strongly encouraged.
l:	Chest X-ray (2 view) will be performed once per cycle for patients with a history of pleural effusion because methotrexate can accumulate in pleural effusions. For patients with adequate chest imaging with re-staging CT prior to cycle, it is permitted to omit Chest X-ray imaging. C1D1 chest X-ray may be omitted by the treating physician if baseline chest imaging (completed within 30 days of C1D1) is deemed adequate.
m:	Off-study evaluation. There is no requirement to repeat imaging at off-study visit if patient comes off for adverse event, although it is strongly encouraged.

10. MEASUREMENTS

10.1 Primary Outcome Measurement

To determine the progression-free survival (PFS) of the oral combination of regorafenib and methotrexate for patients with pre-treated metastatic *KRAS* mutated non-squamous NSCLC

- **Title**: Progression-free survival
- **Time Frame**: See Study Calendar (Section 9)
- **Safety Issue**: This outcome is not a safety issue.
- **Relevant Subset**: All patients treated with at least one dose of regorafenib and methotrexate.
- **Measurement Definition**
    - Progression free survival is measured from the time of first study treatment until objective tumor progression as determined by RECIST 1.1 criteria or death from any cause, whichever comes earlier. Refer to Section 12.4 for censoring rules.
- **Measurement Methods**
    - RECIST version 1.1 criteria (Appendix B)[121] Investigators on the trial will perform tumor measurements based on RECIST v1.1 criteria. There will be no independent review.
    - Death will be indicated as occurred or not occurred. Study personnel may use public records to check for mortality for any patients, especially those considered lost-to-follow-up.
    - Measurement Timepoints
    - Refer to Study Calendar Section 9

10.2 Secondary Outcome Measurements

10.2.1 To determine the objective response rate using RECIST v1.1 criteria of the oral combination of regorafenib and methotrexate for patients with pre-treated metastatic *KRAS* mutated non-squamous NSCLC

- Relevant Subset: All patients treated with at least one dose of regorafenib and methotrexate.

- Measurement Definition
    - Proportion of complete responses + partial responses as determined by RECIST v1.1 criteria
- Measurement Methods
    - RECIST version 1.1 criteria (Appendix B)
- Measurement Timepoints
    - Refer to Study Calendar Section 9

10.2.2 To determine the <u>disease control rate at 8 weeks</u> using RECIST v1.1 criteria of the oral combination of regorafenib and methotrexate for patients with pre-treated metastatic *KRAS* mutated non-squamous NSCLC

- Relevant Subset: All patients treated with at least one dose of regorafenib and methotrexate.
- Measurement Definition
    - Proportion of complete responses + partial responses + stable disease as determined by RECIST v1.1 criteria at 8 weeks
- Measurement Methods
    - RECIST version 1.1 criteria (Appendix B)
- Measurement Timepoints
    - Refer to Study Calendar Section 9

10.2.3 To determine the <u>safety</u> using CTCAE v4.03 criteria of the oral combination of regorafenib and methotrexate for patients with pre-treated metastatic *KRAS* mutated non-squamous NSCLC

- Relevant Subset: All patients treated with at least one dose of regorafenib and methotrexate.
- Measurement Definition
    - Incidence of adverse events
- Measurement Methods
    - Common Terminology Criteria for Adverse Events (CTCAE v4.03)
- Measurement Timepoints
    - From time of first study treatment through the end-of-treatment visit

10.2.4 To determine the <u>pharmacokinetic parameters of methotrexate</u> when combined with regorafenib (i.e., Cmax)

- Relevant Subset: For trough level, all patients with at least one evaluable baseline pharmacokinetic sample and one follow-up trough pharmacokinetic sample treated with at least one dose of regorafenib and methotrexate. For Cmax level, all patients with at least one evaluable pharmacokinetic sample at the time that approximates the Cmax (1 hour-post dose) treated with at least one dose of regorafenib and methotrexate.

- Measurement Definition
  - Concentration of methotrexate (measured generally as micromolar/L)
    - Cmax is measured one hour (±15 minutes) after ingestion of regorafenib and methotrexate
    - Trough concentrations are measured at least ~2 days after a dose of oral methotrexate
- Measurement Methods
  - Methotrexate assay is a fluorescent polarization immunoassay
- Measurement Timepoints
    - Refer to Study Calendar Section 9

10.3 Exploratory Endpoint

10.3.1 To correlate *KRAS* circulating tumor DNA (ctDNA) using CAPP-Seq[1] pre-treatment and throughout treatment with clinical outcome measures, including response rate and progression-free survival

- *Relevant Subset*: All patients treated with at least one dose of regorafenib and methotrexate, have at least one evaluable ctDNA measurement, and at least one follow-up imaging.
- *Measurement Definition*
  - ctDNA measured as percentage of total circulating free DNA and/or as ctDNA titer (continuous variable in units pg/mL)
- Measurement Methods
  - ctDNA levels measured using CAncer Personalized Profiling by deep Sequencing (CAPP-Seq)
  - Radiographic tumor assessments using RECIST v1.1 criteria (Appendix B)[121]
  - See primary outcome and secondary outcome definitions of objective response rate and progression-free survival
- Measurement Timepoints
  - Refer to Study Calendar Section 9

11. REGULATORY CONSIDERATIONS

This protocol was submitted to FDA as IND 136973, and determined to be IND-exempt. FDA noted that an eligibility criteria specifying the use of non-approved diagnostic tests, eg, the *KRAS*-mutation test, without an IDE submission to the FDA requires the investigator's judgment that the use is non-significance risk (NSR), and the IRB's agreement as indicated by IRB approval of the protocol.

Patients with non-squamous non-small cell lung cancer undergo molecular testing of their tumor tissue and/or circulating tumor DNA at the time of diagnosis and/or at variable times throughout their disease and treatment course as part of their routine clinical care. This testing generally include testing of the KRAS gene. There are many platforms available for molecular testing. At

this time, none of these are formally approved by the FDA to make treatment determinations for KRAS-mutated lung cancer patients. KRAS mutation testing on tissue includes but is not limited to the following CLIA certified assays: Stanford in-house assay STAMP (Solid Tumor Actionable Mutation Panel) and Foundation Medicine. KRAS mutation testing on blood (ie, circulating tumor DNA) includes but is not limited to the following CLIA certified assays: Foundation Medicine, Guardant 360, Biodesix. Patients will enroll in the study if they have a known KRAS mutation as by detected by one of the aforementioned assays and/or similar assays that were performed as part of their routine clinical care. This study usage is considered by the investigators to be of non-significant risk.

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. When necessary, an extension, amendment or renewal of the IRB approval should be obtained and also forwarded to Bayer. The Protocol Director will disseminate the protocol amendment information to all participating investigators. Modifications to the study protocol should be discussed and agreed on by Bayer when possible unless a modification is required to eliminate an immediate hazard(s) to the trial patient .

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. **The DSMC will meet after the first 5 patients have completed the DLT period, and again after 10 patients have completed the DLT period.** The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the OnCore database system and will be maintained by the research team. CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

This is a prospective open-label non-randomized single-arm phase II study of oral regorafenib in combination with oral methotrexate in metastatic KRAS mutated non-squamous NSCLC patients who have received at least 1 systemic therapy, with a primary endpoint of progression free survival (PFS).

12.1.1 Randomization

This is a single arm study and randomization will not be performed.

12.2 Interim Analyses - "safety stopping rule"

Given the combination of oral regorafenib and oral methotrexate has never been studied together but each individual compound has been studied extensively, we will monitor the number of patients with dose limiting toxicity (DLT), defined in Section 6.1, **based on repeated significance testing**, to ensure there is not excessive toxicity. **If 2 or more of the first 6 patients have a DLT, or if 3 or more out of the first 12 patients have a DLT,** we will suspend enrollment to the trial and reconsider the dosing strategy (including dose and schedule of regorafenib and/or methotrexate). Finally, if at the final analysis, 3 or more patients have DLT, we will take this as an indication that the regimen may be too toxic and additional dose and schedule may be considered for future development of the combination. The table below shows the probability of stopping early (pstop) and the probability of declaring the regimen too toxic at an interim look or at the final analysis (pcross), for various value of the probability of DLT (ptox); the expected sample size under each scenario is also shown (ess).

ptox	pcross	pstop	ess
0.05	0.012	0.004	18.0
0.10	0.102	0.033	17.7
0.14	0.243	0.089	17.2
0.18	0.417	0.175	16.5
0.22	0.588	0.284	15.5
0.26	0.731	0.406	14.5
0.30	0.838	0.530	13.3

12.3 Descriptive Statistics and Exploratory Data Analysis

Demographic variables (such as age) and baseline tumor and treatment characteristics will be summarized by descriptive statistics (i.e., proportions for binary or categorical variables; mean, standard deviation and range for symmetrically distributed continuous characteristics; medians, quartiles and ranges for characteristics with skewed distributions). The correlation of dichotomies will be assessed using Fisher's exact test. The correlation of continuous or ordinal variables with a dichotomy will be made using Wilcoxon rank sum test. The correlations of two continuous variables will be performed using Kendall correlation. Time-to-event categories will

be calculated with Kaplan-Meier method. Correlation involving a time-to-event variable with a dichotomy will be made using the log-rank test and displayed using Kaplan-Meier. All analyses will use a two-sided significance level of 5%. No adjustment for multiplicity will be carried out.

12.4 Primary Analysis

The primary analysis is to determine the progression-free survival (PFS) of the oral combination of regorafenib and methotrexate for patients with pre-treated metastatic *KRAS* mutated non-squamous NSCLC. Progression-free survival is measured from the time of first study treatment until objective tumor progression as determined by RECIST 1.1 criteria or death from any cause, whichever comes earlier. The primary analysis population will include all patients treated with at least one dose of regorafenib and methotrexate. Progression-free survival will be calculated using the Kaplan-Meier method along with 95% confidence interval.

For analysis purposes:

- 1) **Recorded progression date** is the date of the protocol-scheduled clinic visit closest after all protocol-scheduled radiological assessments and/or physical exam assessments (which collectively document progression) have been done.
- 2) **Recorded censoring date** is defined in patients with no documented progression before data cutoff or dropout as the last date on which progression status was adequately assessed (i.e., date of the protocol-scheduled clinic visit corresponding to the last adequate radiological assessments and/or physical exam assessment that showed no documented progression). For instance, patients going off-study for undocumented clinical progression, treatment toxicity or other reason, change of cancer treatment, or decreasing performance status will be censored at the last adequate tumor assessment.

For the primary analysis, progression events and censoring events are detailed below.

Situation	Date of Progression or Censoring	Outcome
No baseline tumor assessment	First day of study treatment	Censored
Progression documented between protocol scheduled visits/radiological assessments	Date of next scheduled protocol-visit	**Progressed**
No progression	Date of last visit with adequate assessment	Censored
Treatment discontinuation for undocumented progression	Date of last visit with adequate assessment	Censored
Treatment discontinuation for toxicity or other reason	Date of last visit with adequate assessment	Censored
New anticancer treatment started	Date of last visit with adequate assessment	Censored
Death before first PD assessment	Date of death	**Progressed**
Death between adequate assessment visits	Date of death	**Progressed**
Death or progression after more than one missed visit	Date of last visit with adequate assessment	Censored

Sensitivity analyses for primary endpoint of PFS may be performed:

- 1) PFS may be calculated with the investigator's claim of clinical progression, noted as a progression event, with date of progression at the protocol-scheduled visit (or next protocol-scheduled visit if in between visits).
- 2) PFS may be calculated with "dropouts" (i.e., clinical progression, treatment discontinuation for toxicity or other event, change of cancer treatment, or decreasing performance status) as progression events, with date of progression at the protocol-scheduled visit (or next protocol-scheduled visit if in between visits).

12.5 Secondary Analysis

- **Objective response rate (ORR):** The ORR will be assessed using RECIST v1.1 criteria. This analysis will include all patients treated with at least one dose of regorafenib and methotrexate. Percent responding will be tabulated by response category (complete response, partial response, stable disease, and progressive disease) and summarized as responding (CR+PR) or not, with exact 95% confidence intervals based on a binomial distribution. Both confirmed and unconfirmed ORR will be reported per RECIST v1.1 criteria.
- **Disease control rate at 8 weeks (DCR@8 weeks):** The DCR@8 weeks will be assessed using RECIST v1.1 criteria. This analysis will include all patients treated with at least one dose of regorafenib and methotrexate. Percent responding will be tabulated by response category (complete response, partial response, stable disease, and progressive

disease) and summarized as having disease control (CR+PR+SD) or not at the week 8 (±1 week) follow-up scan, with exact 95% confidence intervals.

- **Safety:** Safety will be assessed using Common Terminology Criteria for Adverse Events (CTCAE v4.03). This analysis will include all patients treated with at least one dose of regorafenib and methotrexate. Adverse events will be tabulated by category and severity. Dose limiting toxicities will also be tabulated.
- **Pharmacokinetic parameters of methotrexate when combined with regorafenib (i.e., trough and Cmax).** PK for methotrexate will be assessed by a fluorescent polarization immunoassay. For analysis of methotrexate trough levels, patients with at least one evaluable baseline pharmacokinetic sample and one follow-up trough pharmacokinetic sample treated with at least one dose of regorafenib and methotrexate will be included. For analysis of methotrexate Cmax levels, all patients with at least one evaluable Cmax pharmacokinetic sample treated with at least one dose of regorafenib and methotrexate will be included. Descriptive statistics will be used.

12.5.1 Secondary Analysis Population

In general, except where noted elsewhere in statistical plan:

- Efficacy population: All patients enrolled on study treated with at least one dose of study treatment.
- Safety Population: All patients treated with at least one dose of study treatment.

12.6 Sample Size

Sample size is 22 patients, with at least 18 evaluable for PFS.

12.6.1 Accrual Estimates

We estimate accrual of 2-3 patients per month based on prior accrual to *KRAS* specific NSCLC trials in the second line setting and beyond. If accrual falls short of expectations, we will enhance our efforts to discuss the trial with our patients and will consider advertising to local community oncologists.

12.6.2 Sample Size Justification

Our null hypothesis is that the median PFS is 2 months. We wish to have 80% power at the alternative hypothesis of a median PFS of 8 months. Assuming a horizon for PFS of 2.0 months and exponential PFS, the probability of being PFS-free at that time is 50.0% under the null hypothesis and 8% under the alternative hypothesis. With 18 informative patients, we would have approximately 80% power to reject the null hypothesis at a one-sided significance level of 5%.

12.6.3 Effect Size Justification

The null hypothesis for this study is based on two major references: 1) Median PFS with regorafenib alone in pre-treated metastatic NSCLC (*KRAS* mutation not specified) was 84 days (approximately 2-3 months), and 2) Median PFS with a similar agent to regorafenib, sorafenib, in pre-treated metastatic *KRAS* mutated NSCLC was 2.3 months (95% CI 1.6-3.0)[2]. A clinically meaningful alternative hypothesis would be 8 months.

12.7 Criteria for Future Studies

If the study meets its primary endpoint with acceptable toxicity profile, future studies with the combination of oral regorafenib and methotrexate may be pursued in KRAS driven cancers, including but not limited to lung cancer.

12.8 Exploratory Objective

The exploratory objective is to correlate *KRAS* circulating tumor DNA (ctDNA) using CAPP-Seq[1] pre-treatment and throughout treatment with clinical outcome measures such as objective response rate and progression-free survival. ctDNA levels will be measured using CAncer Personalized Profiling by deep Sequencing (CAPP-Seq). This exploratory analysis will be identified retrospectively in terms of patients and timepoints selected. The analysis may include patients treated with at least one dose of regorafenib and methotrexate, have at least one evaluable ctDNA measurement, and at least one follow-up imaging. ctDNA may be measured as percentage of total circulating free DNA and/or as ctDNA titer (continuous variable in units pg/mL). Suggested analyses are outlined below. Changes in ctDNA may be analyzed by transforming absolute value of ctDNA (pg/ml) using base-2 logarithm base-2. The distribution of change will be summarized as percent change from baseline in a waterfall plot (cumulative distribution function). The correlation of percent ctDNA (and/or DNA titer) with sum of longest diameter of tumor target lesions (continuous variable in mm or cm, RECIST 1.1) will be calculated using Kendall correlation. The correlation of ctDNA (and/or DNA titer) with overall response category (RECIST v1.1) will be calculated using the Wilcoxon rank sum test. Significance of the change in the biomarker (ctDNA) will be assessed using a paired t-test; however, if normal quantile plots indicate a departure from normality, the assessment will be made using the Wilcoxon signed-rank test. Baseline ctDNA will be correlated with PFS by means of a Cox regression model (ctDNA on log scale). Changes in ctDNA will be correlated with PFS by means of a Cox model with ctDNA as a time-varying covariate and baseline ctDNA as an adjustment variable. P-values will be reported without correction for multiple testing, however the number of statistical tests planned and/or performed will be included in reports of this study.

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APPENDICES

Appendix A: ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

*As published in Am. J Clin Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix B: RECIST Version 1.1 Criteria
Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009 Jan;45(2):228-47.

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

Measurable:

- Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
  - 10 mm by CT, MRI or PET scan (slice thickness no greater than 5 mm)
  - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
  - 20 mm by chest X-ray
- Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT, MRI or PET scan (slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable:

- All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging

Guidance:

- 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 should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.
- Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥ 10mm diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.
- Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.
- CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less.
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement.
- Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised.
- Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.
- Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol.

Tumor Response Evaluation

Baseline documentation of 'target' and 'non-target' lesions

- When more than one measurable lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

- A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Systemic Response Criteria

Complete Response (CR) requires ALL of the following:

- Disappearance of all target and non-target lesions
- All pathological lymph nodes, whether target or non-target, must have reduction in short axis to < 10 mm.
- No new lesion

Partial Response (PR) requires ALL of the following:

- At least 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters.
- No unequivocal progression of existing non-target lesions
- No new lesion

Progression of Disease (PD) requires ANY of the following:

- At least 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.
- Unequivocal progression of existing non-target lesions
- Appearance of one or more new lesions

Stable Disease (SD) requires ALL of the following:

- Not CR
- Not PR

Appendix C: CYP3A4 Inhibitors/Inducers

References:

- Huang, S. Drug Development and Drug Interactions. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm *Accessed [01-22-2017]*.
    - ***Please see this site for a comprehensive and up to date list.***

Definitions:
Strong, moderate, and weak inhibitors are drugs that increase the AUC of sensitive index substrates of a given metabolic pathway ≥5-fold, ≥2 to <5-fold, and ≥1.25 to <2-fold, respectively.

CYP3A4 INHIBITORS

Enzyme	Strong Inhibitors	Moderate inhibitors	Weak inhibitors
CYP3A	boceprevir, cobicistat, conivaptan, danoprevir and ritonavir, elvitegravir and ritonavir, grapefruit juice, indinavir and ritonavir, itraconazole, ketoconazole, lopinavir and ritonavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, troleandomycin, voriconazole, clarithromycin, diltiazem, idelalisib, nefazodone, nelfinavir, suboxone	aprepitant, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, verapamil	chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor

Note, most p-GP inhibitors also inhibit CYP3A4 to some degree: amiodarone, carvedilol, clarithromycin, dronedarone, itraconazole, lapatinib, lopinavir and ritonavir, propafenone, quinidine, ranolazine, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, verapamil.

CYP3A4 INDUCERS

Definitions:
Strong, moderate, and weak inducers are drugs that decreases the AUC of sensitive index substrates of a given metabolic pathway by ≥80%, ≥50% to <80%, and ≥20% to <50%, respectively.

CYP Enzymes	Strong Inducers	Moderate Inducers	Weak Inducers
CYP3A	carbamazepine, enzalutamide, mitotane, phenytoin, phenobarbital, rifampin, St. John's Wort	bosentan, efavirenz, etravirine, modafinil	armodafinil, rufinamide

Appendix D: Examples of Clinical Substrates for the Cytochrome Enzymes CYP2B6, CYP2C8, and CYP2C9

Please see these sites for a comprehensive and up to date list

References:

- Huang, S. Drug Development and Drug Interactions. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm *Accessed [01-22-2017]*.

- *Flockhart DA. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007). "http://medicine.iupui.edu/clinpharm/ddis/clinical-table/" Accessed [01-22-2017]*.

Cytochrome Enzyme	Substrates
CYP2B6	Artemisinin, bupropion, cyclophosphamide, efavirenz, ifosfhamide, ketamine, meperidine, methadone, nevirapine, propafol, selegiline, sorafenib
CYP2C8	Amodiaquine, cerivastatin, montelukast, paclitaxel, pioglitazone, repaglinide, rosiglitazone, sorafenib, torsemide
CYP2C9	***NSAIDS***: diclofenac, ibuprofen, lornoxicam, meloxicam, S-naproxen, piroxicam, suprofen ***Oral hypoglycemic agents***: glipizide, tolbutamide ***Angiotensin II Blockers***: irbesartan, losartan ***Sulfonylureas***: glyburide, glibenclamide, glipizide, glimepiride, tolbutamide ***Others***: amitriptyline, celecoxib, fluoxetine, fluvastatin, glyburide, nateglinide, phenytoin, rosiglitazone, tamoxifen, torsemide, valproic acid, S-warfarin, zakirlukast

Appendix E: Participant Eligibility Checklist3.1 Inclusion Criteria

In order to be eligible for participation in this trial, the patient must meet *ALL* of the following criteria (i.e., mark "yes" or "N/A" to all criteria):

YES	NO	INCLUSION CRITERIA	SUPPORTING DOCUMENTATION
		1. Histologic or cytologic confirmed diagnosis of non-squamous non-small cell lung cancer that is recurrent or metastatic. Adenosquamous is allowed provided the patient has confirmed adenocarcinoma component.	
		2. Documentation of pathogenic KRAS mutation	
		3. Previous receipt of at least one systemic therapy for recurrent or metastatic disease **OR** previous receipt of adjuvant systemic therapy within 6 months of enrollment. There is no limit on number of prior therapies allowed.	
		4. Prior systemic therapy must be completed at least 2 weeks prior to study treatment, with either improvement of clinically significant treatment-related toxicities to grade 0 to 1 **OR** stabilized to a new baseline.	
		5. Previously treated **OR** asymptomatic non-progressing < 1 cm untreated brain metastases are allowed	
		6. Measurable disease based on RECIST version 1.1 criteria (Appendix B)	
		7. Ability to understand and the willingness to sign a written informed consent document	
		8. Age ≥ 18 years-old	
		9. ECOG performance status of 0 or 1 (Appendix A)	
		10. Adequate bone marrow, liver and renal function as assessed by the following laboratory requirements: a. Absolute neutrophil count (ANC) ≥ 1500/mm^3 b. Platelet count ≥ 100,000 /mm^3 c. Hemoglobin (Hb) ≥ 9 g/dL d. Serum creatinine ≤ 1.5x upper limit of normal (ULN) **OR** calculated (Cockcroft-Gault formula) *or* measured creatinine clearance ≥ 50 mL/min for patients with creatinine levels > 1.5x ULN e. Total bilirubin ≤ 1.5x ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5x ULN f. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3x ULN (≤ 5x ULN for patients with liver involvement of their cancer)	
		11. Must be able to swallow and retain oral medication.	

		12. Women patients of childbearing potential and men patients with women partners of childbearing potential must agree to use adequate contraception or agree to abstain from heterosexual activity beginning at the time of signing informed consent until at least 3 months after the last dose of study treatment. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not considered childbearing.	

3.2 Exclusion Criteria

In order to be eligible for participation in this trial, the patient must *NOT* meet the following criteria (i.e., mark "no" or "N/A" to all criteria):

YES	NO	EXCLUSION CRITERIA	SUPPORTING DOCUMENTATION
		1. Previously treated with regorafenib	
		2. Known allergy to regorafenib or methotrexate	
		3. Currently receiving another systemic standard or investigational anti-cancer therapy. Prior investigational therapy must be completed within 4 half-lives (if known) or 2 weeks, whichever is longer. The maximal washout of investigational therapy will not exceed 4 weeks prior to study treatment. Bone medications such as bisphosphonates and RANK ligand inhibitors permitted.	
		4. Leptomeningeal disease as documented by CSF cytology.	
		5. Clinically significant cardiovascular-related disease including: a. Uncontrolled hypertension (systolic pressure >150 mm Hg or diastolic pressure > 90 mm Hg on *repeated* measurements that does not resolve prior to study treatment on C1D1 despite optimal medical management b. Congestive heart failure – New York Heart Association (NYHA) Class III or greater c. Active coronary artery disease (i.e., unstable or new onset angina within 3 months of study treatment; myocardial infarction within 6 months of study treatment) d. Clinically significant cardiac arrhythmias other than atrial flutter/fibrillation e. Stroke, including transient ischemic attacks, within 6 months of study treatment f. Other clinically significant arterial events, *except* for controlled asymptomatic pulmonary embolism, within 6 months of study treatment	

		6. Clinically significant hemorrhage or bleeding event within 1 month of study treatment	
		7. Uncontrolled symptomatic pleural effusion or ascites	
		8. Known active additional malignancy that is undergoing or expected to undergo systemic treatment during duration of study participation.	
		9. Known history of human immunodeficiency virus (HIV) infection or known current active hepatitis B (i.e., Hep B DNA positive in prior 3 months) or hepatitis C infection (i.e., Hep C RNA positive in prior 3 months, with the exception of patients who have completed curative therapy and are Hep C RNA negative on retest).	
		10. Major surgical procedure (e.g., involving the opening of a major body cavity) within 4 weeks of study treatment. This does *not* apply to low-risk procedures (i.e., thoracentesis; paracentesis; chest tube/PleurX catheter placement; line placement; needle biopsy of tumor; and bronchoscopy).	
		11. Presence of a clinically significant non-healing wound or non-healing ulcer	
		12. Concomitant therapy required at time of first dose of study treatment, including: a. Strong CYP3A4 inhibitors and CYP3A4 inducers (Appendix C) b. Regular use of NSAIDs, proton pump inhibitors, and probenecid	
		13. Women who are pregnant or breast-feeding.	
		14. Any condition which, in the investigator's opinion, including substance abuse, medical, psychological or social conditions that makes the patient unsuitable for trial participation or may interfere with the patient's participation in the study.	

By signing this form of this trial, I verify that this patient is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

Appendix F: Cellworks Modeling Overview

The methodology entails predictive simulation via computational biology modeling to create virtual tumor models based on tumor genomics to determine disease characteristics and identify therapy responses. Computational biology modeling (CBM) was previously outlined and published in studies of glioblastoma multiforme, multiple myeloma, myelodysplastic syndrome, acute myeloid leukemia and myeloproliferative neoplasm [1–4]. Based on over 10,000 published PubMed references, this model incorporates signaling, metabolic and epigenetic pathway interactions important in cancer including growth factor signaling cascades, cytokines, chemokines, mTOR regulators, cell cycle regulators, oxidative and ER stress responses, cancer metabolism, autophagy and proteosomal degradation, DNA damage repair, apoptosis cascades and p53 signaling to predict a patient's response to a single drug or a combination of drugs. The CBM cancer network includes comprehensive coverage of the kinome, transcriptome, proteome, and metabolome.

Virtual lung cancer models in CBM are created via genomic profiling information of KRAS positive NSCLC cell lines. Genomic information of 5 NSCLC cell lines [TABLE 1] were taken from publicly accessible cBioPortal and COSMIC databases. For each NSCLC cell line available genomic information (i.e., cytogenetic abnormalities and DNA sequencing data) was entered into the CBM, which utilized PubMed, STRING, HumanNet, and PathwayCommons online resources to determine whether the cell lines gene mutation generated an activated or inactivated protein. Unique NSCLC cell line models with their individualized disease network maps of dysregulated activated and inactivated protein pathways were created via simulation. The genomic aberration information derived from cytogenetics and sequencing data was used to create a list of genes with mutations and CNV in the cell lines' genome. The genes found on the loci of the affected regions of the chromosomes were extracted from the human reference genome at ENSEMBL, and the complete list of genes is matched with the Cancer Technology Network to determine the subset of genes to be represented in the model.

Key assumptions are made when representing the aberrations input to the model network: gain of function or amplification of tumor promoter genes and loss of tumor suppressor genes dynamically creates the neoplastic disease state upon simulation [5]. Gene variants with therapeutic implications are searched using public domain to determine each mutation's functionality, represented as either a loss or gain of function. However, genes with mutations of unknown significance are parsed through a suite of variant calling algorithms to determine if the mutation is deleterious. For a deleterious mutation of unknown significance, a tumor promoter gene is assumed to have gain of function while a tumor suppressor gene is assumed to have loss of function at the protein activity level. Frameshift and nonsense mutations are assumed to cause a loss of gene function [6].

For CNV interpretation, amplifications are represented as an increase of gene expression while deletions are represented as knockdown of gene expression. Additionally, amplifications of tumor suppressor genes have lower contribution to the disease when compared to amplification of tumor promoter genes. A deletion of tumor suppressor genes has a higher dominance in the disease network when compared to deletion of tumor promoter genes.

Dynamic disease models of the NSCLC cell line profiles with their individualized protein network maps were created for each KRAS mutant NSCLC cell line based on their mutanome data. A digital drug library of 67 FDA approved drugs including Methotrexate and Regorafenib was created for CBM by programming each individual agent's mechanism of action, as well as effects on specific protein targets and pathways determined from published literature. Using the drug models, virtual applications of these drugs were applied to each cell line model individually, and in combination, via computer simulation, amounting to thousands of simulation studies for testing multiple drugs in combination and at different doses. The efficacy of each therapy in the genetically varying NSCLC KRAS cell lines was measured as a function of relative growth inhibition score – the degree to which crucial cancer signaling pathways and phenotypes were repressed.

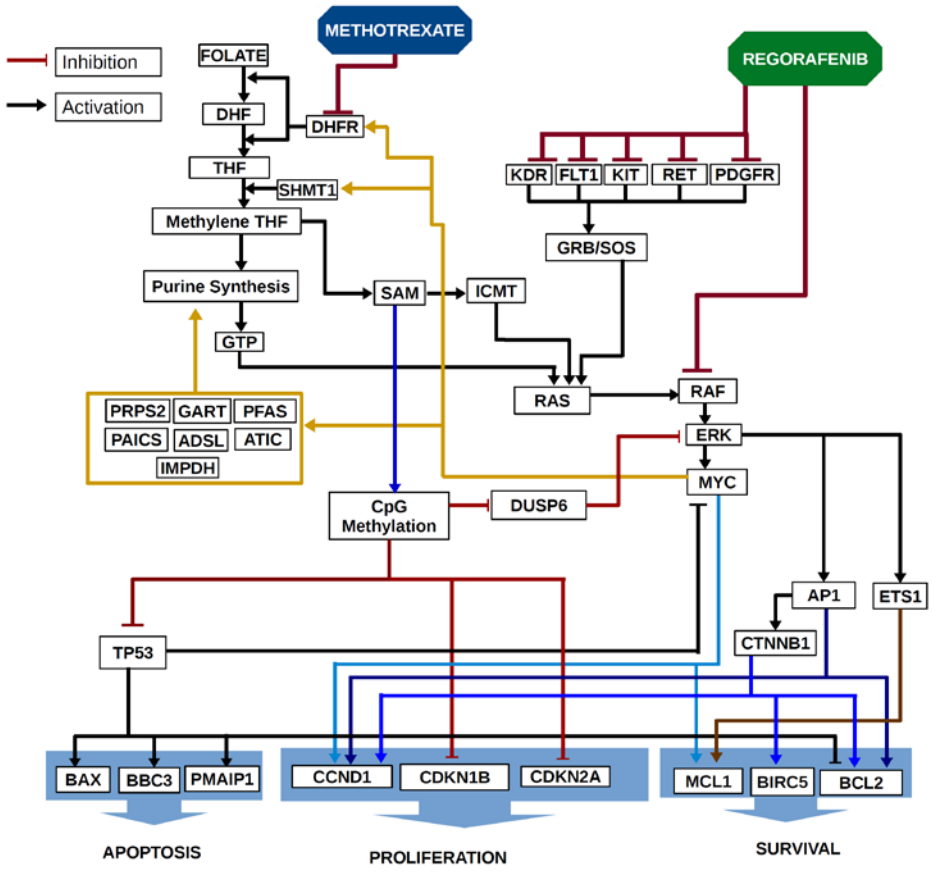
Relative Growth Inhibition Score = Proliferation/Viability=Proliferation/(Survival/Apoptosis)

The proliferation index is an average function of the active CDK-cyclin complexes that define cell cycle checkpoints: CDK4-CCND1, CDK2-CCNE, CDK2-CCNA, and CDK1-CCNB1. A viability index based on survival and apoptosis is also generated for each cell line model. The biomarkers constituting the survival index include AKT1, BCL2, MCL1, BIRC5, BIRC2, and XIAP, while the apoptosis index includes BAX, CASP3, NOXA, and CASP8. The overall viability index of a cell is calculated as a ratio of survival index/apoptosis index, and the weightage of each biomarker is adjusted to achieve a maximum correlation with the experimental trends for the endpoint. Reduction on relative growth characteristic (proliferation, viability, apoptosis) was monitored in the NSCLC cell lines with every drug simulation study to shortlist and identify the most efficacious combination of Methotrexate and Regorafenib in KRAS driven NSCLC profiles.

TABLE 1
5 following NSCLC Cell lines used for predicting response for Methotrexate and Regorafenib combination.

Cell lines	KRAS status
H358	mut
H2122	mut
A549	mut
Calu3	wt
H1650	wt

Figure 1 Scientific Rationale for Combining Methotrexate with Regorafenib



In Conclusion – Why Regorafenib and Methotrexate are Synergistic and Predicted by the Simulation Approach

This is established relevant known biological knowledge coded in the computational model

- KRAS undergoes sequential post-translational modifications (PTM) which makes its membrane interaction more stable [7].
- The process involves prenylation of KRAS, proteolysis of the prenylated CAAX by RCE1 (Ras Converting CAAX Endopeptidase 1), followed by carboxymethylation of the prenylated cysteine by ICMT (Protein-S-isoprenylcysteine-O-methyltransferase) [7].
- KRAS interaction with membrane is important for KRAS mediated signaling. Disruption of PTM events modulates KRAS functionality [7].
- RAF/ERK is one of the dominant pathway under KRAS mutant signaling [7].

Hence synergistic reasons –

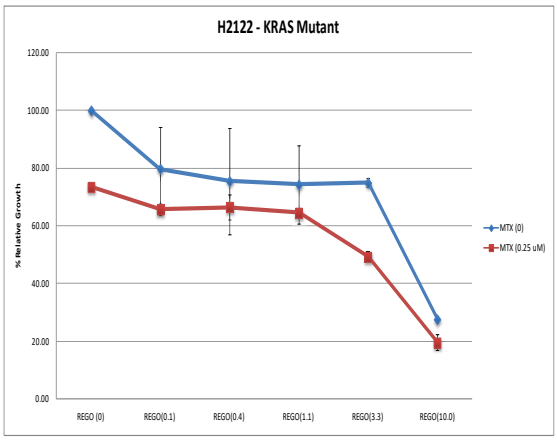
- Methotrexate (MTX) interferes with the carboxymethylation step by inhibiting ICMT via S-adenosyl methionine (SAM) [8]. In this way MTX can show synthetic lethality in KRAS mutant profiles [9].
- MTX also inhibits SAM mediated DNA CpG methylation resulting in increased expression of DUSP6 [10]. DUSP6 inhibits activated ERK [11].
- KRAS profiles have higher level of activated MYC due to up-regulation of KRAS/RAF/ERK/MYC pathway [12]. Regorafenib inhibits RAF/ERK pathway and will inhibit KRAS signaling [16]. Regorafenib indirectly inhibits MYC which makes Methotrexate effective and decreases DHFR. Low DHFR will further sensitize MTX to have a higher impact [17].
- Activated MYC causes resistance to MTX by transcriptional up-regulation of DHFR [13-15].

Of note, the predictive results were subsequently validated through wet lab experiments (see Figure 2 on next page).

Figure 2 Dose Response Curve Regorafenib (0-10 uM) with Methotrexate (0.25 uM) in (A) laboratory and (B) simulation model of H2122 KRAS Mutated NSCLC, and in (C) laboratory and (D) simulation model of CALU3 KRAS Wild Type NSCLC. (E) Synergy confirmed with Regorafenib (3.3 uM) and Methotrexate (0.25 uM) with Bliss Ratio in H2122 KRAS mutated NSCLC but not in CALU3 KRAS Wild Type NSCLC.

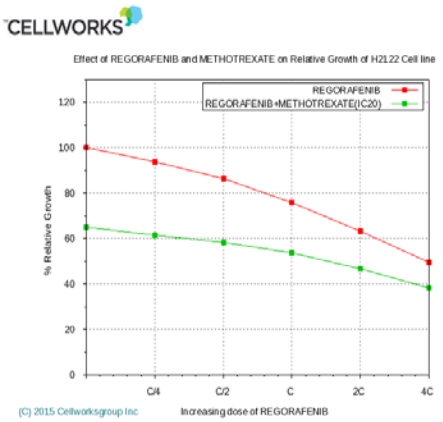
LABORATORY

A



SIMULATION

B



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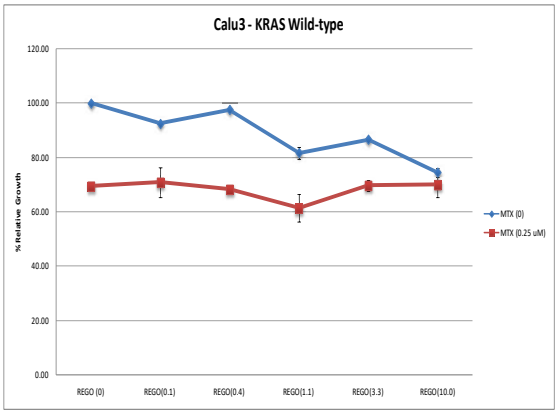

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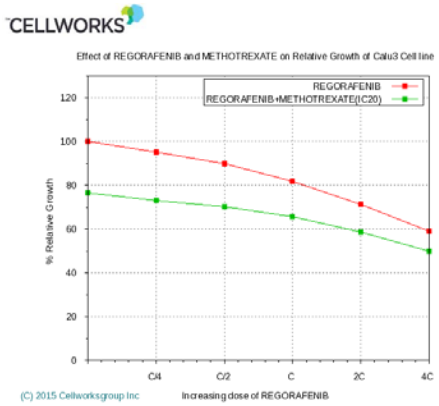
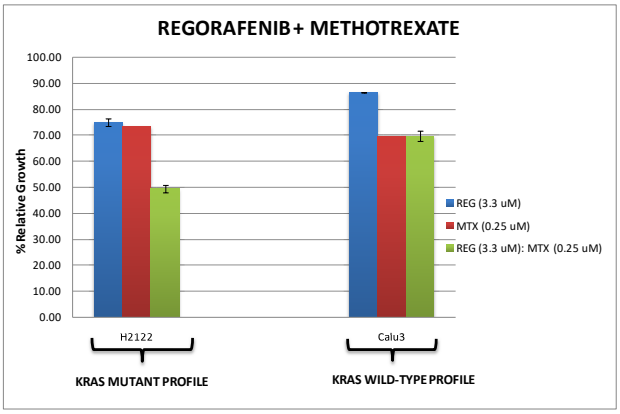


E



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