A Phase II Randomized Therapeutic Optimization Trial for Subjects with Refractory Metastatic colorectal Cancer using ctDNA: Rapid 1 Trial

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Coordinating Center:	University of Florida
Principal Investigator:	Sherise Rogers. MD, MPH University of Florida 1600 SW Archer Road Gainesville, FL 32608 Telephone: (352) 273-7828 Email: Sherise.Rogers@medicine.ufl.edu
Statistician:	Dr. XiangYang Lou, PhD University of Florida Health Cancer Center
Protocol Manager:	CRO Project Management Office pmo@cancer.ufl.edu
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ABBREVIATIONS

AE	adverse event
AESI	adverse event of special interest
ALT	alanine transaminase (also SGPT)
ANC	absolute neutrophil count
AST	aspartate transaminase (also SGOT)
AUC	area under curve
BSA	body surface area
BUN	blood urea nitrogen
CBC	complete blood count
CRC	Colorectal Cancer
CL	clearance
Cl_{cr}	Creatinine clearance
CR	complete remission
CRF	case report form
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	clinical trials management system
CRO	Clinical Research Office
CtDNA	Circulating Tumor DNA
CTO	Clinical Trials Office
DISC	Data Integrity and Safety Committee
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DPD	Dihydropyrimidine dehydrogenase
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EOT	End of Treatment
FDG	fluorodeoxyglucose positron emission
FFPE	Formalin-Fixed Paraffin-Embedded
FSH	follicle stimulating hormone
5FU	fluorouracil
GCP	Good Clinical Practice
H&E	Haemotoxylin and Eosin (stained slides)

HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonization
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	intravenous
kg	kilogram(s)
LDH	lactic dehydrogenase
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
NSAE	non-serious adverse event
ORR	overall response rate
OS	overall survival
PCR	polymerase chain reaction
PD	progressive disease
PET	positron emission tomography
PFS	progression free survival
PMO	Project Management Office
PI	principal investigator
PK	pharmacokinetics
PR	partial remission
PS	performance status
PT	prothrombin time
PTT	partial thromboplastin time
RBC	red blood cells
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	ribonucleic acid
SAE	serious adverse event
SC	subcutaneous
SD	stable disease

SGOT	serum glutamic oxaloacetic transaminase (also AST)
SGPT	serum glutamic pyruvate transaminase (also ALT)
T_{max}	time to maximum plasma concentration
UF	University of Florida
UFHCC	University of Florida Health Cancer Center
ULN	upper limit of normal
US	United States
WBC	white blood cell
WHO	World Health Organization
WOCBP	women of childbearing potential

Protocol Signature Page

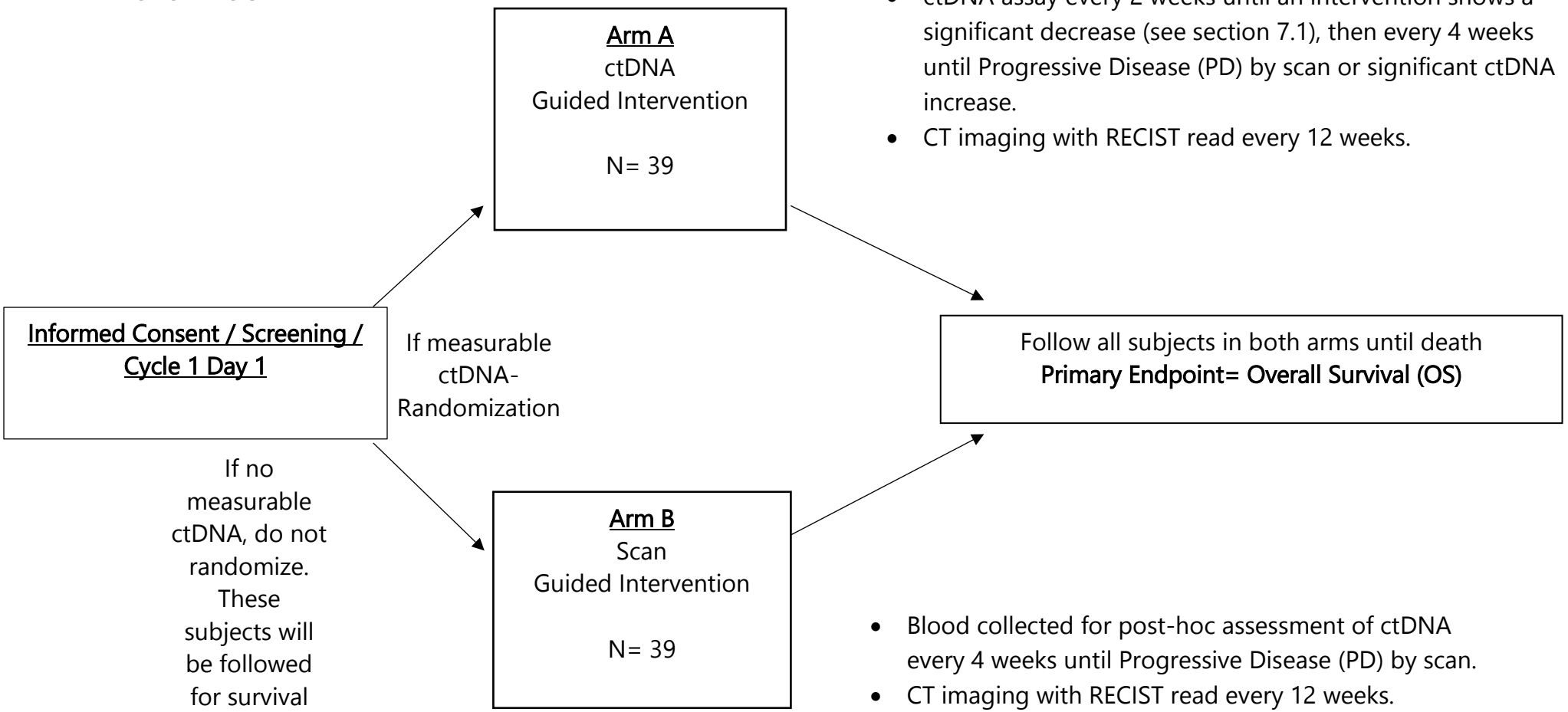
A Phase II Randomized Therapeutic Optimization Trial for Subjects with Refractory Metastatic colorectal Cancer using ctDNA: Rapid 1 Trial

Study Principal Investigator:

Signature of Investigator	Date
Printed Name of Investigator	
Name of Facility	
Location of Facility (City/State)	

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

STUDY SCHEMA



- ctDNA assay every 2 weeks until an intervention shows a significant decrease (see section 7.1), then every 4 weeks until Progressive Disease (PD) by scan or significant ctDNA increase.
- CT imaging with RECIST read every 12 weeks.

- Blood collected for post-hoc assessment of ctDNA every 4 weeks until Progressive Disease (PD) by scan.
- CT imaging with RECIST read every 12 weeks.

Accrual Goal: 78 randomized

PROTOCOL SYNOPSIS

Title:	A Phase II Randomized Therapeutic Optimization Trial for Subjects with Refractory Metastatic colorectal Cancer using ctDNA: Rapid 1 Trial
Funding Source(s):	University of Florida
Rationale:	Patients with advanced CRC after progressing through first line therapy, have a number of FDA-approved therapeutic options that are associated with a clinical benefit for a substantive minority of patients. Present clinical practice is to try these various interventions in a step-wise empiric fashion using scans every 3 months to determine the effectiveness of each intervention. This process requires 3-4 months between therapeutic interventions from which the patient may derive no clinical benefit, unless the selected therapeutic agent by chance happens to provide some level of benefit. As is most often the case, a patient's performance status may decline and thus limit the number of possible interventions to only 1 or 2 before further intervention is precluded. This empiric approach that is dependent on relatively infrequent scans (every few months) not only limits the number of possible therapeutic attempts, but often misses the intervention that may provide meaningful benefit due to the limited number of possible therapeutic attempts. Additionally, the 3 months of exposure to each non-beneficial therapeutic result in entirely unnecessary toxicity. An alternative approach makes use of frequent ctDNA monitoring after each given intervention to gage the effectiveness of the intervention and thus abbreviates the interval between therapeutic attempts to 2-3 weeks rather than 4 months as required using scans. This rapid assessment by ctDNA allows each patient to be exposed to each potential beneficial intervention for a relatively brief period of time, thus limiting toxicity, while allowing a far larger number of interventions to be attempted to find the intervention or interventions that provide the greatest clinical benefit for each patient. This is essentially a means to "personalize" available therapeutics for each patient.

Objectives:	Primary: To determine the difference in overall survival (OS) of adults with metastatic colorectal cancer after treatment with oxaliplatin, between use of the ctDNA guided assay (Signatera Test) and routine scan-guided treatment. Secondary: To measure and compare progression free survival (PFS) and best overall response (OR) in subjects with ctDNA-guided treatment compared to routine scan-guided treatment. To investigate the dynamics of ctDNA vs scan evidence of disease status. Exploratory: To investigate the relationship of the microbiome and other novel biomarkers relative to patient outcomes.
Study Design:	This is a prospective, single center, two-arm randomized trial.
Accrual Goal:	A total of 78 randomized subjects, with a maximum enrollment cap of 100 subjects to account for any withdrawals or screen failures.
Inclusion Criteria:	Individuals eligible for study participation must meet the following criteria: A. A histologic confirmation of adenocarcinoma of the colon or rectum with RECIST measurable metastatic disease not currently candidates for oligometastatic definitive management (i.e., potentially curative surgery or ablation). B. Must have at least received first-line oxaliplatin-based therapy for metastatic disease, or a clinically acceptable and documented reason they did not, and progressed or were intolerant to the therapy. Individuals who recurred within 6 months of completion of oxaliplatin based adjuvant chemotherapy are also eligible. Subjects

	may enroll at any line of therapy past this first line so long as the patient's next clinically reasonable prescribed treatment would be FOLFIRI + Bevacizumab/biosimilar, Anti-EGFR therapy (with or without irinotecan), OR Lonsurf. C. Subjects must have tissue from either the primary and/or metastatic deposit available for submission at enrollment. Tissue can be from either a biopsy or resection surgery, whichever is most recent, but must be from the past five years. See Section 9.3.1 for tissue block and slide specifications. D. Subjects must have tissue and blood shipped to Natera at least 10 days prior to the anticipated treatment start date. E. Subjects must have had molecular profiling to determine tumor RAS, BRAF and MMR/MSI status. F. Subjects with known or suspected Gilbert's disease must be formally tested for UGT1A1*28 with results available to study team prior to treatment initiation. G. Any clinically relevant (as deemed by the PI) adverse events related to prior therapies must have resolved to Grade 1 or less (CTCAE 5.0) at study enrollment. H. Both males and females ≥ eighteen years of age. I. An ECOG Performance Status less than or equal to 2. J. A life expectancy of at least 6 months. K. Adequate organ function as defined as: • Absolute neutrophil count (ANC) ≥ 1,500/µL • Hemoglobin ≥ 9g/dL • Platelets ≥ 100,000/µL • Total bilirubin ≤ 1.5 ULN or direct bilirubin ≤ 1 x ULN • Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN; if liver metastases present, then AST and ALT must be ≤ 5 x ULN

	• Serum creatinine ≤ 1.5 x ULN or calculated creatinine clearance ≥ 59 mL/min/1.73m using Cockcroft-Gault equation L. Subjects must not have more than one active malignancy at the time of enrollment (Subjects with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen [as determined by the treating physician and approved by the PI] may be included). M. Written informed consent obtained from the subject and the subject agrees to comply with all the study-related procedures. N. Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for at least 12 weeks after the last dose of the protocol-specified treatment to minimize the risk of pregnancy. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. WOCBP includes any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal. Post-menopause is defined as: • Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or • For women with irregular menstrual periods who are taking hormone replacement therapy (HRT), a documented serum follicle-stimulating hormone (FSH) level of greater than 35 mIU/mL.

	O. Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (*e.g.*, abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for 12 weeks following the last dose of the protocol-specified treatment.
Exclusion Criteria:	Subjects with any of the following will not be eligible for study participation: A. Subject with colorectal cancer known to be Microsatellite High (MSI-H), deficient in DNA mismatch repair genes (dMMR), or BRAF (V600E) mutated. B. Females or males of childbearing potential who are **unwilling or unable** to use an acceptable method to avoid pregnancy for the entire study period and for at least 12 weeks after the last dose of the protocol-specified treatment. C. Females who are pregnant or breastfeeding. D. History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician. E. Prisoners or subjects who are involuntarily incarcerated, or subjects who are compulsorily detained for treatment of either a psychiatric or physical illness. F. Prior radiation therapy must have been completed 14 days prior to study entry. G. Prior chemotherapy or biologic therapy must have been completed 21 days prior to study entry.

	H. Subject has known Dihydropyrimidine Dehydrogenase (DPD) deficiency.
Efficacy Assessments:	RECIST (version 1.1), ctDNA assay, overall survival assessments
Statistical Considerations:	A total of 39 randomized patients in each arm randomized 1:1 will provide an 80% power to detect a HR of 0.55 (target median OS of 11 months) using an alpha of 0.1. We will use Overall Survival (OS) as the primary endpoint and assume a median OS of 6 months with the standard approach based on the experimental arm of the CORRECT Trial (Grothey et al, Lancet 2013) using single agent regorafenib in the same population as is being enrolled in the present trial. An interim futility analysis will occur after 26 patients are enrolled onto each arm. The trial would be stopped if the futility boundary is exceeded or an additional 13 patients would be enrolled onto each arm if futility is not exceeded.
Estimated Enrollment Period:	36 months
Estimated Study Duration:	48 months

1. BACKGROUND

1.1 Metastatic Colorectal Cancer

Colorectal cancer (CRC) accounts for about 150,000 new cases annually in the US, of which about 50,000 patients succumb to their cancer. For patients with metastatic CRC, the 5-year survival is on the order of 10% with only a small minority of patients able to undergo surgical resection with curative intent. For the overwhelming majority of patients, palliative treatment with systemic chemotherapy represents the standard of care with an associated median survival of about 30 months. Patients with advanced disease are typically treated with the combination of a fluoropyrimidine (5-FU or capecitabine) with oxaliplatin or irinotecan plus bevacizumab for the first 2 lines of therapy. In cases of left-sided primary disease, anti-EGFR antibodies (cetuximab or panitumumab) may be employed instead of bevacizumab. After progressing through the first 2 lines of therapy, patients have a number of NCCN supported/FDA approved therapeutic options that are associated with a clinical benefit for a substantive minority of patients. Present clinical practice is to try these various interventions in a stepwise empiric fashion using scans every 3 months to determine the effectiveness of each intervention. This process requires 3-4 months between therapeutic interventions from which the patient may derive no clinical benefit unless the selected therapeutic agent by chance happens to provide some level of benefit. As is most often the case, a patient's performance status may decline and thus limit the number of possible interventions to only 1 or 2 before further intervention is precluded. This empiric approach that is dependent on relatively infrequent scans not only limits the number of possible therapeutic attempts but often misses the intervention that may provide meaningful benefit due to the limited number of possible therapeutic attempts. Additionally, the 3 months of exposure to each non-beneficial therapeutic will result in entirely unnecessary toxicity, both physical and financial.

An alternative approach makes use of frequent ctDNA monitoring performed 2 weeks after a given intervention to gage the effectiveness of the intervention and thus abbreviating the interval between therapeutic attempts to 2-3 weeks rather than 3-4 months as required with the present approach using imaging scans. This rapid assessment by ctDNA allows each patient to be exposed to each potential beneficial intervention for a relatively brief period of time, thus limiting toxicity, while allowing a far larger number of interventions to be attempted to find the intervention or interventions that provide the greatest clinical benefit for each patient. This is essentially a means to "personalize" available therapeutics for each patient.

1.2 Circulating Tumor DNA (ctDNA)

Using a single tumor mutation to assay for ctDNA, Tie and colleagues (Ann Oncology 2015) demonstrated a close relationship between pretreatment ctDNA levels and imaging using the sum of the longest tumor diameters as shown in Figure 1 below.

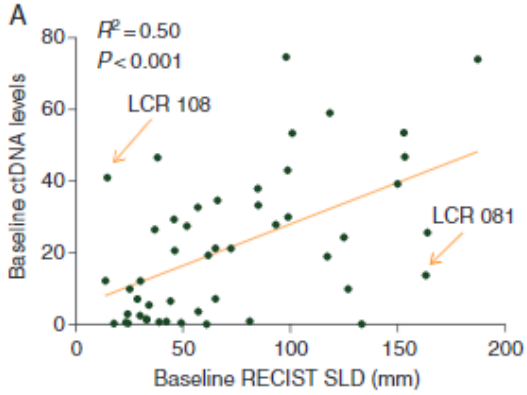


Figure 1

In this same investigation it was found that a 10-fold or greater decrease in ctDNA was associated with a RECIST response in 64% of patients whereas a less than 10-fold decrease was associated with a response in 25% of patients.

Osumi and colleagues (Nature 2019) used mutations in 14 colon-cancer associated genes to assay for ctDNA in 29 patients with metastatic CRC undergoing second line therapy. They found that changes in ctDNA levels at 2 and 8 weeks closely mirrored image detected disease status as shown in Figure 2 below.

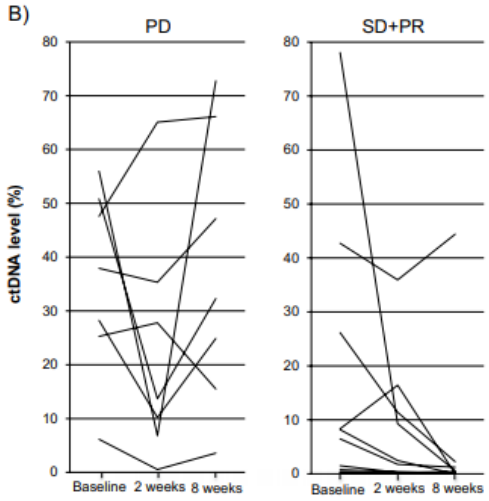


Figure 2

Taken together, these data support a strong relationship between ctDNA levels and disease status as measured by conventional imaging in patients with metastatic CRC.

The Signatera ctDNA assay (Natera Inc San Carlos, CA) is CLIA-approved and utilizes paraffin embedded tumor tissue from either the primary CRC or a metastatic site subjected to NGS to identify 16 truncal mutations specific for a given individuals cancer. These mutations are then assayed in the blood using PCR technology and serve as a quantitative measure of tumor burden that may be followed over time as an indicator of benefit associated with any given therapeutic intervention. In patients with active metastatic CRC the assay detected ctDNA in 100% of 25 patients investigated whereas patients achieving a PR (15 patients) or NED (12 patients) following chemotherapy had ctDNA positivity rates of 60% and 33% respectively (Kasi et.al. ASCO 2020). In patients with oligometastatic CRC, the Signatera assay was positive in all patients at pre-op at a median level of 36.5 mean tumor molecules (MTM) per ml. This % dropped to 49% of patients post-op with a median MTM of 5.6 with a further decrease during adjuvant therapy to 33% of patients with a median MTM of 2.2 supporting the role of ctDNA monitoring as a metric of disease burden (Hook et.al. ESMO 2020). A second study presented at ESMO 2020 by Loupakis and colleagues enrolled 100 patients with advanced CRC undergoing resection with curative intent and followed with the Signatera ctDNA assay. As shown in Figure 3, patients who were ctDNA negative following resection had a far superior outcome relative to those who remained ctDNA positive. This lends further support to the use of ctDNA as a valid measure of tumor burden in patients with metastatic CRC.

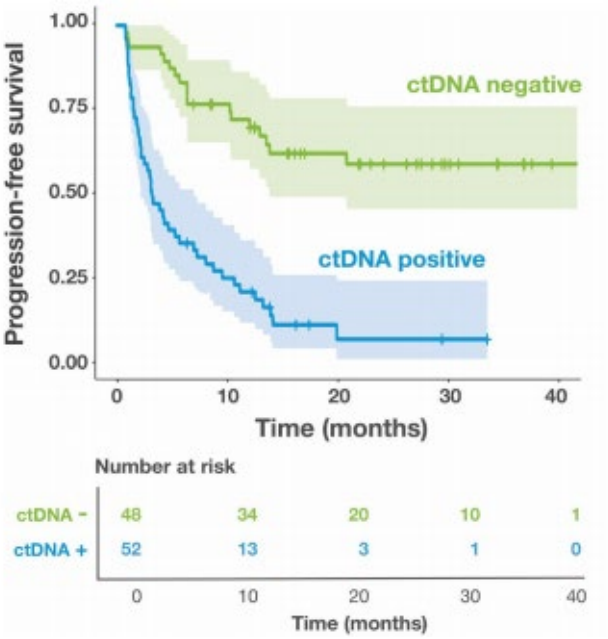


Figure 3

1.3 Current Study

We propose a prospective randomized phase 2 study design comparing the usual empiric scan-driven approach vs interventions guided by ctDNA assessments, both arms using a pre-specified order of treatment. Eligible patients will have progressed or have demonstrated intolerance to first-line oxaliplatin -based therapies, or had a clinically acceptable and documented reason why they did not receive the therapy. Overall Survival (OS) will be the primary endpoint assuming a median OS of 6 months with the standard approach based on the experimental arm of the CORRECT Trial (Grothey et al Lancet 2013) using single agent regorafenib in a similar patient population as is being enrolled in the present trial.

Subjects may start treatment once all eligibility criteria are confirmed (Section 4). However, subjects will not be randomized until and unless Natera results from the baseline assay results in measureable ctDNA. In the event no ctDNA is measured, the subject will NOT be randomized and instead will receive routine care outside the study and be followed for survival.

Patients randomized to Arm A, the ctDNA guided arm, will have ctDNA assayed every 2 weeks real-time until a given intervention demonstrates a significant decrease in ctDNA, at which time, testing frequency would be decreased to every 4 weeks until either scans demonstrate PD or the ctDNA levels rise significantly. Once a patient progresses on a given intervention, they will initiate therapy in a pre-specified order as listed in Appendix E as soon as any significant toxicity from the prior therapy allow the next treatment to safely begin, as determined by the treating physician. This sequential approach will continue until all interventions are exhausted or the patient's performance status declines to a point where further interventions are not feasible, or the patient withdraws from the study.

For patients randomized to Arm B, the control arm, ctDNA samples will be obtained every 4 weeks until they are off study for retrospective batch analysis. All patients will be scanned at 12-week intervals. Based on the results of scans, therapeutic interventions will be administered in a pre-specified order with the agents listed in Appendix E.

2. OBJECTIVES AND ENDPOINTS

2.1 Primary

2.1.1. Primary Objective

To determine the difference in overall survival (OS) of adults with metastatic colorectal cancer after treatment with oxaliplatin, between use of the ctDNA guided assay (Signatera Test) and the routine scan-guided approach.

2.1.2 Primary Endpoint

Overall Survival (OS), defined as time between date of randomization and death

2.2 Secondary

2.2.1 Secondary Objectives

- To measure and compare progression free survival (PFS) and best overall response (OR) in subjects with ctDNA-guided treatment compared to routine scan-guided treatment.
- To investigate the dynamics of ctDNA vs scan evidence of disease status.

2.2.2 Secondary Endpoints

- Progression Free Survival (PFS), defined as time between date of randomization and disease progression or death (whichever comes first)
- Best Overall Response (OR) during the entire period of study participation

2.3 Exploratory

To investigate the relationship of the microbiome and other novel biomarkers relative to patient outcomes.

3. STUDY DESIGN

3.1 Study Overview

This is a prospective, single center, two-arm randomized trial. A total of 78 subjects are planned, with a maximum enrollment cap of 100 subjects to account for any withdrawals, screen failures, or subjects with no measurable ctDNA. Subjects in Arm A will be administered drugs/regimens in a pre-specified order as listed in Appendix E, as appropriate. Subjects may enroll at second or subsequent lines of therapy so long as the patient's next clinically reasonable prescribed treatment would be FOLFIRI+ Bevacizumab/biosimilar, Anti-EGFR therapy (with or without irinotecan), OR Lonsurf; subjects will enter the pre-specified treatment order (Appendix E) anywhere between T1-T3, as appropriate given mutation status and prior lines of therapy. These subjects will be treated with a different agent based on Signatera's ctDNA assay, moving to a new drug/regimen when the assay indicates non-response and prior relevant toxicity for the next treatment has resolved to an acceptable point per the treating physician. If an intervention is identified that shows a significant decrease in ctDNA, frequency of testing would be decreased to approximately every four weeks until either (a) scans demonstrate progressive disease, or (b) ctDNA levels rise significantly. Each therapeutic intervention will use the most recent ctDNA as the new baseline. These subjects will continue until all interventions are exhausted, further interventions are not feasible, or subject withdraws from the study.

Subjects in Arm B will also be administered drugs/regimens in a pre-specified order, identical to the process in Arm A, as listed in Appendix E. These subjects will be treated based on imaging results every 12 weeks, moving to a new drug when scans show PD, as is the current routine practice. ctDNA samples will be obtained from these patients approximately every 4 weeks for retrospective batch analysis. All subjects will undergo imaging scans at the usual 12-week intervals for comparison to the ctDNA assays. Evaluations will be taken at baseline and at each of the study visits. All subjects will be followed for overall survival.

In the event that ctDNA results (Arm A) or RECIST reads (Arm B) are not received in time to make a treatment decision at a study visit, clinical judgement of the treating physician will be followed until the next study visit or opportunity to modify the treatment plan.

Furthermore, In the event initial ctDNA results are not available from Natera within the randomization window, the subject will remain on their first regimen until the results are received and subject is able to be randomized. In this event, the pre-randomized subject will have their ctDNA tested at 2 weeks past Cycle 1 Day 1, but results will not be released to the study team unless the subject is confirmed to be randomized to Arm A upon receipt of initial screening results.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study, however, only subjects with measurable ctDNA will be randomized. Others will receive routine care outside the study and only be followed for survival.

Total duration of subject enrollment will be approximately 36 months. Total duration of the study is expected to be approximately 48 months.

4. SELECTION OF SUBJECTS

Subjects with a diagnosis of Metastatic Colorectal Cancer who meet the following inclusion and exclusion criteria will be eligible for participation in this study. **Per UFHCC guidelines, exceptions to inclusion and exclusion criteria are not permitted.** For questions concerning eligibility, please contact the PMO (pmo@cancer.ufl.edu).

4.1 Number of Subjects

78 subjects are expected to participate in this study, with a maximum enrollment cap of 100 subjects to account for any withdrawals or screen failures.

4.2 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for study participation:

A. A histologic confirmation of adenocarcinoma of the colon or rectum with RECIST measurable metastatic disease not currently candidates for oligometastatic definitive management (i.e., potentially curative surgery or ablation).
B. Must have at least received first-line oxaliplatin-based therapy for metastatic disease, or a clinically acceptable and documented reason they did not, and progressed or were intolerant to the therapy. Individuals who recurred within 6 months of completion of oxaliplatin based adjuvant chemotherapy are also eligible. Subjects may enroll at any line of therapy past this first line so long as

the patient's next clinically reasonable prescribed treatment would be FOLFIRI + Bevacizumab/biosimilar, Anti-EGFR therapy (with or without irinotecan), OR Lonsurf.

C. Subjects must have tissue from either the primary and/or metastatic deposit available for submission at enrollment. Tissue can be from either a biopsy or resection surgery, whichever is most recent, but must be from the past five years. See Section 9.3.1 for tissue block and slide specifications.
D. Subjects must have tissue and blood shipped to Natera at least 10 days prior to the anticipated treatment start date.
E. Subjects must have had molecular profiling to determine tumor RAS, BRAF and MMR/MSI status.
F. Subjects with known or suspected Gilbert's disease must be formally tested for UGT1A1*28 with results available to study team prior to treatment initiation.
G. Any clinically relevant (as deemed by the PI) adverse events related to prior therapies must have resolved to Grade 1 or less (CTCAE 5.0) at study enrollment.
H. Both males and females ≥ eighteen years of age.
I. An ECOG Performance Status less than or equal to 2.
J. A life expectancy of at least 6 months.
K. Adequate organ function as defined as:
   • Absolute neutrophil count (ANC) ≥ 1,500/µL
   • Hemoglobin ≥ 9g/dL
   • Platelets ≥ 100,000/µL
   • Total bilirubin ≤ 1.5 ULN or direct bilirubin ≤ 1 x ULN
   • Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN; if liver metastases present, then AST and ALT must be ≤ 5 x ULN
   • Serum creatinine ≤ 1.5 x ULN or calculated creatinine clearance ≥ 59 mL/min/1.73m using Cockcroft-Gault equation
L. Subjects must not have more than one active malignancy at the time of enrollment (Subjects with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen [as determined by the treating physician and approved by the PI] may be included).
M. Written informed consent obtained from the subject and the subject agrees to comply with all the study-related procedures.

N. Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for at least 12 weeks after the end of protocol-specified treatment to minimize the risk of pregnancy. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy.

   WOCBP includes any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal. Post-menopause is defined as:

   - Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or
   - For women with irregular menstrual periods who are taking hormone replacement therapy (HRT), a documented serum follicle-stimulating hormone (FSH) level of greater than 35 mIU/mL.

O. Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (*e.g.*, abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for12 weeks following the last dose of the protocol-specified treatment.

4.3 Exclusion Criteria

Subjects with any of the following will not be eligible for study participation:

A. Subject with colorectal cancer known to be Microsatellite High (MSI-H), deficient in DNA mismatch repair genes (dMMR), or BRAF (V600E) mutated.

B. Females or males of childbearing potential who are **unwilling or unable** to use an acceptable method to avoid pregnancy for the entire study period and for at least 12 weeks after the last dose of the protocol-specified treatment.

C. Females who are pregnant or breastfeeding.

D. History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician.

E. Prisoners or subjects who are involuntarily incarcerated, or subjects who are compulsorily detained for treatment of either a psychiatric or physical illness.
F. Prior radiation therapy must have been completed 14 days prior to study entry.
G. Prior chemotherapy or biologic therapy must have been completed 21 days prior to study entry.
H. Subject has known Dihydropyrimidine Dehydrogenase (DPD) deficiency.

4.4 Inclusion of Women and Minorities

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

5. REGISTRATION PROCEDURES

All consented subjects must be entered into the University of Florida's Clinical Trial Management System (OnCore) prior to assignment of a subject identification number. The study team must submit the completed study specific eligibility checklist, supporting source documentation and a copy of the signed informed consent document(s) to the UFHCC Project Management Office (PMO; PMO@cancer.ufl.edu) or their assigned Project Manager. Upon receipt of a completed eligibility packet, the designated Project Manager will review the source to verify eligibility and assign a subject number. No subject may be enrolled until his/her eligibility packet is complete and eligibility is verified by PMO. If eligibility cannot be confirmed, the project manager will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be able to participate in the trial.

All eligible and enrolled subjects will receive a unique subject number. This number will be used to identify the subject throughout the study. Subjects withdrawn from the study will retain their subject number.

6. STUDY PROCEDURES

Written informed consent must be obtained prior to performing any study-specific evaluations or tests. Tests or evaluations performed as standard of care within the specified screening period, but prior to informed consent, may be accepted for this study and need not be repeated. Screening measurements will be obtained within 30 days prior to start of treatment.

In the event a subject must be re-screened, tissue already shipped to Natera for purposes of this trial need not be re-shipped. However, a new blood sample must be sent to Natera if the last ctDNA blood collection >14 days from the start of treatment.

Unless a new consent is provided that requires re-consent, re-screened subjects need not sign a new, duplicate consent form prior to re-screening procedures.

Additionally, a second microbiome collection will not be required for subjects undergoing re-consent at screening, so long as the collection occurred within 90 days prior to treatment start and there have been no significant changes in patient status or treatment.

6.1 Schedule of Events

Visit: **Procedure:**	SCREENING **(≤ 30 days prior to C1D1)**	TREATMENT BASELINE **Treatment X, Cycle 1, Day 1**[11]	CYCLE 2, DAY 1 AND ONWARD (SEE APPENDIX E) **(+/- 3 days)**	EVERY12WEEKS **POST CYCLE 1, Day 1** **(+/- 7** days)	END OF TREATMENT (EOT)[7]	FOLLOW UP **(30 ± 7 DAYS AFTER LAST DOSE OF TREATMENT)**[8]	SURVIVAL FOLLOW UP EVERY 3 MONTHS (**90 ± 7 DAYS)**
Informed Consent (not subject to 30-day screening window)	X						
Demographic Information	X						
Medical History	X						
Complete Physical Exam	X						
Brief Physical Exam		X	X		X		
Vital Signs (VS)	X	X	X		X		
ECOG PS	X						
Research Blood, including buffy coat and plasma collection[10]	X	X	X		X		
Safety labs including CMP, CBC w/diff[1]	X	X	X		X		
Pregnancy Test (Urine or Serum)[2]	X		X[12]				
Microbiome Samples[14]	X[14]			X[14]			
Diagnostic Imaging Scan/TA[3]	X			X	X		
FFPE / H&E Tumor Tissue Submission[4]	X						
Tumor Marker Assessment (Carcinoembryonic Antigen (CEA) test)	X	X	X		X		
Randomization[13]		X					
Administration of Drug[5]		X	X				
ctDNA assessment[6]	X	X	X		X		
Concomitant Medication Review[9]	X	X	X		X	X	
Adverse Event Review[9]		X	X		X	X	
Survival Assessment[15]							X

Abbreviations: VS=vital signs (blood pressure, temperature, pulse and respiratory rates, weight and height); CBC/diff=complete blood count and white blood cell differential; CMP=12 item complete metabolic profile (sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, alkaline phosphatase, AST, ALT, total bilirubin);

1 Safety labs will be performed as per institutional standards while subject is on each agent, if more or additional labs are required to monitor safety

2 Women of child bearing potential only

3 TA= Tumor Assessment using RECIST 1.1 criteria. A baseline tumor evaluation must be performed within 4 weeks before a subject begins protocol-specified treatment.

4 Tumor tissue sample from previously routine biopsy or resection, whichever is most recent, required at enrollment, and must be shipped no fewer than 10 days prior to start of therapy. Details on specifications, including alternative submission requirements, can be found in section 9.3.1.

5 **Arm A-** Subjects will be administered drugs/regimens in a pre-specified order as listed in Appendix E. Subjects will be treated with one of these agents moving to a new drug/regimen when the ctDNA assay or scan indicates non-response. **Arm B-** Subjects will be administered drugs/regimens in a pre-specified order as listed in Appendix E. Subjects will be treated with one of these agents moving to a new drug/regimen when CT scan indicates non-response.

6 ctDNA collection should occur as close as possible prior to dosing. In the event a subject's screening ctDNA occurred within 14 days of their baseline visit (C1D1 of their first on-study regimen), a second ctDNA test at baseline is not required. **Arm A-** ctDNA collected every 2 weeks as per Appendix E. If an intervention is identified that shows a significant decrease in ctDNA, testing frequency will be decreased to every four weeks until either scans demonstrate progressive disease or ctDNA levels rise significantly. **Arm B-** ctDNA collected for post-hoc assessment every 4 weeks per Appendix E until Progressive Disease (PD) by scan. At screening for both arms, schedule ctDNA sample collection as early as possible to allow for confirmation of sufficient quantity of samples for assay creation prior to Cycle 1 Day 1. Per Natera, turnaround time for the initial assay is up to twenty (20) business days. In addition, per Natera, turnaround time for subsequent assays is up to ten (10) business days. In the event that ctDNA results (Arm A) or RECIST reads (Arm B) are not received in time to make a treatment decision at a study visit, clinical judgement of the treating physician will be followed until the next study visit or opportunity to modify the treatment plan. **No Measurable ctDNA at Baseline**- No further ctDNA will be collected

7 Subjects will continue until all interventions are exhausted, further interventions are not feasible, or subject withdraws from the study. EOT visit will be completed within 30 days of discontinuation.

8 If EOT visit is done 30 days after last dose of treatment, follow up visit may be omitted.

9 ConMeds include use of probiotics, nutritional supplements, antibiotics, and other holistic supplements to inform the planned correlative analyses on microbiota samples. ConMeds and AE assessments should also occur on any PRN visit to oncology during study period. Adverse events to be collected from randomization to follow up visit, for events deemed to be associated with the study

10 Research blood to be collected at same visits that ctDNA is collected; See laboratory manual for more details. "Visits" refer to the table header "visit" in the SOE- i.e. the visit "Screening" would encompass all 30 days prior to C1D1, so blood draws occurring during that time period count as part of that "visit", and do not necessarily need to be collected on the same day.

11 Subject may begin initial treatment (i.e., Treatment 1 Cycle 1 Day 1) while awaiting initial assay results from Natera. However, subjects may not be randomized until confirmation is received from Natera that the subject has measureable ctDNA. Randomization should begin within 14 days of treatment start. In the event a subject has assessments (Physical Exam, ECOG, Labs, and other procedures per protocol) collected at screening within 7 days of their C1D1 of their first regimen overall, those procedures will not need to be repeated at their C1D1 of their first regimen overall.

12 As clinically indicated and per institutional practice

13 Randomization should begin prior to or within 14 days after treatment start. In the event initial ctDNA results are not available from Natera within this 14-day randomization window, the subject will remain on their first regimen until the results are received and subject is able to be randomized. In this event, the pre-randomized subject will have their ctDNA tested at 2 weeks past Cycle 1 Day 1, but results will not be released to the study team unless the subject is confirmed to be randomized to Arm A upon receipt of initial screening results. Subjects without measurable ctDNA will not be randomized and will only be followed for survival

14 Microbiome samples will be collected at two timepoints: screening (sample must be received prior to C1D1 dosing) and 12 weeks after C1D1 (sample must be received within (+/-) 14 days of first scan). There will be no reportable protocol deviation for microbiome specimens not collected or collected outside of collection window. Such variance poses no risk to patient safety or primary study endpoints.

15 Subjects who did not have measurable ctDNA, will only receive quarterly survival follow-up visits. These may be completed via telephone or in person.

6.2 End of Treatment (EOT) Evaluations

Subjects will continue until all interventions are exhausted, further interventions are not feasible, subject withdraws from the study, or when the physician and/or subject decides it is not appropriate to continue based on subject disease status. EOT visit will be completed within 30 days of protocol-specified treatment completion. End of treatment evaluations will also be performed within 30 days if the subject is discontinued as per section 8.2.

6.3 Follow up/Survival Evaluations

Subjects will have a follow-up visit for the evaluation of adverse events 30 days (± 7 days) after the last dose of study medication.

If the subject is unavailable to travel to the institution, the visit may be made via telephone or telehealth interview to review adverse event resolution or the occurrence of any new adverse events. Additionally, external or internal medical records may be consulted with permission of the subject to complete remote visit reporting.

If a subject is removed from the study for reasons other than disease progression (per imaging and/or ctDNA), imaging/disease assessments will continue per protocol until documented progression or until, at the investigator's discretion, further off-protocol treatment is begun. All subjects will continue to be followed for survival. Following discontinuation of the study for any reason, report the date the first off-protocol anti-cancer therapy is administered.

If a subject becomes unreachable during the course of the study, the investigator or study team will make a reasonable effort to contact the subject and document each attempt (in accordance with Section 4.3.4 of the ICH E-6 GCP). If these attempts are not successful, the subject may be documented as “lost to follow up.” For subjects lost to follow-up, the termination date will be the date of last contact with the subject. Vital statistics will still be monitored through public reporting databased for survival status.

7. PROTOCOL-SPECIFIED TREATMENT

7.1 Treatment Schedule/Administration

All subjects will be treated with agents/regimens in a pre-specified order with the drugs/regimens listed in Appendix E. Subjects may enroll at second or subsequent lines of therapy, so long as the patient's next clinically reasonable prescribed treatment would be FOLFIRI+ Bevacizumab/biosimilar, Anti-EGFR therapy (with or without irinotecan), OR Lonsurf. Accordingly, subjects will begin treatment on agents T1-T3 and proceed in the pre-specified order in Appendix E. All visits will be recorded as Treatment X (sequential number) Cycle Y Day Z. For example, the first day of treatment after moving past the first treatment in Appendix E will be represented as T2C1D1.

Subjects on arm A will move through the treatment algorithm based on the results of Signatera's ctDNA assay, moving to a new drug/regimen when the assay indicates non-response. Routine imaging, approximately every 12 weeks per treatment regimen, and clinical assessments for toxicity may also be used to indicate a non-response or need for new treatment and the latter will override ctDNA results in the event of a discrepancy (i.e., ctDNA going down, scans showing overt progression). Prior toxicities relevant to the next treatment must have first resolved to an acceptable point per the treating physician before starting the next treatment. If an intervention is identified that shows a significant decrease in ctDNA (or scan), testing frequency will be decreased to every four weeks until either scans demonstrate progressive disease or ctDNA levels rise significantly, defined as a 10% increase from baseline or 50% increase from nadir. Each therapeutic intervention will use the most recent ctDNA as the new baseline. These subjects will continue on the algorithm until all interventions are exhausted, further interventions are not feasible, or subject withdraws from the study.

For Arm B, subjects will be treated with the same agents/regimens in the same pre-specified order as subjects in Arm A and as listed in Appendix E. However, these subjects will have treatment changes based on traditional imaging results every 12 weeks or unacceptable toxicity, moving to a new drug when scans show progressive disease or treatment intolerance, as is the current routine practice. Prior toxicities relevant to the next treatment must have first resolved to an acceptable point per the treating physician before starting the next treatment. These subjects will continue on the algorithm until all interventions are exhausted, further interventions are not feasible, or subject withdraws from the study. Blood will be collected for ctDNA every 4 weeks from these subjects throughout study participation, but analyses will be performed post-hoc.

Subjects without measurable ctDNA will receive standard of care treatment management and be followed only for overall survival.

7.1.1 Dose Calculations

Dose calculations will be performed per institutional and treating physician practice, in consultation with the published literature, package insert and good clinical practice. Appendix E includes general, standard dosing for each regimen but should not overrule institutional standard practice.

7.2 Supportive Care

7.2.1 Supportive Care Guidelines

Full supportive care may be provided as is routine for patients receiving these agents, including transfusions of blood and blood products, antibiotics, antiemetics, growth factors, antidiarrheals, analgesics, etc., when appropriate. Bisphosphonates or denosumab are allowed for subjects with bone metastases. These should follow institutional guidelines.

7.2.2 Concomitant Therapy

Relevant medical history should be obtained at screening and include prior medications and treatment history. All medications taken within four weeks prior to screening, regardless of indication, should be recorded. All anti-cancer agents previously taken should be recorded. Additionally, special care will be taken to

record use of probiotics, nutritional supplements, antibiotics, and other holistic supplements to inform the planned correlative analyses on microbiota samples.

Any therapy or medication (except the protocol-specified treatment), administered from screening until thirty days after the last dose of any the protocol-specified treatment, is considered a concomitant therapy or medication. However, if another course of anti-cancer therapy is initiated prior to the thirty-day follow-up period visit, a record of concomitant medications will no longer be performed. If the use of any concomitant treatments (medications or procedures) becomes necessary, the treatment must be recorded, including the name of the drug or treatment, dose, route, date, indication for use, expected duration, and frequency of treatment. Assessment and documentation of concomitant medications will be done at each visit.

7.2.3 Prohibited Concomitant Therapy

Supportive care measures consistent with optimal subject care will be permitted throughout the study, as long as the therapy is not included in this section as prohibited.

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy not specified in this protocol
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than the protocol-specified treatment in this trial. Participation in other clinical trials may be allowed with approval by the PI
- The use of palliative RT for symptom management is allowable consistent with good clinical practice, but continued participation in the study will be determined on a case-by-case basis with the PI or study leadership team. Once a lesion is irradiated, it will no longer be included in the RECIST assessment for response. In general, a requisite to radiate a lesion may indicate progression of disease. This decision will be made on a case by case basis by discussion of the treating physician and the study investigators.
- Cancer-Related Surgery, except those allowed at discretion of the PI
- Medications listed in the eligibility criteria and in the package insert for the anti-

cancer agent being administered (see section 10 for a summary).

7.3 Dose Modifications

The National Cancer Institute (NCI) Common Toxicity Criteria (CTCAE Version 5.0) for Adverse Events (CTCAE) will be used to grade toxicity (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Dose modifications based on toxicity will be performed per institutional guidelines, as all are standard of care/ FDA approved treatments.

8. TREATMENT DISCONTINUATION

8.1 Screen Failures

Subjects who sign informed consent, but do not meet eligibility criteria, or withdraw prior to eligibility being verified, and undergo at least some of the screening procedures will be considered screen failures. Subjects who sign informed consent and are verified as eligible, but do not proceed to formal registration will also be considered screen failures. A record of screen failures will be maintained by the study site.

8.2 Criteria For Protocol-Specified Treatment Discontinuation

Subjects who discontinue participation in the clinical study on their own or subjects who are withdrawn by the investigator, for reasons other than completion of treatment, disease progression or toxicity, will be defined as premature withdrawals.

A subject will be discontinued from protocol therapy under the following circumstances:

- Any adverse event which, in the Investigator's opinion, requires termination of the study.
- The subject becomes pregnant. Pregnancy will be reported along the same timelines as a serious adverse event.
- The subject uses illicit drugs or other substances, or takes part in activities that may, in the opinion of the Investigator, have a reasonable chance of contributing to toxicity or otherwise interfering with results.
- The development of a second malignancy that requires treatment, which would interfere with this study.
- The subject is lost to follow-up.

- Development of an intercurrent illness or situation which would, in the judgment of the investigator, affect assessments of clinical status and study endpoints to a significant degree.
- Administration of prohibited anti-cancer therapy or medication (Section 7.2.3) administered from screening until 30 days following EOT.
- Substantial non-compliance with the requirements of the study.
- Interruption in treatment for greater than twenty-eight continuous days, due to toxicity, or sixty continuous days for any other reason.
- Subject desire to discontinue therapy.

The Investigator will make every reasonable effort to keep each subject in the study unless it is in the subject's best interests to discontinue participation. If a subject is removed from the study or declines further participation, all EOT evaluations should be performed if the subject is willing and able to be assessed. A description of the reason(s) for withdrawal from the study must be recorded on the case report form (CRF). The Investigator should also ensure that all subjects are followed up for survival status after their final visit.

Subjects who discontinue following entry will have relevant information completed and recorded on the CRF. All subjects who discontinue because of adverse events or clinically significant laboratory abnormalities should be followed up until they recover or stabilize, and the subsequent outcome recorded. If any subject should experience a serious adverse event during the trial or within 30 days of stopping the study, the Investigator will inform the UF Health Data Integrity and Safety Committee.

8.3 Replacement of Subjects

Subjects may be replaced if they are not screen failures, have not completed any doses of treatment for any reason, and are not discontinued for disease progression, toxicity, or any of the reasons listed in section 8.2. This includes subjects who otherwise meet all eligibility criteria, are randomized, but fail to have measurable ctDNA reported by the Signatera assay. All subjects who fail to have measurable ctDNA will be observed for survival and included in the final study results.

Only subjects who received at least one dose of protocol treatment will be included in the primary analysis.

9. BIOLOGICAL SPECIMENS AND CORRELATIVES

9.1 Source of Specimens

Correlative study specimens will be collected from patients per the schedule of events. Processing and short-term storage for secondary and exploratory analyses will follow the instructions outlined in the Study Laboratory or Procedure Manual for this study.

Biospecimen collection and submission is required as a component of subject participation in the study.

9.2 Correlative Studies of Biospecimens

Each subject will be asked to provide stool, oral and urine microbiome samples at each of two time points. This should provide a maximum of 312 samples. Processing, storage and analyses will be conducted through the UFHCC Microbiome Biorepository. Microbiota samples will be examined for their microbiota content with the intention of making associations between the microbiota and each clinical outcome measure including OS, PFS, RR and toxicity. Research blood collection will be obtained at the time points indicated for post-hoc analysis including alternate assay validation for tumor circulating DNA, germline genetic and genomic profiling, and other biomarkers.

9.3 Preparation, Shipment and Storage of Specimens

See Study Laboratory or Procedure Manual for collection, processing and shipping instructions for all tissue, stool, oral swab, urine and blood specimens.
All tissue, stool, urine, oral swab, and blood samples will be labelled with the subject's unique study number and physically stored. During the life of the study, these biospecimens remain available only to the study team. For analyses that require research collaborations outside of UF, only de-identified specimens will be shared. Samples will be stored for the life of the study and will then be destroyed or transferred to a biospecimen bank for general research use, if subject agrees to enroll in the separate banking study.

At study enrollment, all subjects will be given the option to provide permission for all biospecimens collected and stored during this study, including those residual specimens remaining after completion of all planned exploratory analyses, to remain available for long-term research use (i.e., beyond the life of this study) (IRB# 201901513). Thus, future research on remaining biospecimens after the study-specific analyses have been completed can only be considered by subjects providing their additional consent on an IRB- approved study consent form for optional biobanking. Future specific use of those biomaterials will require secondary IRB approval. At the closure of this treatment trial, any biospecimens remaining from subjects who have provided consent for long-term storage/use will be transferred to a biobank (or destroyed if samples or resources are inadequate). For subjects who decline secondary long-term storage, their biomaterials will be destroyed at the completion of the treatment trial and will not be transferred.

9.3.1 Tumor Tissue

Subjects must have tissue from either the primary and/or metastatic deposit available for submission at enrollment. Tissue may be provided from either a biopsy or surgical resection, whichever is most recent, and should have been collected within the past 5 years prior to enrollment. To create the assay, Natera requires an FFPE tissue block with >25mm^2 of surface area and one H&E stained slide (preferred). However, 6-10 (10-micron) slides OR 12-20 (5-micron) slides and one H&E stained slide (10-micron thickness) is acceptable.

9.3.2 Blood Samples

Natera

Natera requires two 10mL Streck tubes and one 6mL EDTA tube for initial assay creation. These will be collected at screening. For subsequent ctDNA testing timepoints, Natera requires two 10mL Streck tubes only.

Research Samples

EDTA tubes will be collected and processed for research buffy coat and plasma samples at each visit specified in the Schedule of Events.

9.3.3 Microbiota Samples

Urine and oral microbiome specimens will be collected in clinic. Kits will be provided to patients for self-collection of stool. These will be distributed to patients prior to the collection date indicated by the Schedule of Events. Instructions for collection and kit return will be included as well as self-addressed and pre-paid postage. Subjects may also choose to bring the collection and kit to their study doctor at their scheduled visit.

10. PROTOCOL-SPECIFIED TREATMENT INFORMATION

All agents prescribed as part of this study are FDA-approved for colorectal cancer. Complete prescribing information can be found in the product's package insert. The information below is a summary of that information.

10.1 FOLFIRI + Bevacizumab

FOLFIRI is a combination of folinic acid (leucovorin), fluorouracil (5FU), and irinotecan (Camptosar). Bevacizumab is the generic name for Avastin.

10.1.1 Identification and Physical Description

All three medications that compose FOLFIRI are liquid and administered via intravenous infusion. Bevacizumab is administered as an intravenous infusion, as a clear to slightly opalescent, colorless to pale brown solution in a single-dose vial.

10.1.2 Administration

FOLFIRI is administered as an intravenous infusion. Folinic acid (leucovorin) is given over two hours before fluorouracil on Day 1 and irinotecan is given over 90 minutes on Day 1. Fluorouracil (5-FU) continuous infusion is given via an at-home infusion pump over 46 hours, beginning Day 1 and ending on Day 3. A cycle lasts 14 days.

Bevacizumab is administered as an intravenous infusion. The first infusion is administered over 90 minutes. The subsequent infusion is administered over 60 minutes if the first infusion is tolerated. Then, administer all subsequent infusions over 30 minutes if second infusion over 60 minutes is tolerated. A cycle last 14 days.

10.1.3 Storage, Handling, and Dispensing

Leucovorin: Prior to reconstitution, store below 40 °C (104 °F), preferably between 20 and 25 °C (68 and 77 °F), unless otherwise specified by manufacturer. Protect from light.

Irinotecan: Store at controlled room temperature 15°C to 30°C (59° to 86°F). Protect from light.

Fluorouracil (5-FU): Store at room temperature 15° to 30°C (59° to 86°F). Protect from light.
Bevacizumab: Store diluted Avastin solution at 2°C to 8°C (36°F to 46°F) for up to 8 hours. Discard any unused portion left in a vial, as the product contains no preservatives.

10.1.4 Contraindications
Leucovorin is contraindicated for pernicious anemia and other megaloblastic anemias secondary to the lack of vitamin B12.

Avoid the use of strong CYP3A4 inducers with FOLFIRI if possible; substitute non-enzyme–inducing therapies at least 2 weeks prior to initiation of nal-IRI. Avoid the use of strong CYP3A4 or UGT1A1 inhibitors, if possible; discontinue strong CYP3A4 inhibitors at least 1 week prior to starting therapy.

The following drugs are noted in the irinotecan prescribing information as interacting with irinotecan, and should be avoided: St. John's Wort, CYP3A4 inducing anticonvulsants (phenytoin, phenobarbital, and carbamazepine), ketoconazole, itraconazole, troleandomycin, erythromycin, diltiazem and verapamil. Because 5-FU interacts with warfarin, caution should be exercised if concomitant use is necessary.

None for Bevacizumab

10.1.5 Special Warnings and Precautions for Use
FOLFIRI: Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of treatment.

Bevacizumab: Gastrointestinal Perforations and Fistula, Surgery and Wound Healing Complications, Hemorrhage, Arterial Thromboembolic Events (ATE), Venous Thromboembolic Events (VTE), Hypertension, Posterior Reversible Encephalopathy Syndrome (PRES), Renal Injury and Proteinuria, Infusion-Related Reactions, Embryo-Fetal Toxicity, Ovarian Failure, and Congestive Heart Failure.

10.1.6 Adverse Effects

The most common adverse effects with FOLFIRI (>15%) include nausea/vomiting, diarrhea, neutropenia, mucositis, hair loss, anemia, hand-foot syndrome, thrombocytopenia, weakness, and fever. Supportive care will be administered per routine care.

Bevacizumab: Most common adverse reactions incidence (incidence > 10%) are epistaxis, headache, hypertension, rhinitis, proteinuria, taste alteration, dry skin, hemorrhage, lacrimation disorder, back pain and exfoliative dermatitis.

10.2 Cetuximab or Panitumumab

Cetuximab is the generic name for the U.S. Brand name drug Erbitux. Other names are C225, IMC-C225, MOAB C225. Eli Lilly and Co.

Panitumumab is the generic name for the brand name Vectibix, which is manufactured by Amgen, Inc.

10.2.1 Identification and Physical Description

Cetuximab is a recombinant human/mouse chimeric monoclonal antibody used to treat cancers of the colon and rectum. It is also used to treat head and neck cancer.

Panitumumab is an epidermal growth factor receptor antagonist indicated as a single agent for the treatment of metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens.

10.2.2 Administration

Cetuximab is given as an intravenous infusion. The recommended initial dose is 500 mg/m2 administered as a 120-minute intravenous infusion. The recommended subsequent dosage is 250 mg/m2 weekly as 60-minute infusion every 14 days until disease progression or unacceptable toxicity.

Panitumumab is given as an intravenous infusion. The recommended dose is 6 mg/kg over 60 minutes every 14 days.

10.2.3 Storage, Handling and Dispensing

Cetuximab: Store intact vials at 2°C to 8°C (36°F to 46°F); do not freeze or shake. Preparations in infusion containers are stable for up to 12 hours refrigerated at 2°C to 8°C (36°F to 46°F) and up to 8 hours at room temperature of 20°C to 25°C (68°F to 77°F).

Panitumumab: Store vials in original carton under refrigeration at 2°C to 8°C (36°F to 46°F) until time of use. Protect from direct sunlight. The diluted infusion solution should be used within 6 hours of preparation if stored at room temperature, or within 24 hours of dilution if stored at 2°C to 8°C (36°F to 46°F).

10.2.4 Contraindications

None

10.2.5 Special Warnings and Precautions for Use

Cetuximab:

Subjects may be pre-treated with an H1 receptor antagonist if there is concern for an infusion reaction. Serum electrolytes will be monitored during and after administration as outlined in the FDA package insert.

Lack of benefit with cetuximab has been observed in patients with colorectal tumors harboring a KRAS mutation. Thus, as noted in Appendix E, this treatment will be skipped if subject is harboring a KRAS mutation.

Panitumumab:

Limit sun exposure. Not indicated for use in combination with chemotherapy. Discontinue Vectibix in patient developing interstitial lung disease, pneumonitis, or lung infiltrates. Monitor electrolytes during and for 8 weeks after completion of Vectibix therapy and institute appropriate treatment.

10.2.6 Adverse Effects

Cetuximab: The most common adverse reactions (incidence ≥25%) include: cutaneous adverse reactions (including rash, pruritus, and nail changes), headache, diarrhea, and infection. Supportive care will be administered as per routine care.

Panitumumab: The most common adverse reactions (≥20%) are skin toxicities (i.e. erythema, dermatitis acneiform, pruritus, exfoliation, rash, and fissures), paronychia, hypomagnesemia, fatigue, abdominal pain, nausea, diarrhea, and constipation.

10.3 TAS-102

Drug Name: TAS-102 (Lonsurf), TAIHO Oncology, Inc.

10.3.1 Identification and Physical Description

Lonsurf is a combination of trifluridine and tipiracil, indicated for the treatment of patients with metastatic colorectal cancer. The drug is provided as 15mg or 20mg round white or red tablets.

10.3.2 Administration

Lonsurf is a tablet that is taken orally. The recommended dose is 35 mg/m2 twice daily on Days 1 through 5 and Days 8 through 12 of each 28-day cycle. Lonsurf should be taken within 1 hour after completion of morning and evening meals.

10.3.3 Storage, Handling and Dispensing

Tablets should be stored at room temperature (68°F to 77°F).

10.3.4 Contraindications

None

10.3.5 Special Warnings and Precautions for Use

Lonsurf may cause severe myelosuppression, consisting of anemia, neutropenia, thrombocytopenia, and febrile neutropenia. Obtain complete blood counts prior to and on Day 15 of each cycle, and more frequently as clinically indicated. Lonsurf will be withheld for febrile neutropenia, Grade 4 neutropenia, or platelets less than 50,000/mm^3 and resumed at a reduced dose, as per the FDA package insert.

10.3.6 Adverse Effects

The most common adverse reactions (≥10%) include anemia, neutropenia, asthenia/fatigue, nausea, thrombocytopenia, decreased appetite, diarrhea, vomiting, abdominal pain, and pyrexia. Supportive care will be administered as per routine care.

10.4 Regorafenib

Regorafenib is the generic name for the brand name Stivarga, which is manufactured by Bayer.

10.4.1 Identification and Physical Description

Regorafenib is a kinase inhibitor used for patients with metastatic colorectal cancer and liver cancer. Tablets are supplied in bottles of 28 tablets.

10.4.2 Administration

Regorafenib is taken orally once per day. The usual adult dose for colorectal cancer is 160 mg orally once a day for the first 21 days of each 28-day cycle until disease progression or unacceptable toxicity. This drug should be taken at the same time each day with food.

10.4.3 Storage, Handling and Dispensing

Store at room temperature (approximately 77°F). To protect from moisture, keep tablets in the original bottle and do not remove the desiccant. Discard any unused tablets 7 weeks after opening the bottle.

10.4.4 Contraindications

None.

10.4.5 Special Warnings and Precautions for Use

If subjects have wild type KRAS mutation, they should be first treated with an anti-EGFR therapy consistent with Appendix E.

Subjects should avoid taking strong CYP3A4 inducers or CYP3A4 inhibitors or inhibitors while on this medication.

10.4.6 Adverse Effects

The most common adverse reactions (≥30%) include asthenia/fatigue, decreased appetite and food intake, hand-foot skin reaction (HFSR) [palmar-plantar erythrodysesthesia (PPE)], diarrhea, mucositis, weight loss, infection, hypertension, and dysphonia. Supportive care will be provided as per routine care.

10.5 Capecitabine

The brand name of capecitabine is Xeloda.

10.5.1 Identification and Physical Description

Capecitabine is a nucleoside metabolic inhibitor with antineoplastic activity used to treat multiple cancers, including colorectal cancer. The drug is supplied as film-coated oblong tablets.

10.5.2 Administration

Capecitabine is taken twice daily for the first 14 days of each 21-day cycle. Food has been shown to reduce both the rate and extent of absorption of capecitabine.

10.5.3 Storage, Handling and Dispensing

Capecitabine should be stored in a tight container at room temperature (approximately 77°F). The bottle must remain tightly closed. Tablets should not be cut or crushed.

10.5.4 Contraindications

Contraindications for use include known hypersensitivity to capecitabine or any ingredient in the formulation.

Additionally, the following drugs are known to interact with capecitabine: anticoagulants, phenytoin, leucovorin, and CYP2C9 substrates.

10.5.5 Special Warnings and Precautions for Use

Do not initiate capecitabine therapy if baseline ANC $<1500/mm^3$ and/or baseline platelet count $<100,000/mm^3$.

Patients receiving capecitabine who also take oral coumarin-derivative anticoagulants, such as warfarin and phenprocoumon, should have their anticoagulant response (PT/INR) monitored frequently in order to adjust the anticoagulant dose accordingly.

Cardiotoxicity is common in patients with a prior history of coronary artery disease.

Severe mucocutaneous reactions, Steven-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN), have been reported. XELODA should be permanently discontinued in patients who experience a severe mucocutaneous reaction during treatment.

There is an increased risk of severe or fatal adverse reactions to capecitabine in patients with low or absent Dihydropyrimidine Dehydrogenase (DPD) activity.

10.5.6 Adverse Effects

Most common adverse reactions (≥30%) were diarrhea, hand-and-foot syndrome, nausea, vomiting, abdominal pain, fatigue/weakness, and hyperbilirubinemia. Supportive care will be provided as per routine care.

10.6 FOLFIRI

FOLFIRI is a combination of folinic acid (leucovorin), fluorouracil (5FU), and irinotecan (Camptosar). See details for FOLFIRI noted above in section 10.1

10.7 FOLFOX/CAPOX

FOLFOX is a combination of folinic acid (leucovorin), fluorouracil (5FU), and oxaliplatin (Eloxatin).

CAPOX is a combination of Capecitabine (Xeloda) and Oxaliplatin (Eloxatin).

10.7.1 Identification

All three medications that compose FOLFOX are liquid and administered via intravenous infusion. Eloxatin is a platinum-based drug used in combination with infusional 5-fluoracil/leucovorin, which is indicated for adjuvant treatment of Stage III colon cancer in patients who have undergone complete resection of the primary tumor, and treatment of advanced colorectal cancer.

For CAPOX, Oxaliplatin is administered via intravenous infusion, while Capecitabine is administered orally.

10.7.2 Administration

FOLFOX: Folinic acid 400mg/m2 IV over two hours before fluorouracil (Day 1), Oxaliplatin IV 85mg/m2 over two hours on Day 1, commonly at the same time as folinic acid. Fluorouracil (5-FU) 400mg/m2 IV push day 1, then 1,200mg/m2/day ×

2 days (total 2,400mg/m2 over 46–48 hours) IV continuous infusion, via an at-home infusion beginning Day 1 and ending on Day 3. The time between doses of oxaliplatin should be 14 days.

CAPOX: Day 1: Capecitabine 850 – 1000 mg/m2, by mouth, twice daily, for the first 14 days of each 21-day cycle. Oxaliplatin IV 130 mg/m2 over 2 hours on Day 1.

10.7.3 Storage, Handling, and Dispensing

FOLFOX: Oxaliplatin: Unopened vials should be stored at 15°C -30°C; protect from light. Leucovorin: Prior to reconstitution, store below 40 °C (104 °F), preferably between 20 and 25 °C (68 and 77 °F), unless otherwise specified by manufacturer. Protect from light. Fluorouracil (5-FU): Store at room temperature 15° to 30°C (59° to 86°F). Protect from light.

CAPOX: Oxaliplatin: Unopened vials should be stored at 15°C -30°C; protect from light. Capecitabine: Store in a tight container at room temperature (approximately 77°F). The bottle must remain tightly closed. Tablets should not be cut or crushed.

10.7.4 Contraindications

FOLFOX: Known allergy to Eloxatin or other platinum compounds.

CAPOX: Capecitabine: Known hypersensitivity to capecitabine or any ingredient in the formulation. Additionally, the following drugs are known to interact with capecitabine: anticoagulants, phenytoin, leucovorin, and CYP2C9 substrates. Oxaliplatin: Nephrotoxic drugs (e.g. aminoglycosides, amphotericin, cisplatin, contrast dye, frusemide, NDSAIDs), Neurotoxic drugs (e.g. vincristine, paclitaxel).

10.7.5 Special Warning and Precautions

FOLFOX: Allergic Reactions: Monitor for development of rash, urticaria, erythema, pruritis, bronchospasm, and hypotension. Neuropathy: Reduce the dose or discontinue Eloxatin if necessary. Pulmonary Toxicity: May need to discontinue Eloxatin until interstitial lung disease or pulmonary fibrosis are excluded. Hepatotoxicity: Monitor liver function tests.

CAPOX:

Oxaliplatin: Patients should be warned about cold avoidance prior to receiving oxaliplatin; ice for mucositis prophylaxis should not be used. Oxaliplatin may cause dizziness or visual disturbances in some patients; exercise caution when driving or operating machinery. Patients with risk factors for dehydration, pre-existing renal dysfunction or on nephrotoxic agents. Patients with a history of cardiovascular disease. Patients taking anticoagulants such as warfarin.
Capecitabine: Do not initiate capecitabine therapy if baseline ANC <1500/mm^3 and/or baseline platelet count <100,000/mm^3. Patients receiving capecitabine who also take oral coumarin-derivative anticoagulants, such as warfarin and phenprocoumon, should have their anticoagulant response (PT/INR) monitored frequently in order to adjust the anticoagulant dose accordingly. Cardiotoxicity is common in patients with a prior history of coronary artery disease. Severe mucocutaneous reactions, Steven-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN), have been reported. XELODA should be permanently discontinued in patients who experience a severe mucocutaneous reaction during treatment.

10.7.6 Adverse Effects

FOLFOX: Most common adverse reactions (incidence ≥ 40%) were peripheral sensory neuropathy, neutropenia, thrombocytopenia, anemia, nausea, increase in transaminases and alkaline phosphatase, diarrhea, emesis, fatigue and stomatitis.

CAPOX: Hypersensitivity reaction, Laryngo-pharyngeal dysesthesia, Cardiotoxicity, Nausea and vomiting, Taste and smell alternation, Neutropenia, Thrombocytopenia, Diarrhea, Oral mucositis, Palmer-planter erythrodysaesthesia (PEE) – hand-foot syndrome (HFS), Peripheral neuropathy, Fatigue, Actinic keratoses flare, Photosensitivity, Ocular changes, Abdominal pain, Anemia, Nail changes, Alopecia-partial, and Hyperbilirubinaemia. Capecitabine: Most common adverse reactions (≥30%) were diarrhea, hand-and-foot syndrome, nausea, vomiting, abdominal pain, fatigue/weakness, and hyperbilirubinemia.

11. ADVERSE EVENTS

11.1 Definitions

11.1.1 Adverse Event

The term "adverse event" (AE) includes any sign, symptom, syndrome, or illness that appears or worsens in a subject during the period of observation in the clinical study and that may impair the wellbeing of the subject. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically significant (*e.g.*, that requires unscheduled diagnostic procedures or treatment measures, or result in withdrawal from the study). An AE is therefore any unfavorable and unintended symptom associated with the study, whether or not related to a study procedure.

The adverse event may be:

- A new illness/condition;
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness/condition;
- An effect of a protocol-specified procedure; or
- A combination of 2 or more of these factors.

No causal relationship with the clinical study is implied by the use of the term "adverse event."

The Investigator or his/her designee will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. AEs will be recorded in the subject CRF. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to the study, or if unrelated, the cause.

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition(s) for which the surgery is required may be an adverse event. Planned surgical measures permitted by the clinical study protocol and the condition(s) leading to these measures are not adverse events.

When a clear diagnosis is available that explains the abnormal objective findings, this diagnosis will be recorded as an adverse event and not the abnormal objective findings (*e.g.*, viral hepatitis will be recorded as the adverse event and not the transaminase elevation). If a definitive diagnosis is not available, then the sign(s) (*e.g.*, clinically significant elevation of transaminase levels) or symptom(s) (*e.g.*, abdominal pain) will be

recorded as the adverse event.

Adverse events fall into the categories "serious" and "non-serious."

11.1.2 Serious Adverse Event

A serious adverse event is one that at any time during the period of observation:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. "Medically important" should be marked only if no other serious criteria are met.

An "unexpected SAE" is any SAE for which the nature, specificity or severity is not consistent with the currently known adverse event profile of the disease under study or the investigational agent(s).

NOTE: The following hospitalizations are not considered SAEs in UFHCC clinical studies:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- admission encountered for another life circumstance that carries no bearing on

health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Clarification of the difference in meaning between "severe" and "serious"

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). Any grade ≥ 3 adverse events per CTCAE is generally considered severe AE. This is not the same as "serious," which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

11.1.3 Non-Serious Adverse Event

A non-serious adverse event is any adverse event not meeting any of the serious adverse event criteria.

11.2 Period of Observation

Following randomization, all SAEs must be collected. Collection of all SAEs must continue for **30 days** after the last study visit. The investigator should notify the DISC of any SAE occurring after this time period that is believed to be related to a protocol-specified procedure. Adverse events will not be collected for subjects who do not have measurable ctDNA, or prior to documentation of the presence of measurable ctDNA at baseline, because they will move straight into routine care and only be contacted every approximately 3 months for survival status.

The investigator will also begin collecting non-serious adverse event (NSAE) information once randomized, for those who have measurable ctDNA. Randomized subjects, including those who were prematurely discontinued from the study, will be followed for any adverse events that occur during the study until 30 days following the last study visit (i.e., until the Follow-up Visit). However, if another course of anti-cancer therapy is initiated outside the context of this study and prior to the 30-day follow-up period visit, collection of adverse events will no longer be performed, with the exception of events that may be possibly, probably, or definitely related to the study or are clinically significant.

11.3 Documenting and Reporting of Adverse Events by Investigator

All adverse events at least possibly related to the study and occurring after randomization, until the Follow Up Visit, must be fully recorded in the subject's case record form.

Documentation must be supported by an entry in the subject's file. A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

Every attempt should be made to describe the adverse event in terms of a diagnosis that encompasses the component signs and symptoms. If only nonspecific signs or symptoms are present, then these should be recorded as separate diagnoses on the pages of the case report form.

All subjects who have adverse events must be monitored to determine the outcome. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the Principal Investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

11.3.1 Assessment of Causal Relationship to the Study

The Investigator will provide an assessment of the potential causal relationship between adverse events and study by determining whether or not there is a reasonable possibility that the event was caused by the study. Because all treatments used in this study are standard of care, and would be provided in the same order at the same dose outside the study as part of routine care, side effects deemed to be associated with treatment will not be recorded or collected. The relationship or association of the adverse event to the study will be characterized as not related, unlikely related, possibly related, probably related, or related:

Not Related: There is not a temporal relationship to the study or the adverse event is clearly due only to the progression of the underlying disease state, intercurrent illness, concomitant medication, concurrent therapy or other known cause.

Unlikely Related: There is little or no chance that the study caused the adverse event; the event is most likely due to another competing cause, including intercurrent illness, progression or expression of the disease state, or a reaction to a concomitant medication or concurrent therapy appearing to explain the reported adverse event.

Possibly Related: The association of the adverse event with the study is unknown; however, the adverse event is not reasonably attributed to any other condition.

Probably Related: When a reasonable temporal relationship exists between the adverse event and the study; significant symptoms abate upon discontinuation of the study and there is a reasonable explanation based on known characteristics of thestudy and there is no clear association with preexisting disease or therapy, intercurrent illness, concurrent therapy or other factor(s).

Related: When the adverse event is a known side effect of a protocol-specified procedure or there is a temporal relationship to the suspected protocol-specified procedure; or the adverse event reappears upon re-entry into the study ; or the significant symptoms of the adverse event abate upon discontinuation of the study.

11.3.2 Intensity of Adverse Events

The intensity of adverse changes in physical signs or symptoms will be graded according to the most up-to-date CTCAE version. For all other adverse events not described in the CTCAE, the intensity will be assessed by the Investigator using the following categories:

Mild (Grade 1) – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.

Moderate (Grade 2) – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required.

Severe (Grade 3) – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible.

Life-threatening (Grade 4) – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

Death (Grade 5) – the event resulted in death.

11.3.3 Action Taken with Drug(s)

The action the Investigator took with protocol-specified treatment as a result of the event should be recorded as one of the following:

None – No action was taken with regard to the protocol-specified treatment as a result of the adverse event.

Interrupted – protocol-specified treatment was stopped due to the adverse event, but

was later resumed at the same dose.

Dose decreased – The dose of protocol-specified treatment was decreased as a result of the adverse event.

Permanently discontinued – The subject was withdrawn from the study due to the adverse event.

Only one item should be chosen. If multiple actions apply, the following "worst case" scenario hierarchy should be used to determine the preferred entry: permanently discontinued > dose decreased > interrupted.

11.3.4 Definition of Outcome

The outcome of the AE should be recorded as one of the following:

Resolved without sequelae – The subject fully recovered from the adverse event with no observable residual effects.

Resolved with sequelae – The subject recovered from the adverse event with observable residual effects.

Not resolved – The adverse event was present at the time of last observation.

Death – The subject died as a result of the adverse event.

11.4 Immediately Reportable Events

11.4.1 Serious Adverse Events

Serious adverse events (SAEs) during the period of observation must be reported to the UFHCC CRO Project Management Office (PMO; pmo@cancer.ufl.edu), and entered into OnCore, within **24 hours** of discovery of the event. All SAEs regardless of "relatedness" will need to be reported to DISC by PMO within 5 business days of discovery of the event. If only limited details are known, these should be reported within that time frame and follow up reports can be submitted for elaboration, clarifications, or corrections. Any email correspondence must be kept in the trial file at the study site. The site investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

Follow-up information will be submitted to the UFHCC CRO PMO (pmo@cancer.ufl.edu), stating that this is a follow-up to a previously reported SAE and giving the date of the

original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the participant continued or withdrew from study participation. PMO will confirm that the event and any necessary follow-ups are reported to the UFHCC Data and Safety Integrity Committee (DISC), the sponsor, and any other regulatory authorities as required (FDA, etc.)

11.4.2 Other Events Requiring Immediate Reporting

All pregnancies, regardless of outcome, must be reported to the UFHCC CRO Project Management Office (PMO; pmo@cancer.ufl.edu), including pregnancies that occur in the female partner of a male study subject. All pregnancies must be followed to outcome. Pregnancies and follow-ups will be submitted by PMO to DISC, the sponsor if required, and any other regulatory authorities required.

Although overdose and cancer are not always serious by regulatory definition, these events should also be reported to the UFHCC CRO Project Management Office (PMO; pmo@cancer.ufl.edu), in addition to other institutional officials per safety reporting requirements, in an expedited manner. In case the overdose did not result in any adverse event, the Investigator should report this as "overdose, no adverse event" and provide the intended amount, as well as the actual amount, of drug administered. In the event of overdose or exaggerated response, appropriate supportive measures should be employed. Actual treatment should depend on the severity of the clinical situation and the judgment and experience of the treating physician. Overdose events and follow-ups will be submitted by PMO to DISC, the study sponsor if required, and any other regulatory authorities required.

Pregnancies and overdoses should be documented and reported per the reporting guidelines below.

Institution Immediate Reporting Information
SAEs and pregnancies must be reported to the UFHCC CRO Project Management Office (PMO), or assigned designee at: Email: pmo@cancer.ufl.edu As well as entered in OnCore, the study CTMS.

11.4.3 Reporting to Natera

The UFHCC CRO Project Management Office (PMO) will forward any SAE to Natera as agreed upon in the contract. PMO will forward both initial and follow-up versions of each report. Reports will be marked with the study ID, product, and description of event. Only SAEs that are related to the procedure (blood draw) will be reported, and only those that are unexpected.

Natera AE Reporting Information
Contact: Dr. Alexey Aleshin Email: aaleshin@natera.com

12. STATISTICAL METHODS

The sections below provide an overview of the statistical considerations and analyses.

12.1 Data collection and management plan

Data will be collected via paper source documents and the medical record, and will subsequently be entered into a validated EDC (REDCap) which will be monitored and audited on a regular basis to ensure accuracy. Data will be accessible only to certified members of the research team, under the supervision of the PI, and will be fully de-identified prior to analysis. All paper records will be archived for a pre-specified length of time as per applicable regulations and federal laws.

12.2 Stratification

Eligible patients will be randomized 1:1 between the two arms with stratification for RAS status (Mutated vs. non-mutated), line of therapy (2^{nd} line vs. 3^{rd} line or later) and initial stage at diagnosis (stage IV at diagnosis vs. less than stage IV).

12.3 Sample Size Justification

A sample size of 26 in each group in stage 1 and 39 in final stage can detect a hazard ratio of 0.55 comparing ctDNA-guided treatment (median survival times: 11 months) and scan-guided treatment group (median survival times: 6 months) assuming 3 years for patient accrual and 1 year for follow up to achieve 0.1 type-1 error rate and 80% power. The calculation is based on one-sided Z-test group sequential testing via simulation, equally incremented 2 stages, two-sided symmetric futility boundary,

O'Brien-Flemming beta spending function and binding futility. The futility boundaries of *p*-value are in the table below:

Stage	Sample Size (ctDNA-guided)	Sample Size (scan-guided)	Upper Futility Boundary	Lower Futility Boundary
1	26	26	0.46225	0.46225
2	39	39	0.05268	0.05268

12.4 Analysis of Primary Endpoint

The study primary endpoint is Overall Survival (OS) defined as time between date of randomization and death/censoring. Stratified log-rank test or weighted log-rank test will be used to compare OS between ctDNA-guided treatment group and scan-guided treatment group. Stratification factors are described in Section 12.2.

12.5 Analysis of Secondary Endpoints / Safety Data

Secondary endpoints include: Progression Free Survival (PFS), defined as time between date of randomization and disease progression or death (whichever comes first); Best Overall Response (OR), defined as proportion of patients having each category of response (Complete Response, Partial Response, Stable Disease, and Progressive Disease) during the period of study participation. To compare secondary endpoints between ctDNA-guided treatment group and scan-guided treatment group, stratified log-rank test or weighted log-rank test will be used for PFS; Cochran-Mantel-Haenszel (CMH) test will be used for best OR. Weighted kappa will be used to assess the agreement between ctDNA vs. response assessment per RECIST.

The incidence, severity and reversibility of toxicities will be performed on all subjects who receive any dose of study medication. Non-serious adverse events that occur more than 30 days after the administration of the last dose of treatment will not be included. The safety and tolerability of treatment is determined by reported AEs, physical examinations, laboratory tests, and ECGs. AEs will be summarized with the incidence and percentage of subjects with at least one occurrence of a preferred term (NCI - CTCAE Version 5 grade) will be included. The number of AEs reported will also be summarized. Causality (relationship to the protocol-specified treatment) will be summarized separately. Duration of AE will be determined and included in listings along with action taken and outcome.

Laboratory results will be classified according to NCI-CTCAE, Version 5.0. Incidence of laboratory abnormalities will be summarized; laboratory results not corresponding to an NCI- CTCAE Version 5.0 term will not be graded. Laboratory toxicity shifts from baseline to worst grade will also be provided. The results from physical examination and vital sign measurement will be tabulated. Descriptive statistics by arm will be provided as appropriate and monitored by the safety stopping rule.

12.6 Analysis of Exploratory Endpoints

Exploratory analysis of microbiome and other biomarkers will be descriptive in nature.

12.7 Interim Analysis

An interim analysis for futility will be performed after the first 26 subjects are enrolled onto each arm in the trial. The futility boundaries are shown in Section 12.3. The trial will be stopped if the futility boundary is exceeded, otherwise an additional 13 patients will be enrolled onto each arm if futility is not exceeded to enroll the full sample of 78 subjects. Enrollment will not be suspended during futility or safety interim analyses.

An interim analysis for safety will be performed after the first 20 subjects are enrolled in each arm based on clinical considerations. At this time, the proportion of Grade 3 or higher toxicity events in the experimental arm and the control arm will be calculated, and if there are more than 50% Grade 3 or higher toxicity events in either arm, accrual will be held for safety review. If deemed safe and appropriate, enrollment will continue up to 78 subjects.

12.8 Data Integrity and Safety Committee

This protocol will be reviewed and monitored by the University of Florida Health Cancer Center Data Integrity and Safety Committee (UFHCC DISC) in accordance with their policies and procedures. They will review and monitor study progress, toxicity, safety and other data from this trial. Questions about subject safety or protocol performance will be addressed with the sponsor-investigator, statistician and study team members. Should any major concerns arise, the DISC will offer recommendations regarding whether or not to suspend the trial.

UFHCC DISC data and safety monitoring activities include:

- Review of clinical trial conducted for progress and safety
- Review of all adverse events requiring expedited reporting as defined in the protocol
- Review of reports generated by data quality control review process

- Notification of the sponsor-investigator of recommended action
- Notification of sites coordinated by the UFHCC of adverse events requiring expedited reporting and subsequent committee recommendations for study modifications

In compliance with the UFHCC data and safety monitoring plan, the PI will provide a Data Integrity and Safety Committee Report to DISC at the predetermined timelines for the level of risk category assigned during the initial SRMC (Scientific Review and Monitoring Committee) review, which occurs prior to initial IRB approval.

UFHCC investigator-initiated protocols will be classified into one of the following categories of risk by the SRMC (see *SRMC manual* for further details):

Level 1 – **Low risk** Investigator Initiated interventional trials.
Level 2 – **Moderate risk** Investigator Initiated or externally sponsored interventional (such as drug, biologic or device) trials using FDA approved or commercially available compounds or interventions.
Level 3 – High **risk** Investigator Initiated or externally sponsored interventional trials (such as investigator-sponsored INDs, Phase I trials, studies requiring biosafety approval, or other areas that may be designated by NIH as high risk).
Level 4 – Complex trials involving **very high risk** to subjects and a high level of complexity such as first in human or gene transfer studies.

The risk level assigned by SRMC will determine the appropriate level of DISC monitoring required, with increased monitoring required for higher-risk trials.

Findings will be communicated to all study sites by UFHCC CRO.

12.9 Data Monitoring

UFHCC (University of Florida Health Cancer Center) Quality Assurance team and/or project management officers will perform remote monitoring and may make monitoring visits to the trial sites periodically during the trial to confirm that all sites are complying with the protocol. Source documents will be reviewed for completion and validated against data submitted electronically via Electronic Data Capture. The site investigator/institution guarantee access to source documents by UFHCC or its designee and appropriate regulatory agencies. As part of the responsibilities assumed by conducting this study, the Principal Investigator (PI) agrees to maintain and have available for monitoring adequate case records (accurate source documents and CRFs) for the subjects treated under this protocol.

The trial site may also be subject to quality assurance audit by any collaborating sponsors or their designee as well as inspection by appropriate regulatory agencies.

It is important for the site investigator and their relevant personnel to be available during the monitoring visits and possible audits and for sufficient time to be devoted to the process.

12.10 Principal Investigator Responsibilities

Per UF IRB requirements, the PI is personally responsible for conducting and supervising the conduct of human subjects research by "protecting the rights, safety, and welfare of subjects under the investigator's care." The PI also must ensure that all the research conducted is done so in an ethical manner and in accordance with all federal, state, and local laws and regulations, institutional policies, and the requirements of the IRB.

Oversight is defined as "management by overseeing the performance or operation of a person or group; watchful care, superintendence, general supervision". Any person serving as a PI has voluntarily accepted these responsibilities and is expected to fully comply with these requirements, as outlined in the UFHCC Guidance: *Principal Investigator Responsibilities and Oversight.*

The PI will be primarily responsible for continuous monitoring of adverse events, protocol violations, and other immediate protocol issues. The study coordinator will collect information on subjects enrolled through the use of electronic or paper adverse event (AE) forms, CRFs, and Informed Consent forms.

13. EMERGENCY PROCEDURES

13.1 Emergency Contact

In emergency situations, the treating physician should contact the Principal Investigator by telephone at the number listed on the title page of the protocol.

13.2 Emergency Identification of Investigational Products

This is a non-blinded, randomized study. Thus, there will be no need for unmasking procedures.

13.3 Emergency Treatment

During and following a subject's participation in the study, the treating physician and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the study.

14. ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

14.1 Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Principal Investigator and Co-Investigators abide by Good Clinical Practice (GCP), as described in International Conference on Harmonization (ICH) Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki.

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board (IRB) approval before initiation of the study.

The Principal Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

All potential serious breaches must be reported immediately to the UFHCC Project Management Office (PMO, pmo@cancer.ufl.edu, who will then report the breach to UFHCC DISC) and the IRB of record, if applicable. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

14.2 Institutional Review Board

Before study initiation, the investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, information to be provided to subjects, and any updates. The investigator should provide the IRB with reports, updates, and other information (e.g., amendments, and administrative letters) according to regulatory requirements or institution procedures.

14.3 Compliance with Laws and Regulations

It is intended that the proposed study be conducted according to the International Conference on Harmonization E6 Guideline for Good Clinical Practice (GCP) and the

Declaration of Helsinki. Please refer to the International Conference on Harmonization and GCP:

http://www.fda.gov/oc/gcp/guidance.html; Declaration of Helsinki: http://www.fda.gov/oc/health/helsinki89.html; Code of Federal Regulations, Title 21: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch. cfm]

All UF Health Cancer Center investigator-initiated trials, meeting the criteria of the FDAAA's applicable clinical trials, will be registered with ClinicalTrials.gov by the Project Management Officer (PMO) or assigned designee. All studies must be registered prior to enrollment of the first participant. The Project Management Officer or assigned designee will maintain the responsibility of updating trials registered with ClinicalTrials.gov. Per FDA requirement, information must be updated at least every twelve months and the registry must be updated within thirty days of any changes in recruitment status or completion of the study. The PMO will determine if registration and updates to the NCI CTRP are required.

14.4 Delegation of Investigator Responsibilities

The Principal Investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the protocol-specified treatment, and their study-related duties and functions. The Principal Investigator will maintain a list of Co-Investigators and other appropriately qualified persons to whom s/he has delegated significant study-related duties.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment). Systems with procedures that ensure the quality of every aspect of the study will be implemented.

14.5 Subject Information and Informed Consent

Before being enrolled in this clinical trial, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her. An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. This document will contain all ICH, GCP, and locally required regulatory elements. The document must be in a language understandable to the subject and must specify the person who obtained informed consent.

After reading the informed consent document, the subject must give consent in writing. The written informed consent will be obtained prior to conducting any study-related procedures or tests. The subject's consent must be confirmed at the time of consent by the personally dated signature of the person conducting the informed consent discussions and a copy of the consent form (preferably signed) must be given to the subject for their records.

The PI will retain the original signed consent document. The PI will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

14.6 Confidentiality

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

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

Subjects will be told that the IRB, UF Health DISC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection law.

14.7 Protocol Amendments

Once the study has started, amendments should be made only in exceptional cases. Protocol amendments will not be implemented without prior written IRB approval. All amendments will be submitted to the IRB and SRMC (as applicable), and written verification that the amendment was submitted and subsequently approved is to be obtained, and notification will be sent out to the applicable study teams, prior to implementing the amendment.

On an emergency-basis, to eliminate an immediate safety hazard to a subject, a protocol deviation may be implemented immediately, provided the IRB and UFHCC CRO PMO (pmo@cancer.ufl.edu) are notified within **5** business days with a full justification and description of the event.

14.8 Case Report Forms

The Principal Investigator and/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 protocol-required

outcomes for safety monitoring and data analysis. All study data will be entered electronically in an Electronic Data Capture system in accordance with the protocol schedule of events and guidelines developed in the Data Management Plan for the study, using a secure access account.

All protocol data is the sole property of UFHCC and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from UFHCC.

14.9 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

UF Health Cancer Center requires that all study documentation be maintained for at least 6 years from the date of final study publication. No study records may be destroyed without prior authorization from UF.

15. REFERENCES

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16. APPENDICES

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Appendix A: PROTOCOL VERSION HISTORY / SUMMARY OF CHANGES

Protocol Version Number	Protocol Version Date	Affected Section(s)	Summary of Revisions Made
1.0	February 24, 2021	N/A- Initial protocol	
1.1	June 11, 2021	Table of Contents	Update page numbers
		Protocol Synopsis	Inclusion Criteria K Creatinine Clearance Value
		Section 4.2	Inclusion Criteria K Creatinine Clearance Value
1.2	July 14, 2021	Section 3.1	Updated treatment decisions
		Section 6, 6.1	Updated screening period Updated Footnote 6
		Appendix E	Updated proposed timeline
1.3	August 9, 2021	Title Page	PI change
2.0	November 17, 2021	Protocol Synopsis	Inclusion Criteria D
		Section 1.3	Eligible subjects
		Section 3.1	Measurable ctDNA update
		Section 4.2	Inclusion Criteria D
		Section 7.1	Update for non-measurable ctDNA
		Section 12.2	Updated to 3rd line or later
3.0	December 1, 2021	Protocol Synopsis	Updated primary objective language and Inclusion Criteria B
		Section 1.3	Updated eligible subjects language
		Section 2.1.1	Updated primary objective language
		Section 3.1	Included later line of therapy in agent entry
		Section 4.2	Updated inclusion Criteria B
		Section 6	Added re-screen procedures
		Section 6.1	Updated screening window, and abbreviations 4, 6 and 14.
		Section 7.1	Included later line of therapy in agent entry
		Section 11	Updated adverse event reporting, since all treatments are standard of care
		Appendix D	Clarified subject's starting treatment #
3.1	August 2nd, 2022	Protocol Synopsis and Section 4.2	Rephrased inclusion criteria D
		Section 6	Rephrased ctDNA re-collection if >14 days from start of trt

		Section 6.1	Updated Footnote 6 to provide clarification on screening/baseline ctDNA sampling Updated Footnote 10 to clarify "visits" for the research blood collection timepoint Updated Footnote 11 to clarify which procedures need not be repeated for C1D1 assessments in the event the visit occurs in close proximity to the screening visit
		Sections 11.2 and 11.4.1	Updated SAE reporting guidelines
3.2	August 18th, 2022	Section 3.1 and Section 6.1 Footnote 13	Added in the event that the initial ctDNA results are not available within the 14-day randomization window.
3.3	November 15th, 2022	Title Page	Updated biostatistician
		Appendix D	Added subject pill diaries
3.4	July 24th, 2023	Appendix E	Updated to allow the addition of Bevacizumab or biosimilar anti-VEGF therapy to treatment regimen at physician discretion. Corrected typographical error in Appendix lettering throughout protocol.

Appendix B: PERFORMANCE STATUS CRITERIA

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

Appendix C: RECIST GUIDELINES (VERSION 1.1)

Response and progression will be evaluated in this study using the international criteria proposed by the New Response Evaluation Criteria in Solid Tumors (RECIST): Revised RECIST Guideline (version 1.1).

Measurability of Tumor at Baseline

Tumor lesions/lymph nodes will be categorized at baseline as measurable or non-measurable. Measurable disease is defined by the presence of at least 1 measurable lesion.

Measurable

Tumor lesions: Measured in at least 1 dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (slice thickness ≤5 mm)
- 10 mm caliper measurement by clinical exam (non-measurable lesions if cannot be accurately measured with calipers)
- 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 scan (CT scan thickness recommended to be ≤5 mm).

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/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitis involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special Considerations for Lesion Measurability

Bone Lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI, can be considered measurable lesions if the soft tissue component meets the definition of measurability.
- Blastic bone lesions are non-measurable.

Cystic Lesions:

- Simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable)
- Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability. If non-cystic lesions are presented in the same subjects, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment:

- Tumor lesions situated at a previously irradiated area, or in an area subjected to other loco-regional therapy, are non-measurable unless there has been demonstrated progression in the lesion.

Baseline Documentation of Target and Non-Target Lesion

Target Lesions

When more than 1 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. Non-nodal target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and can be reproduced in repeated measurements. Measurable lymph nodes are target lesions if they meet the criteria of a short axis of ≥15 mm by CT scan. All measurements are to be recorded in the CRF in millimeters (or decimal fractions of centimeters).

Non-target Lesions

All other lesions (or sites of disease) are identified as non-target lesions (chosen based on the representativeness of involved organs and the ability to be reproduced in repeated measurements) and should be recorded at baseline. Measurement of these lesions are not required but should be followed as 'present,' 'absent,' or in rare cases 'unequivocal progression.' In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the CRF (for example, multiple liver metastases recorded as 1 liver lesion). Lymph nodes with short axis ≥10 mm but <15 mm should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and are not recorded or followed.

Specifications by Methods of Measurement

All measurements should be recorded in metric notation, using a ruler or calipers if clinically assessed. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessed by clinical exam.

An adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a consistent method is used on subsequent examinations for any given subject. If prior to enrollment it is known a subject is not able to undergo CT scans with intravenous contrast due to allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (with or without intravenous contrast) should be used to evaluate the subject at baseline and follow-up should be guided by the tumor type under investigation and the anatomic location of the disease.

Clinical Lesions: Clinical lesions will be considered measurable only when they are superficial and ≥10 mm diameter as assessed using calipers (for example, skin nodules). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion is recommended. When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray when progression is an important endpoint. lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT and MRI: CT scan is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on CT scan assumes that CT slice thickness is ≤5 mm. When CT scan have slice thickness >5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (for example, for body scans). If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Ultrasound: Ultrasound should not be used to measure lesion size. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.

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

Tumor Markers: Tumor markers alone cannot be used to assess tumor response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete response (CR).

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete response (CR) in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (for example, with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease (SD) in order to differentiate between response (or SD) and progressive disease (PD).

PET Scan (FDG-PET, PET CT): PET is not recommended for lesion assessment. If a new lesion is found by PET, another assessment must be done by CT, unless the PET CT is of diagnostic quality. If CT is done to confirm the results of the earlier PET scan, the date of progression must be reported as the earlier date of the PET scan.

Bone Scan: If lesions measured by bone scan are reported at baseline, it is necessary to repeat the bone scan when trying to identify a complete response (CR) or partial response (PR) in target disease or when progression in bone is suspected.

Response Criteria

Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (including the baseline sum if that is the smallest). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of 1 or more new lesions is also considered progression. For equivocal findings of progression (for example, very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Not Evaluable (NE): When an incomplete radiologic assessment of target lesions is performed or there is a change in the method of measurement from baseline that impacts the ability to make a reliable evaluation of response.

Evaluation of Non-target Lesions

Complete Response: Disappearance of all non-target lesions. All lymph nodes must be non-pathological or normal in size (<10 mm short axis).

Non-CR/ non-PD: Persistence of 1 or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease: Unequivocal progression of existing non-target lesions. The appearance of 1 or more new lesions is also considered progression.

Not Evaluable: When a change in method of measurement from baseline occurs and impacts the ability to make a reliable evaluation of response.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the protocol-specified treatment until the earliest of objective progression or start of new anticancer therapy, considering any requirement for confirmation. The subject's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. The best overall response will be calculated via an algorithm using the assessment responses provided by the investigator over the course of the trial.

Time Point Response

It is assumed that at each protocol-specified time point, a response assessment occurs. (When no imaging/measurement is done at all at a particular time point, the subject is not evaluable (NE) at that time point.) Table 17.2.1 provides a summary of the overall response status calculation at each time point for subjects who have *measurable disease* at baseline.

Table 17.2.1- Time Point Response: Subjects with Target or Measurable Disease

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD

Not all Evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Abbreviations: CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease

Table 17.2.2 is to be used when subjects have *non-measurable* disease only.

Table 17.2.2- Time Point Response: Subjects with Non-Target or Non-Measurable Disease

Non-target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluable	No	NE
Unequivocal	Yes or No	PD
Any	Yes	PD

Abbreviations: CR = complete response; NE = not evaluable; PD = progressive disease; SD = stable disease.

[a] non-CR/non-PD is preferred over SD for non-target disease due to SD being increasingly used as an endpoint for assessment in trials; to assign this category when no lesions can be measured is not advised.

Frequency of Tumor Re-Evaluation

A baseline tumor evaluation must be performed within 4 weeks before a subject begins the protocol-specified treatment. Frequency of tumor re-evaluation while on and adapted to treatment should be protocol-specific and adapted to the type and schedule of treatment. Normally, all target and non-target sites are evaluated at each assessment using the same method. However, bone scans may need to be repeated only when CR is identified in target disease or when progression in bone is suspected.

Duration of Response

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are first met for CR or PR (whichever is first recorded) until the first date that disease is recurrent or objective progression is observed (taking as reference for PD the smallest measurements recorded on study).

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

Duration of Stable Disease

Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for objective progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, that is the reference for calculation of PD).

Appendix D: SUBJECT PILL DIARIES

EXAMPLE:					
	Date	Time		Number of Lonsurf Taken	Comments
Day 1	01/01/2022	8:30	AM	2	*Felt nauseated an hour after taking, did not vomit, took a Zofran*
			PM		

CYCLE # _____ **Cycle Start Date:** _____ **Dose Level:** _____

Cycle End Date:

Weeks 1 and 2	Date	Time		Number of Lonsurf tablets Prescribed AM#: PM#: Number of Lonsurf tablets taken	Comments (Describe any missed or extra doses, vomiting and/or bothersome side effects)
Day 1			AM		
			PM		
Day 2			AM		
			PM		
Day 3			AM		
			PM		
Day 4			AM		
			PM		
Day 5			AM		
			PM		
Day 6			AM		
			PM		
Day 7			AM		
			PM		
Day 8			AM		
			PM		
Day 9			AM		
			PM		
Day 10			AM		
			PM		
Day 11			AM		
			PM		
Day 12			AM		
			PM		

[SUBJECT AND/OR STUDY STAFF VERIFYING SIGNATURE & DATE]

CYCLE # _____	**Cycle Start Date:** _____	**Dose Level:** _____
	Cycle End Date:	

Week 1	Date	Time		Number of Stivarga tablets Prescribed AM#: PM#: Number of Stivarga tablets taken	Comments (Describe any missed or extra doses, vomiting and/or bothersome side effects)
Day 1			AM		
			PM		
Day 2			AM		
			PM		
Day 3			AM		
			PM		
Day 4			AM		
			PM		
Day 5			AM		
			PM		
Day 6			AM		
			PM		
Day 7			AM		
			PM		
Week 2	**Date**	**Time**		**Number of Stivarga tablets taken**	**Comments**
Day 8			AM		
			PM		
Day 9			AM		
			PM		
Day 10			AM		
			PM		
Day 11			AM		
			PM		
Day 12			AM		
			PM		
Day 13			AM		
			PM		
Day 14			AM		
			PM		

Week 3	Date	Time		Number of Stivarga tablets taken	Comments (Describe any missed or extra doses, vomiting and/or bothersome side effects)
Day 15			AM		
			PM		
Day 16			AM		
			PM		
Day 17			AM		
			PM		
Day 18			AM		
			PM		
Day 19			AM		
			PM		
Day 20			AM		
			PM		
Day 21			AM		
			PM		

[SUBJECT AND/OR STUDY STAFF VERIFYING SIGNATURE & DATE]

CYCLE # _____ **Cycle Start Date:** _____ **Dose Level:** _____

Cycle End Date:

Week 1	Date	Time		Number of Xeloda tablets Prescribed AM#: PM#: Number of Xeloda tablets taken	Comments (Describe any missed or extra doses, vomiting and/or bothersome side effects)
Day 1			AM		
			PM		
Day 2			AM		
			PM		
Day 3			AM		
			PM		
Day 4			AM		
			PM		
Day 5			AM		
			PM		
Day 6			AM		
			PM		
Day 7			AM		
			PM		
Week 2	**Date**	**Time**		**Number of Xeloda tablets taken**	**Comments**
Day 8			AM		
			PM		
Day 9			AM		
			PM		
Day 10			AM		
			PM		
Day 11			AM		
			PM		
Day 12			AM		
			PM		
Day 13			AM		
			PM		
Day 14			AM		
			PM		

[SUBJECT AND/OR STUDY STAFF VERIFYING SIGNATURE & DATE]

Appendix E: TREATMENT REGIMEN[a]

Sequence of Treatment Regimen	Medication Name: Generic (Trade)[a]	Dosage, Frequency, and Administration	ctDNA testing timepoints	Cycle Length	Notes and Special Precautions
T1. Subjects may start at T1-T3; regardless of where they start, the T-number corresponds to the drug, NOT line of therapy.	FOLFIRI – Folinic acid (leucovorin), fluorouracil (5FU) and irinotecan (Camptosar) and Bevacizumab	Folinic acid (leucovorin) is given at 400mg/m^2 over two hours and irinotecan 180mg/m^2 IV over 30–90 minutes on Day 1, both prior to 5FU. Fluorouracil (5-FU) 400mg/m2 IV push day 1, then 1,200mg/m^2/day × 2 days (total 2,400mg/m^2 over 46–48 hours) IV continuous infusion, via at-home infusion beginning Day 1 and ending on Day 3. Bevacizumab: Injection of 100 mg/4 mL (25 mg/mL) or 400 mg/16 mL (25 mg/mL) in a single-dose vial. For Metastatic Colorectal Cancer, given 5 mg/kg every 2 weeks.	Day 1	14 days 14 days	Individuals who are known to be homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of treatment. See FDA package insert for dosing specifications. See FDA package insert for specifics on warnings and precautions.
T2	Anti-EGFR therapy [Cetuximab (Erbitux) or Panitumumab (Vectibix)] in combination with Irinotecan[b] at physician discretion	Cetuximab: 500 mg/m^2 IV over 120 minutes every 14 days. First dose requires test dose. Panitumumab: 6 mg/kg, IV over 60 minutes. every 14 days.	Day 1	14 days	SKIP and move to Treatment Regimen #3 (Lonsurf) if subject is harboring a KRAS mutation or subject has already been previously treated with Cetuximab or Panitumumab and irinotecan. Serum electrolytes (magnesium) should be monitored during and after administration as outlined in the FDA package insert.
T3	TAS-102 (Lonsurf) in combination with Bevacizumab at physician discretion	Lonsurf: 35 mg/m^2 twice daily on days 1 to 5 and 8 to 12 of each 28-day cycle.	Day 1 and Day 15	28 days	n/a

		Bevacizumab: Injection of 100 mg/4 mL (25 mg/mL) or 400 mg/16 mL (25 mg/mL) in a single-dose vial. For Metastatic Colorectal Cancer, given 5 mg/kg every 2 weeks.			
T4	Regorafenib (Stivarga)	160 mg orally once a day for the first 21 days of each 28-day cycle.	Day 1 and Day 15	28 days	n/a
T5	Capecitabine (Xeloda)	850–1,250mg/m^2 orally twice daily for the first 14 days of each 21-day cycle.	Day 1	21 days	Patients receiving capecitabine who also take oral coumarin-derivative anticoagulants, such as warfarin and phenprocoumon, should have their anticoagulant response (PT/INR) monitored frequently in order to adjust the anticoagulant dose accordingly. See FDA package insert.
T6	FOLFIRI – Folinic acid (leucovorin), fluorouracil (5FU) and irinotecan (Camptosar)	Folinic acid (leucovorin) is given at 400mg/m^2 over two hours and irinotecan 180mg/m^2 IV over 30–90 minutes on Day 1, both prior to 5FU. Fluorouracil (5-FU) 400mg/m2 IV push day 1, then 1,200mg/m^2/day × 2 days (total 2,400mg/m^2 over 46–48 hours) IV continuous infusion, via at-home infusion beginning Day 1 and ending on Day 3.	Day 1	14 days	Individuals who are known to be homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of treatment. See FDA package insert for dosing specifications.
T7	7a. FOLFOX – Folinic acid (leucovorin), oxaliplatin (Eloxatin), and fluorouracil (5FU) Or 7b. CAPOX – Capecitabine (Xeloda),	FOLFOX: Folinic acid 400mg/m^2 IV over two hours before fluorouracil (Day 1), Oxaliplatin IV 85mg/m^2 over two hours on Day 1, commonly at the same time as folinic acid. Fluorouracil (5-FU) 400mg/m2 IV push day 1, then 1,200mg/m^2/day × 2 days (total 2,400mg/m^2 over 46–48 hours) IV	Day 1	14 days	Monitor for persistent neuropathy / neurosensory events and adjust as necessary.

	and oxaliplatin (Eloxatin)	continuous infusion, via an at-home infusion beginning Day 1 and ending on Day 3. CAPOX: Capecitabine 850 – 1000 mg/m^2, by mouth, twice daily, for the first 14 days of each 21-day cycle. Oxaliplatin IV 130 mg/m^2 over 2 hours on Day 1.		21 days	
Next	Return to Treatment Regimen #1 (FOLIRI and Bevacizumab and repeat[c]				

a Actual dosage should be prescribed per institutional standards; this table only outlines common dosing administration practices.
b Only for RAS wild-type tumors. Irinotecan is routinely dosed at 150-180 mg/m2 IV over 30 minutes every 14 days
c This round of FOLFIRI and Bevacizumab should be administered even if subject was treated with FOLFIRI and Bevacizumab in the past. If sequence is repeated, treatment numbers should be indicated as T2.1, T2.2, T2.3, etc. representing the first, second and third treatments, respectively, of treatment round #2.

Appendix F: PROPOSED TIMELINE

Month	Milestone (Target)	Approximate Date
	Activation	August 1, 2021
1*	First patient, First Visit	September 1, 2021
20*	Safety Interim Analysis Target (N=40, 20 in each arm)	March 31, 2023
26*	Futility Interim Analysis (N=52, 26 in each arm)	October 1, 2023
36	Complete enrollment	August 1, 2024
40	Last Patient, Last Visit	December 1, 2024
48	Final Report Filed	May 1, 2025

*Enrollment is expected to occur at a rate of 2-3 subjects per month