Cover Page for Protocol – J1214

NCT Number:	NCT01639131
Official title of study	A multi-institutional open label, trial evaluating the efficacy of Gemcitabine and Docetaxel in patients with relapsed or refractory metastatic colorectal adenocarcinoma with methylated CHFR and/or microsatellite instability phenotype
Document Date:	November 19, 2015

eIRB# NA_00069666 **/ Local Protocol #:** J1214

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Protocol Type / Version # / Version Date: Version 6, November 19, 2015

Protocol Synopsis

Title

A multi-institutional open label, trial evaluating the efficacy of gemcitabine and docetaxel in patients with relapsed or refractory metastatic colorectal adenocarcinoma with methylated checkpoint with forkhead and ring finger domain (CHFR) promoter and/or microsatellite instability phenotype

Background

- CHFR is a checkpoint protein which causes cell cycle arrest and associated chemotherapy resistance when exposed to microtubule inhibitors
- Epigenetic silencing of CHFR expression via CpG promoter methylation has been shown to increase sensitivity to microtubule inhibitors
- Microsatellite instability (MSI-H) colorectal cancer is associated with sensitivity to gemcitabine
- Methylation of CHFR and/or microsatellite instability is/are present in approximately 25-40% of all colorectal adenocarcinoma tumors, with significant overlap of CHFR methylation with MSI-H
- Gemcitabine and docetaxel have been safely combined in the treatment of non-small cell lung cancer and breast cancer
- Gemcitabine and docetaxel combination therapy has demonstrated significant preclinical activity in colorectal cancer cell lines with CHFR and/or MSI phenotype

Purpose and Objectives

- Determine the efficacy of combination gemcitabine and docetaxel chemotherapy in the treatment of metastatic colorectal cancer with CHFR and/or MSI phenotype

Eligibility

- Adults with histologically documented metastatic colorectal adenocarcinoma with methylated CHFR and/or microsatellite instability phenotype that have relapsed or are refractory to one or more line(s) of standard therapy
- Patients must be off prior chemotherapy, radiation therapy, hormonal therapy, or biological therapy for at least 4 weeks.
- ECOG performance status 0 or 1
- Adequate organ and marrow function

Design

- Patients will receive intravenous gemcitabine 500mg/m^2 on days 1 and 8 and docetaxel 70mg/m^2 on day 8 of each 21 day cycle
- Patients will receive filgrastim (G-CSF) on days 9 through 15 or pegfilgrastim 6mg on day 9 or 10 of each cycle
- Patients will be evaluated for toxicity prior to receiving each cycle and every 6 weeks for response using RECIST criteria 1.1
- A minimum of 10 and a maximum of 40 patients will be enrolled

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APPENDIX D

EORTC QOL-C30 and QOL-CR29

Abbreviation Key

CHFR: Checkpoint with forkhead and RING finger domains
MSI/MSS: Microsatellite instable/stable
MMR: Mismatch repair
CRC: Colorectal cancer
PFS: Progression free survival
OS: Overall survival
5FU: 5-fluouracil
PCR: Polymerase chain reaction
IHC: Immunohistochemistry
ULN: Upper limit of normal
ANC: Absolute neutrophil count
RESIST: Response evaluation criteria in solid tumors
CR: Complete response
PR: Partial response
SD: Stable disease
PD: Progressive disease
AE: Adverse event
SAE: Serious adverse event
CTCAE: Common Terminology Criteria for Adverse Events

1. OBJECTIVES

1.1. Primary Objectives

- Determine the response rate of gemcitabine and docetaxel combination therapy for treatment of relapsed or refractory metastatic colorectal adenocarcinoma with methylation of CHFR and/or microsatellite instability

1.2. Secondary Objectives

- Determine the progression free survival with gemcitabine and docetaxel combination therapy in the selected patient population
- Determine the overall survival with gemcitabine and docetaxel combination therapy in the selected patient population
- Assess CHFR methylation in circulating tumor DNA and compare to CHFR methylation observed in tumor tissue
- Assess changes in CHFR methylation in circulating tumor DNA over the time of therapy to determine if CHFR demethylation occurs as a predictor of progression
- Analyze tumor tissue using a global methylation approach to develop a more robust predictive signature of treatment response
- Evaluate changes in quality of life for patients treated with this regimen by serial measurements using the QLQ-C30 and QLQ-CR29 questionnaire.

2. BACKGROUND

2.1 Colorectal Cancer

Globally, colorectal cancer (CRC) is the third most common malignancy with over 1.2 million estimated new cases and 608,700 deaths in 2008 (1). It is the third most commonly diagnosed and third leading cause of cancer death in the United States. In the US in 2011, it is expected that 141,210 new cases of colon cancer will be diagnosed, with 49,380 deaths attributed to colon cancer. Of the new cases, 90% are over the age of 50 with 94% of the deaths in this age group, indicating that the disease is not only a significant cause of death and a common cancer diagnosis in older patients, but also significant cause of early cancer and death in younger patients. (2)

Treatment of CRC by 5-fluorouracil (5FU) has been a component of the standard chemotherapy given to these patients for decades (3). The additions of oxaliplatin and irinotecan as well as targeted agents including bevacizumab and cetuximab/panitumumab to the prior standard of 5-fluouracil have increased the median overall survival of patients from 10 months to over 2 years. However, primary or acquired chemoresistance causes patients to become refractory to these two or three lines of therapy. Hence, the five year overall survival for metastatic colorectal cancer is only 10%, demonstrating a need for finding novel drug combinations to treat these patients (4).

2.2 Gemcitabine

Background
Gemcitabine is an FDA approved commercially available antimetabolite. It is a nucleoside pyrimidine analogue. Gemcitabine first received FDA approval in 1996. It currently has approval for the treatment of pancreatic cancer as a single-agent, in combination with platinum-based therapy in ovarian and nonsmall cell lung cancer, and in combination with paclitaxel in metastatic breast cancer. The cytotoxic effect is due to the inhibition of DNA synthesis via the actions of its metabolites gemcitabine diphosphate and gemcitibine triphosphate. Gemicitabine diphosphate inhibits ribonucleotide reductase. Gemcitabine triphosphate is incorporated into the DNA, inhibiting DNA polymerase.

Gemcitabine has been utilized for the treatment of unselected colorectal cancer patients previously without significant efficacy. Two partial responses were observed in the initial phase I study of gemcitabine, one being in advanced colorectal cancer. However, gemcitabine as a single agent has subsequently been studied in phase 2 studies in both upfront treatment and refractory disease with minimal activity (5-9). Gemcitabine has been combined with fluouracil with mixed responses. A review article of the available phase 1 and 2 studies was published in 2008 but Merl, et al. with response rates ranging from 3.8% to 38% from 8 studies in both treatment naïve and refractory patients (10). A more recent study demonstrated a partial response in 2 of 22 refractory metastatic CRC patients (11).

Dosing and Toxicity
The dose of gemicitabine ranges from 800mg/m^2 to 1250mg/m^2 intravenous infusion over 30 minutes on days 1 and 8 of a 21 day cycle or days 1, 8, and 15 of a 28 day cycle. The drug is metabolized intracellularly into its two active metabolites gemcitabine diphosphate and gemcitabine triphosphate. The active metabolite intracellular half-life ranges from 1.7 to 19.4 hours. Its excretion is primarily in the urine. It is not significantly protein bound.

The dose limiting toxicity of gemcitabine is myelosuppression. Other prominent toxicities associated with gemcitabine include fever, nausea, vomiting, fatigue, edema, alopecia, rash, and elevated liver enzymes. These primary toxicities associated with gemcitabine usually resolve after cessation of treatment.

2.3 **Docetaxel**

Background
Docetaxel is an FDA approved, commercially available antimicrotubule agent. It is approved for the treatment of locally advanced or metastatic breast cancer, locally advanced or metastatic non-small cell lung cancer, hormone refractory prostate cancer, gastric adenocarcinoma, and squamous cell carcinoma of the head and neck. The cytotoxic effect of docetaxel is due to the formation and stabilization of microtubules which prevents the reorganization necessary during the mitotic phase of the cell cycle.

Docetaxel at a dose of 100mg/m^2 every 3 weeks had minimal benefit in unselected colon cancer patients including three phase 2 studies of 76 total patients (12-14). Of these patients only 3 had objective responses with one complete response and 2 partial responses. An

additional 2 patients experienced a minor response and 9 patients demonstrated stable disease.

Dose and Toxicology

Docetaxel is given intravenously over 1 hour every 3 weeks. It can be dosed from 60mg/m^2 to 100mg/m^2 as a single agent or used in combination with other cytotoxic agents in doses of 60mg/m^2 or 75mg/m^2. It is 94-97% protein bound. The primary metabolism is by the CYP 3A4 isoenyme and its metabolism may be modified by medication which induce, inhibit, or metabolized by cytochrome P450 3A4. The drug is eliminated predominately through feces with minor component in the urine.

The primary dose limiting toxicities are myelosuppression and fluid retention. Other significant toxicities include asthenia, neurosensory symptoms including paresthesia, dysthesia, and pain, hypersensitivity reactions including rash, hypotension, bronchospasm, and rarely anaphylaxis, hepatic injury, alopecia, and cutaneous reactions. Premedication with oral corticosteroids is recommended for all patients to prophylaxis against excessive fluid retention and infusion reactions.

2.4 Rationale for combination therapy in selected colorectal cancer patients

The microsatellite instability phenotype in colorectal cancer is caused by defective mismatch repair (MMR) proteins that lead to double-strand DNA breaks in malignant tissue. The defect can occur either by a genetic mutation in MMR genes, such as hMLH1, hMSH2, and hMSH6, or by epigenetic inactivation via promoter methylation in as in the majority sporadic colon cancer with the MSI phenotype. Approximately 15% of sporadic colon cancer patients have MSI phenotype tumors. (15) Previously it has been shown that DNA polymerase inhibitors such as aphidicolin, gemcitabine and hydroxyurea were more toxic to the hMLH1 deficient HCT116 cell line than HCT116 with proficient-hMLH1(16). [redacted].

Checkpoint with forkhead and RING finger domains (CHFR) is a cell cycle check point protein that protects cells from apoptosis following taxane agent microtubule alteration. CHFR inhibits Plk1 kinase by ubquination. Active Plk1 kinase inhibits the cdc2 protein that delays G2 to M transition (17). When the CHFR gene is unmethylated and expressed it prevents cells treated with taxane agents from proceeding through unregulated mitosis that leads to apoptosis. If the CHFR gene is epigenetically silenced with gene promoter methylation, cancer cells are exquisitely sensitive to taxane chemotherapy (18 - 20)

[redacted]

Gemcitabine and docetaxel have been safely combined in the treatment of non-small cell lung cancer, refractory breast cancer, and ovarian cancer (24-27). The combination of gemcitabine and docetaxel has been reported in CRC patients only in phase 1 studies which included a total of 4 patients, none of which had a response reported. (28-30). [redacted]

Patients for this study must have CHFR methylation or microsatellite instability. CHFR methylation will be assessed using methylation specific PCR or immunohistochemistry (lack of expression). For MSP, DNA is isolated from formalin-fixed, paraffin-embedded (FFPE) specimen. Molecular analysis of the CHFR gene is performed by methylation-specific PCR, which was developed by our collaborator on this study, Jim Herman, and is now the standard technique to assess methylation of a gene. An MSP assay entails initial modification of DNA by sodium bisulfite, converting all unmethylated, but not methylated, cytosines to uracil, and subsequent amplification with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. MSP eliminates the false positive results inherent to previous PCR-based approaches, which relied on differential restriction enzyme cleavage to distinguish methylated from unmethylated DNA. MDXHealth or the Vu Cancer Center in Amsterdam will be conducting these assessments in a CLIA-certified or CCKL-certified laboratory for this technique. Microsatellite instability will be assessed using standard-of-care techniques of PCR-based analysis using the Promega kit or immunohistochemistry. The MSI Analysis System kit (Promega, Madison, WI) consists of five nearly monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) for MSI determination and two polymorphic pentanucleotide markers (Penta C and Penta D) for sample identification. The assay involves fluorescent polymerase chain reaction (PCR) analysis of tumor samples and matching normal samples. The limit of detection of the MSI assay has been determined to be approximately 1 cell in 10-100 total cells (10%). Johns Hopkins is a CLIA-certified site for MSI testing using this technique, and patients may also have documentation of their MSI status at external laboratories to qualify for the study.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed metastatic or unresectable adenocarcinoma of the colon or rectum.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.3 Patients must be either intolerant or refractory to one or more standard line(s) of chemotherapy treatment prior to enrollment. Toxicity from prior regimens must be resolved to less than or equal to grade 1 prior to enrollment. Patients with grade 2 neurotoxicity may be enrolled on a case by case basis at the discretion of the principle investigator. Patients should be off all treatment for at least 4 weeks prior to trial enrollment.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of gemcitabine in combination with docetaxel in patients <18 years of age with colorectal adenocarcinoma, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.5 ECOG performance status 0 or 1 (Karnofsky ≥70%, see Appendix A).

3.1.6 Life expectancy of greater than 12 weeks.

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

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– total bilirubin	≤ 1.5 x ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal or 5X ULN in the presence of liver metastases
– creatinine	within normal institutional limits
OR	
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

3.1.8 Additional eligibility criteria

a. Microsatellite instability phenotype of archival tissue biopsy determined by treating institution by PCR and IHC assay

b. Methylation CHFR gene promoter in archival tissue biopsy
-A patient will be considered to have CHFR methylation if he/she has a methylation specific band on MSP for the CHFR gene or lack of expression by IHCs; MSP primers are publicly reported and developed at Oncomethylome in a CLIA laboratory. Patients who test positive for MSI at any of the 5 loci will be considered MSI+ as per standard convention or who have absent expression of MLH1, MSH2, MSH6, or PMS2 by IHC.
-Results from another institution's CLIA-certified MSI/IHC will be considered for eligibility.
-Patients with microsatellite instability and a family history supportive for a possible diagnosis of hereditary nonpolyposis colorectal cancer will be referred to a genetics counselor for further evaluation and recommendations.

3.1.9 As gemcitabine and docetaxel are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men and women treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 3 months after completion of treatment.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to gemcitabine or docetaxel.

3.2.5 Patients receiving any medications or substances that are inhibitors or inducers of CYP 3A4 are ineligible. Investigator can change to a similar agent that is a non-CYP3A4 inhibitor/inducer with a washout period of 1 week.

Lists including medications and substances known or with the potential to interact with the cytochrome 450 3A4 isoenzyme are provided in appendix C.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects of study medications. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with gemcitabine and docetaxel breastfeeding should be discontinued if the mother is treated. These potential risks may also apply to other agents used in this study including supportive medications.

3.2.8 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with gemcitabine and docetaxel. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study centrally at Johns Hopkins Hospital by the Study Coordinator. All sites should call the Study Coordinator: [redacted] verify dose level availabilities. The required forms can be found in Appendix D.

Following registration, patients should begin protocol treatment within 21 days.* Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed [redacted]

- Copy of required laboratory tests including MSI testing at treating institution (archival tissue biopsy documentation confirming results is acceptable) or documentation of CHFR methylation status
- Signed patient consent form
- HIPAA authorization form
- Eligibility Screening Worksheet
- Registration Form

The research nurse or data manager at the participating site will then call [redacted] r e-mail [redacted] the Study Coordinator to verify eligibility. To complete the registration process, the Coordinator will

- Assign a patient study number
- Confirm tissue sample is positive for CHFR methylation or MSI prior to formal registration
- Register the patient on the study
- Fax or e-mail the patient study number and dose to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment formulation and supportive care will be administered on an outpatient basis, per institutional guidelines. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

REGIMEN DESCRIPTION*					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Gemcitabine	Appropriate antiemetic	500mg/m^2 in 100 ml NS	IV over 30 minutes to 1 hour	Days 1 and 8	21 days (3 weeks)
Docetaxel	Dexamethasone 8mg po bid on days 7, 8, and 9	70mg/m^2 in 250 ml D5W	IV over 1 hr through separate IV line	Day 8	

* This is the protocol used in the coordinating center, and participating sites may have slightly different guidelines for administration for these standard agents, with the dose of the chemotherapeutic agents being equal.

5.1.1 Supportive care for study drugs

a. Dexamethasone should be used for prevention of hypersensitivity reactions, nausea, and extravascular fluid retention from docetaxel. [redacted]

b. Filgrastim 5mcg/kg on days 9 -14 or pegfilgrastim 6mg one time dose on day 9 or 10 should be given as primary prophylaxis.

5.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

5.3 Duration of Therapy

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

- Disease progression,
- Radiation treatment required for palliative management of disease,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

In the event that the patient is deemed to be receiving continued clinical benefit in the face of progressive disease by RECIST criteria, the patient may continue on therapy with agreement of the PI. If progressive disease is confirmed on successive imaging or clinical exam, the date of progression will be marked as the first timepoint that progression was noted.

5.4 Duration of Follow Up

Patients will be followed for 4 weeks after cessation of study treatment for resolution of toxicity, and then every three months until death. Follow-up for survival will be via email, phone call, or by checking the electronic patient record system to verify

subject's status. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for Removal from Study

- Patients will be removed from study when any of the following criteria: Death
- Patient voluntarily chooses to withdraw from the protocol

. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose Level	Gemcitabine Dose	Docetaxel Dose
-2	300 mg/m^{2}days 1 and 8	50mg/m^2 day 8
-1	400 mg/m^2 days 1 and 8	50mg/m^2 day 8
0	500 mg/m^{2}days 1 and 8	70mg/m^2 day 8

Event Name	Nausea/Vomiting*	
Grade of Event	**Management/Next Dose for *Gemcitabine***	**Management/Next Dose for *Docetaxel***
≤ Grade 1 or 2	No change in dose	No change in dose
Grade 3	Hold** until ≤ Grade 2. Resume at one dose level lower, unless toxicity resolved within 3 days.***	Hold** until ≤ Grade 2. Resume at one dose level lower, unless toxicity resolved within 3 days.***
Grade 4	Hold* until ≤ Grade 2. Resume at one dose level lower, unless toxicity resolved within 3 days.***	Hold* until ≤ Grade 2. Resume at one dose level lower, unless toxicity resolved within 3 days.***
* Patients whose symptoms are controlled within three days of onset of grade 3 or 4 toxicity will not require a dose reduction **Patients requiring a delay of >3 weeks should go off protocol therapy. ***If there are greater than 2 dose reductions in single drug patients should discontinue the offending drug and may continue the other drug if desired.		
Recommended management: Intensify prophylactic antiemetics, e.g. addition of aprepitant, benzodiazepine, etc.		

Event Name	Peripheral Neuropathy
Grade of Event	**Management/Next Dose for *Docetaxel***
≤ Grade 1 or 2	No change in dose

Grade 3	Hold* until ≤ Grade 2. Resume at one dose level lower.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >3 weeks should go off protocol therapy. ** If there are greater than 2 dose reductions of docetaxel patients should discontinue docetaxel and may continue gemcitabine if desired.	

Event Name	**Diarrhea***	
Grade of Event	**Management/Next Dose for *Gemcitabine***	**Management/Next Dose for *Docetaxel***
≤ Grade 1 or 2	No change in dose	No change in dose
Grade 3	Hold** until < Grade 2. Resume at one dose level lower, if indicated.***	Hold** until < Grade 2. Resume at one dose level lower, if indicated.***
Grade 4	Off protocol therapy	Off protocol therapy
* Patients whose symptoms are controlled within three days of onset of grade 3 or 4 toxicity will not require a dose reduction **Patients requiring a delay of > 3 weeks should go off protocol therapy. *** If there are greater than 2 dose reductions in single drug patients should discontinue the offending drug and may continue the other drug if desired.		
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.		

Event Name	**Neutropenia/Thrombocytopenia**	
Counts***	**Management/Next Dose for *Gemcitabine***	**Management/Next Dose for *Docetaxel***
ANC ≥ 1200, Platelets ≥ 100,00	No change in dose	No change in dose
ANC < 1200 and ≥ 1000, OR Platelets <100,000 and ≥75,000	Hold until ANC ≥ 1200 and platelets ≥ 100,000. Resume at same dose level.	Hold until ANC ≥ 1200 and platelets ≥ 100,000. Resume at same dose level.
ANC <1000 and ≥ 500, OR Platelets <75,000 and ≥50,000	Hold* until ANC ≥1200 and platelets ≥ 100,000. Resume at one dose level lower.	Hold* until ANC ≥1200 and platelets ≥ 100,000. Resume at one dose level lower.
ANC <500 OR Platelets < 50,000	Hold* until ANC ≥1200 and platelets ≥ 100,000. Resume at one dose level lower.	Hold* until ANC ≥1200 and platelets ≥ 100,000. Resume at one dose level lower.
ANC = Absolute neutrophil count *Patients requiring a delay of >3 weeks should go off protocol therapy. ** If there are greater than 2 dose reductions in single drug patients should		

discontinue the offending drug and may continue the other drug if desired. ***Counts on treatment days only (day 1 and 8)

Event Name	**Hypersensitivity**
Grade	**Management for *Docetaxel***
Grade 1 (e.g. mild flushing/pruritis)	Complete infusion. Supervision at bedside. Subsequent cycles may be given with diphenhydramine and H-2 blocker in addition to dexamethasone at the discretion of treating provider.
Grade 2 (e.g. moderate flushing, rash, dyspnea, chest discomfor	Stop infusion. Give supportive medications including dexamethasone, diphenhydramine. Restart infusion at lower rate once symptoms have resolved. If moderate symptoms during first cycle then dexamethasone should be increased to 20mg 12 hours and 6 hours before treatment in addition to pretreatment 10mg IV dose. Diphenhydramine and H2-blocker premedication should be added 30 minutes prior to docetaxel infusion.
Grade 3 or 4	Administer supportive care including dexamethasone and diphenhydramine in addition to bronchodilation therapy and vasopressors as indicated. Discontinue docetaxel and continue gemcitabine per provider and patient preference.

Event Name	**Fluid retention**
Severity	**Management/Next Dose for *Docetaxel***
Asymptomatic peripheral edema or pleural effusion	Initiate diuretic if considered appropriate by patient provider. Continue docetaxel. No change in dose.
Symptomatic peripheral edema	Initiate or intensify diuretic therapy. Continue docetaxel. No change in dose
Symptomatic pleural effusion	Discontinue docetaxel. May continue gemcitabine per provider and patient preference.
*Patients should have thoracentesis if effusion is suspected to represent progressive disease.	

Event Name	**General Toxicity**	
Grade of Event	**Management/Next Dose for *Gemcitabine***	**Management/Next Dose for *Docetaxel***
Grade 1	No change in dose	No change in dose
Grade 2 or 3,	Hold* until ≤ Grade 1.	Hold* until ≤ Grade 1.

clinically significant	Resume at one dose level lower, except electrolyte abnormalities noted below**	Resume at one dose level lower, except electrolyte abnormalities noted below**
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of > 3 weeks should go off protocol therapy. ** If there are greater than 2 dose reductions in single drug patients should discontinue the offending drug and may continue the other drug if desired.		
1) Electrolyte abnormalities can and should be corrected as clinically indicated and do not require dose adjustments unless correction is not possible. 2) If single offending drug is highly suspected then it should be dose reduced while maintaining dose of non-offending drug. If specific offending drug is not clear then dose reduction of both drugs is advised.		

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Adverse events associated with treatment drugs

7.1.1 **Gemcitabine**

1. Hematologic: Neutropenia, anemia, thrombocytopenia, and leukopenia are reported.
2. Dermatologic: A rash is seen in about 25% of patients and is associated with pruritus in about 10% of patients. The rash is usually mild, not dose-limiting, and responds to local therapy. Desquamation, vesiculation, and ulceration have been reported rarely. Alopecia is usually minimal. Injection-site reactions.
3. Gastrointestinal: Nausea and vomiting are reported in about two-thirds of patients and requires therapy in about 20% of patients. It is rarely dose limiting, and is easily manageable with standard antiemetics. Diarrhea, constipation, mucositis.
4. Hepatic: Abnormalities of hepatic transaminase enzymes occur in two-thirds of patients, but they are usually mild, nonprogressive, and rarely necessitate stopping treatment. However, gemcitabine should be used with caution in patients with impaired hepatic function.
5. Pulmonary: Bronchospasm and/or dyspnea within a few hours of infusion of the drug, cough, rhinitis, pneumonitis.
6. Neurologic: Somnolence, insomnia, paresthesia, pain.
7. Cardiovascular: A few cases of hypotension were reported. Some cases of myocardial infarction, congestive heart failure, and arrhythmias have been reported. Peripheral edema is reported in about 30% of patients. Some cases of facial edema have also been reported. Edema is usually mild to moderate, rarely dose-limiting, sometimes painful, and reversible after stopping gemcitabine treatment.
8. Other: Flu-like symptoms are reported for about 20% of patients. This includes fever, headache, back pain, chills, myalgia, asthenia, and anorexia. Malaise and sweating are reported.

7.1.2 **Docetaxel**

1. Hematologic: The major toxic effect of docetaxel, which limits dose, is neutropenia which may lead to fever and infection. Other toxic effects, which may be seen, include leukopenia, thrombocytopenia, anemia. Disseminated intravascular coagulation often in association with sepsis or multiorgan dysfunction has been reported. Very rare cases of myeloid leukemia or myelodysplasia have occurred in docetaxel, doxorubicin, and cyclophosphamide treated patients
2. Infusional/Allergic reactions: Severe anaphylactoid reactions, characterized by a flush associated with hypo- or hypertension, with or without dyspnea, may occur requiring immediate discontinuation of the docetaxel infusion and aggressive therapy. All patients should be premedicated with an oral
corticosteroid prior to the initiation of the infusion of docetaxel. If minor reactions such as flushing or localized skin reactions occur, interruption of therapy is not required. Other hypersensitivity reactions including flushing, pruritis, fever, chills, rigors, lower back pain are reported.
3. Dermatologic: skin rash, desquamation following localized pruriginous maculopapular eruption, skin erythema with edema, nail changes, alopecia, extravasation reaction (erythema, swelling, tenderness, pustules), nail changes and conjunctivitis.
4. Respiratory: dyspnea with restrictive pulmonary syndrome, pleural
effusions
5. Cardiovascular: arrhythmias, pericardial effusions, fluid retention syndrome. Rare cases of congestive heart failure have been reported with docetaxel used in combination with other chemotherapy agents.
6. Gastrointestinal: nausea, vomiting, oral mucositis, diarrhea, anorexia, increase in liver function tests, hepatic failure. Patients with SGOT (AST) > 1.5 times normal and alkaline phosphatase > 2.5 times normal appear to have decreased docetaxel clearance and appear to be more likely to suffer severe toxicity, including drug-related death.
7. Neurotoxicity: reversible dysesthesias or paresthesias, peripheral neuropathy, seizure, headache, lethargy or somnolence.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Adverse Event and Deaths on Study Reporting

7.3.1 Expedited Reporting on Adverse Event Form to PI within 48 hours:

a. Serious adverse events (SAEs) are occurrences possibly, probably or definitely related to the research. An SAE is defined as an untoward medical occurrence that:

- resulted in a death;
- was life-threatening;
- required or prolonged hospitalization;
- caused persistent or significant disability/incapacity;
- resulted in congenital anomalies or birth defects; or
- required intervention to prevent permanent impairment or death.

b. All other deaths not included in the SAE category above.

c. All grade 3 and 4 (CTCAE) events that are not in the consent and that are possibly, probably or definitely related to the research, but not included in the SAE category above.

7.3.2 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions. Adverse event submission for routine AEs must be submitted by CRF within 28 days of the AE being noted by the study team.

7.4 Secondary Malignancy

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

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

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

7.5 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy).

8. PHARMACEUTICAL INFORMATION

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

8.1 Gemcitabine

8.1.1 Other Names
2'-Deoxy-2',2'-difluorocytidine monohydrochloride, Gemzar

8.1.2 Classification
Antimetabolite (nucleoside pyrimidine analogue)

8.1.3 Mode of Action
Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S phase) and also blocking the progression of cells through the G1/S phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

8.1.4 Storage and Stability
Unreconstituted drug vials are stored at controlled room temperature (15ºC to 30ºC, 59ºF to 86ºF). Reconstituted solution should be stored at controlled room temperature and used within 24 hours. Solutions of gemcitabine should not be refrigerated; as crystallization may occur. The unused portion should be discarded.

8.1.5 Dose Specifics

800 mg/m2 IV over 30 minutes on days 1, 8 of each cycle.

8.1.6 Preparation
Reconstitute the 200 mg vial with 5ml and the 1 gm vial with 25 ml preservative free normal saline to make a solution containing 38 mg/ml. Shake to dissolve.
Gemcitabine may be further diluted with NS as per institutional standards.

8.1.7 Route of Administration
IV infusion.

8.1.8 Incompatibilities
No information available.

8.1.9 Availability
Gemcitabine is commercially available in 200 mg and 1 gm vials.

8.1.10 Side Effects

1. Hematologic: Neutropenia, anemia, thrombocytopenia, and leukopenia are reported.
2. Dermatologic: A rash is seen in about 25% of patients and is associated with pruritus in about 10% of patients. The rash is usually mild, not dose-limiting, and responds to local therapy. Desquamation, vesiculation, and ulceration have been reported rarely. Alopecia is usually minimal. Injection-site reactions.
3. Gastrointestinal: Nausea and vomiting are reported in about two-thirds of patients and requires therapy in about 20% of patients. It is rarely dose limiting, and is easily manageable with standard antiemetics. Diarrhea, constipation, mucositis.
4. Hepatic: Abnormalities of hepatic transaminase enzymes occur in two-thirds of patients, but they are usually mild, nonprogressive, and rarely necessitate stopping treatment. However, gemcitabine should be used with caution in patients with impaired hepatic function.
5. Pulmonary: Bronchospasm and/or dyspnea within a few hours of infusion of the drug, cough, rhinitis, pneumonitis.
6. Neurologic: Somnolence, insomnia, paresthesia, pain.
7. Cardiovascular: A few cases of hypotension were reported. Some cases of myocardial infarction, congestive heart failure, and arrhythmias have been reported. Peripheral edema is reported in about 30% of patients. Some cases of facial edema have also been reported. Edema is usually mild to moderate, rarely dose-limiting, sometimes painful, and reversible after stopping gemcitabine treatment.
8. Other: Flu-like symptoms are reported for about 20% of patients. This includes fever, headache, back pain, chills, myalgia, asthenia, and anorexia. Malaise and sweating are reported.

8.1.11 Nursing/Patient Implications

1. If the patient reports burning at the injection site, slow down rate to allow the

dose to run in over 1 hour.
2. Rash can be treated with topical therapy or the administration of diphenhydramine prior to administration.
3. Flu-like symptoms can be treated with acetaminophen.

8.2 Docetaxel

8.2.1 Other names: Taxotere, 4-acetoxy-2α-benzoyloxy-5β, 20-epoxy-1, 7β, 10β-trihydroxy-
9-oxotax-11-ene-13α-yl-(2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate

8.2.2 Classification: Antimicrotuble

8.2.3 Mode of action:
In vitro, docetaxel promotes tubulin assembly into microtubules and inhibits depolymerization thus stabilizing microtubules, which is different from the action of other spindle poisons in clinical use. This can lead to bundles of microtubules in the cell, which by blocking cells in the M phase of the cell cycle, results in the inability of the cells to divide.

8.2.4 Storage and stability: Store between 2 and 25°C (36 and 77°F). Retain in the original
package to protect from bright light. Freezing does not adversely affect the product. The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.
Docetaxel infusion solution, if stored between 2 and 25°C (36 and 77°F) is stable for 4 hours. Fully prepared docetaxel infusion solution (in either 0.9% Sodium Chloride solution or 5% Dextrose solution) should be used within 4 hours (including the 15 - 30 minute IV administration).

8.2.5 Dose specifics
70mg/m^2 IV infusion on day 8 of 21 day cycle

8.2.6 Preparation:
Preparation of docetaxel for infusion requires two separate dilutions.
First dilution: Gather the appropriate number of vials of docetaxel for Injection Concentrate and diluent (13% Ethanol in Water for Injection). If the vials were refrigerated, allow them to stand at room temperature for approximately 5 minutes. Aseptically withdraw the entire contents of the
appropriate diluent vial into a syringe by partially inverting the vial and transfer it to the appropriate vial of docetaxel for Injection Concentrate. **If the procedure is followed as described, an initial diluted solution of 10 mg docetaxel/ml will result.** Mix the initial diluted solution by repeated inversion for at 45 seconds to assure full mixture of the concentrate and diluent. Do not shake.

The initial diluted docetaxel solution (10 mg docetaxel/ml) should be clear; however, there may be some foam on top of the solution due to the polysorbate 80. Allow the solution to stand for a few minutes to allow any foam to dissipate. It is not required that all foam dissipate prior to continuing the preparation process. The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.
Second dilution for infusion: Aseptically withdraw the required amount of initial diluted docetaxel solution (10 mg docetaxel/ml) with a calibrated syringe and inject into a 250 ml infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74 mg/ml. If a dose greater than 200 mg of docetaxel is required, use a larger volume of the infusion vehicle so that a concentration of 0.74 mg/ml docetaxel is not exceeded. Thoroughly mix the infusion by manual rotation. As with all parenteral products, docetaxel should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If the docetaxel for Injection initial diluted solution or final dilution for infusion is not clear or appears to have precipitation, these should be discarded.

8.2.7 Route of administration: IV infusion

8.2.8 Incompatibilities:
Contact of the docetaxel concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP (di-2-ethylhexyl phthalate), which may be leached from PVC infusion bags or sets, the final docetaxel dilution for infusion should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

8.2.9 Availability: Two dosing vials are available, 80mg/2ml for 80mg dose and 20mg/0.5ml for 20mg dose, each with diluent.

8.2.10 Side effects: Please refer to the package insert for complete product instructions and toxicity.

1. Hematologic: The major toxic effect of docetaxel, which limits dose, is neutropenia which may lead to fever and infection. Other toxic effects, which may be seen, include leukopenia, thrombocytopenia, anemia. Disseminated intravascular coagulation often in association with sepsis or multiorgan dysfunction has been reported. Very rare cases of myeloid leukemia or myelodysplasia have occurred in docetaxel, doxorubicin, and cyclophosphamide treated patients
2. Infusional/Allergic reactions: Severe anaphylactoid reactions, characterized by a flush associated with hypo- or hypertension, with or without dyspnea, may

occur requiring immediate discontinuation of the docetaxel infusion and aggressive therapy. All patients should be premedicated with an oral corticosteroid prior to the initiation of the infusion of docetaxel. If minor reactions such as flushing or localized skin reactions occur, interruption of therapy is not required. Other hypersensitivity reactions including flushing, pruritis, fever, chills, rigors, lower back pain are reported.
3. Dermatologic: skin rash, desquamation following localized pruriginous maculopapular eruption, skin erythema with edema, nail changes, alopecia, extravasation reaction (erythema, swelling, tenderness, pustules), nail changes and conjunctivitis.
4. Respiratory: dyspnea with restrictive pulmonary syndrome, pleural effusions
5. Cardiovascular: arrhythmias, pericardial effusions, fluid retention syndrome. Rare cases of congestive heart failure have been reported with docetaxel used in combination with other chemotherapy agents.
6. Gastrointestinal: nausea, vomiting, oral mucositis, diarrhea, anorexia, increase in liver function tests, hepatic failure. Patients with SGOT (AST) > 1.5 times normal and alkaline phosphatase > 2.5 times normal appear to have decreased docetaxel clearance and appear to be more likely to suffer severe toxicity, including drug-related death.
7. Neurotoxicity: reversible dysesthesias or paresthesias, peripheral neuropathy, seizure, headache, lethargy or somnolence.

Potential Drug Interactions: In in vitro screening assays, docetaxel was a significant inhibitor of CYP3A4 and a moderate to weak inhibitor of CYP2D6, 2C19, 2C9 and 1A2. These preliminary data indicate that there may be some potential for interactions with co-administered drugs that are metabolized by these isozymes although the results should be treated with caution until definitive studies are conducted.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Changes in promoter methylation

We have recently designed a highly-sensitive, novel nano-enabled methylation based technology, termed Methylation-on-Beads (MOB), as a tool that has enormous potential for detection of methylation changes in circulating DNA. Candidate gene methylation (CHFR and MMR genes) will be evaluated in circulating tumor DNA in plasma samples obtained pretreatment and every cycle thereafter. DNA will be isolated from blood and treated with sodium bisulfite under denaturing conditions. Bisulfite modified DNA from each post treatment specimen will be analyzed for changes in DNA methylation at specific gene promoters (to include CHFR, p16, mLH1, MSH6 and O6-MGMT). PCR primers for these have been described and are in frequent use in the Ahuja/Herman laboratories. Conditions for all stage I multiplexes will be optimized through primer design and PCR conditions to achieve equal product intensity. These optimal conditions will ensure a similar

sensitivity for the detection of methylated alleles across genes in the stage 2 MSP assays. All stage 2 PCR reactions will be conducted at annealing temperatures (68-70ºC) that exceed the melting temperature of the primers to ensure the highest specificity for amplification of only methylated alleles present in the DNA sample. For each gene, separate PCR reactions will be performed with methylated-specific primers and unmethylated specific primers (as used in conventional gel based MSP analysis). Each reaction will be run in triplicate. Mixing experiments utilizing DNA from cell lines methylated at a given locus and a completely unmethylated DNA source (normal DNA) will be performed to determine accuracy of quantification. Assays will be performed on the Bio-Rad I-cycle. Two methods of detection will be compared: non-specific quantification of PCR products using Syber Green, and specific detection using molecular beacons. A ratio of the inverse of the cycle threshold for the methylated gene of interest over the inverse of the cycle threshold for beta-actin will be calculated. This 'methylation index' will be compared pre- and post-treatment. If changes in methylation pattern are observed by MSP, this result will be further validated by bisulfite sequencing of selected genes.

Changes in candidate gene promoter methylation will be assessed to determine if there is correlation with clinical response to treatment, i.e. progression during treatment.

9.1.2 Advances in array technologies now allow for a comprehensive methylome analysis in cancer samples at low cost. We will also study the global methylation pattern using an Infinium Methylation Assay that surveys genome-wide DNA methylation profiles. Using this technology, approximately 482,420 CpGs are queried, including 99% of RefSeq genes; there are 17 CpGs studied on average per gene. Results are analyzed using BeadStudio software which allows for data analysis, as well as integration of methylome data with mRNA expression array studies. As little as 500ng of input DNA is required for the BeadChip. We will investigate if the Illumina Infinium chip can be used to define more robust epigenetic signatures between responders and non-responders to our therapy.

9.2 Collection and Handling of Specimens

9.2.1 Blood

Blood samples for methylation analysis will be collected from all participants into the enclosed purple tubes (4 mL EDTA BD Vacutainer cell preparation tubes, Becton Dickinson, Franklin Lakes, NJ). These tubes are designed to separate plasma and mononuclear cells from whole blood. Each collection will remove 18 mL of blood from the patient (6 tubes of 3mL each). Each purple tube holds near 3mL of blood. Tubes will be collected and transported on ice to each center's research blood processing and storage area **within 30 minutes**.

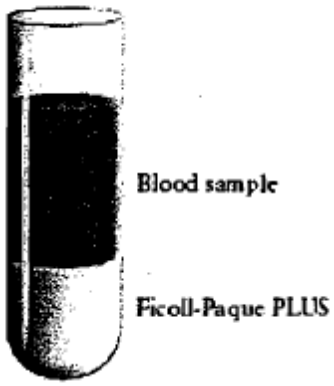
Processing of the blood:

1. Take six enclosed 15 mL conical tubes.
2. Add 3 mL of Ficoll-Paque PLUS buffer to each conical tube. (BUFFER MUST BE KEPT IN 4ºC for storage)
    a. The volume of buffer should be roughly equal to the volume of blood in the purple tops.
3. Layer the blood of a purple top tube above the ficoll buffer.

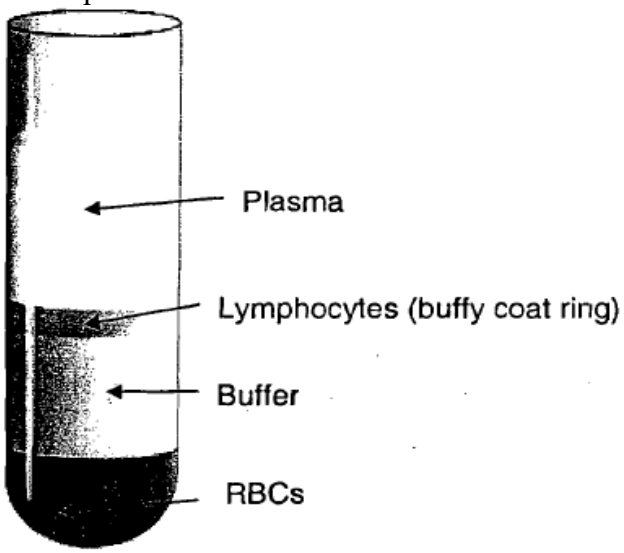
a. To do this, carefully pour the blood into the plasma conical tubes by tilting the conical tube at an angle close to horizontal and letting the blood from the purple-top tube run very slowly down the side of the conical tube. THIS IS IMPORTANT. If done correctly, the blood should sit right on top of the buffer as shown below.
Layer the rest of the blood samples.

4.

Fig 3.



5. Next spin the samples in a centrifuge for 10 minutes at 2000 rpm at 4ºC. Be sure that the centrifuge is balanced.The layers will now separate as shown below:



Carefully pipet the plasma layer (the clear yellow layer on top) from the conical tube into the enclosed 1.5 mL sterile eppendorf tubes. Add no more than 1.2mL of plasma to each eppendorf tube to give the plasma room to expand during freezing.

6. **Pay careful attention that you do not draw from the white buffy coat.**

7. Pipet the white buffy coat (white layer or monocytes) into the

enclosed 1.5mL eppendorf tubes. Similarly, leave room for expansion.
a. When collecting the white blood cells (WBC), keep the tip of the pipette close to the wall of the tube, this is where the wbc accumulate
b. Try not to pipette ficoll with the WBC.
8. Transfer plasma and WBC for each spun conical tube to corresponding eppendorf tubes. Depending on the patient, you may have from 8-10 eppendorf tubes filled with plasma and 3-6 tubes filled with WBC.
9. Freeze plasma and WBC at least -70ºC.
10. At time of shipping, ship on dry ice to [redacted] (Specimen Accessioning Core) Lab.
11. All tubes should be labeled with the unique patient identifier, Cycle number, Day on cycle on the tube.

9.2.2 Archival Tissue

Archival tissue will be utilized to test for CHFR methylation in order to determine eligibility. We will also possibly explore other predictive methylation biomarkers or a biomarker panel that may be more predictive of clinical benefit than CHFR methylation or MSI alone. These exploratory analyses are dependent on observing some clinical benefit. Whole methylome expression arrays using the Illumina Infiunium platform would be utilized for these exploratory analyses, with which we have significant experience and is appropriate for FFPE tissues.

Archival tissues will be requested for each patient from all priorsurgeries. Paraffin blocks **containing tumor sections** will be requested. Paraffin blocks will be sectioned in 10micron cuts with total 25 cuts. In addition, one stained hematoxylin and eosin slide and two unstained slides will be requested.

9.2.3 Shipping of correlative samples

Blood samples will be shipped from the centers to the Specimen Accessioning Core Lab at Johns Hopkins.

[redacted]

Biopsy samples will be shipped from the centers to [redacted] lab at Johns Hopkins.

[redacted]

9.2.4 Handling of specimen(s)
A unique patient identifier will be assigned to each patient by the coordinating center. The same unique patient identifier linked to the tumor biopsies will be used to shield the archived blood samples. The protocol scientific investigator(s) handling the samples will be blinded as to the patient identification, patient data and outcome. After shipping, samples, and associated data, will be stored at Johns Hopkins unless the patient withdraws consent.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 21 days prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-enrollment	Day 1 of each cycle	Day 8 of each cycle	Time of progression[e]
Treatment				
Gemcitabine		X	X	
Docetaxel			X	
Tests and Observations				
H & P	X	X		X
CHFR and MSI testing[a]	X[a]			
Vital signs	X	X	X	X
Height	X			
Weight/BSA	X	X		X
Performance status	X	X		X
Tumor measurements	X	***A***		X
QOL assessment [(screening and q 2 cycles) C3D1, C5D1, etc] [d]	X [d]	X [d]		X
Laboratory Studies				
CBC with differential	X	X	X	X
Complete Metabolic	X[b]	X[b]	X[b]	X[b]

panel[b]				
Serum or urine HCG	***B***			
CEA	X	X		
Urinalysis	X			
Staging				
CT scan chest, abdomen, pelvis	X	***C***		X
Laboratory Correlates				
Research Blood[c]	X	X[c]		X[c]
Archival Tissue[a]	X[a]			

A – Within 2 days of treatment if accessible by physical exam
B – For women of child-bearing potential
C – CT scan required every 2 cycles beginning prior to start of cycle 3

a: MSI testing via IHC and PCR will be assessed and 10 unstained slides are needed for CHFR analysis and 20 unstained slides from archival tissue for other planned methylation analyses.
b: Sodium, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, albumin, alkaline phosphatase, total bilirubin, total protein, SGOT [AST], SGPT [ALT]
c: Research blood: 6 purple top tubes, 3ml each processed per protocol section 9.2.1
d: The quality of life assessments will be completed at screening, after every two cycles (C3D1, C5D1, C7D1, etc), and at time of progression (EOT)
e: Per Section 5.4, subjects will be followed for 4 weeks after cessation of study treatment for resolution of toxicity, and then every three months until death. Follow-up for survival will be via email, phone call, or by checking the electronic patient record system to verify subject's status.

11. MEASUREMENT OF EFFECT

Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 6 (not less 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1 Definitions

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

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

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

11.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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 slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

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

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

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

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

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

11.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to

the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

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

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

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible. **This study will only use standard CT scans, and not MRI, or PET-CT / FDG PET scans.**

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are

identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

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

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

11.4 Response Criteria

11.4.1 Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD):	At least a 20% increase in the sum of the

	diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.4.2 Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
Non-CR/Non-PD:	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD):	Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

11.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as

"*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

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

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

11.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

11.7 Response Review

All responses will be reviewed by experts independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images will be performed.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

This is a DSMP Level I study under the SKCCC Data Safety Monitoring Plan (9/22/2011). The Clinical Research Office QA Group will perform an audit at the end of the first year and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee.

12.1 **Data Reporting**

12.1.1 Method

This study will be monitored by Johns Hopkins SKCCC Clinical Research Office (CRO). Cumulative protocol- and patient-specific data will be recorded electronically in the SKCCC CRMS web-based database with study specific electronic CRFs. Data must be submitted with 28 days of each study visit. All sites will have access to the CRMS database.

Non-lead sites will submit all source documents for eligibility and registration verification for monitoring by the SKCCC. In addition, every third patient will be monitored by the lead site with full research chart review to be submitted, deidentified, and on site monitoring may be applicable as well.

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting data and/or data forms to either the Coordinating Center. The date for submission to the Coordinating Center is described above (28 days from patient visit).

12.2 **Multicenter Guidelines**

This protocol will adhere to the policies and requirements of the Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.
- Each institution will be responsible for ordering treatment drugs.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Statistical Methods

Patients with early colorectal cancer can often undergo curative resection. Metastatic disease, however, is common at the time of diagnosis, and prognosis in these cases is poor. Gene mutations and other tumor biomarkers have recently been identified for screening and treatment selection. This study will evaluate the combination of gemcitabine and docetaxel for treatment in late stage colon cancer patients after resistance to at least first line therapy. Patients with either microsatellite instability (MSI) or CHFR methylation will be chosen for this targeted therapy. It is estimated that 20-30% of colon cancers will be positive for one or the other of these markers.

Primary objective: The primary objective for this study will be the response rate.

Secondary objectives:

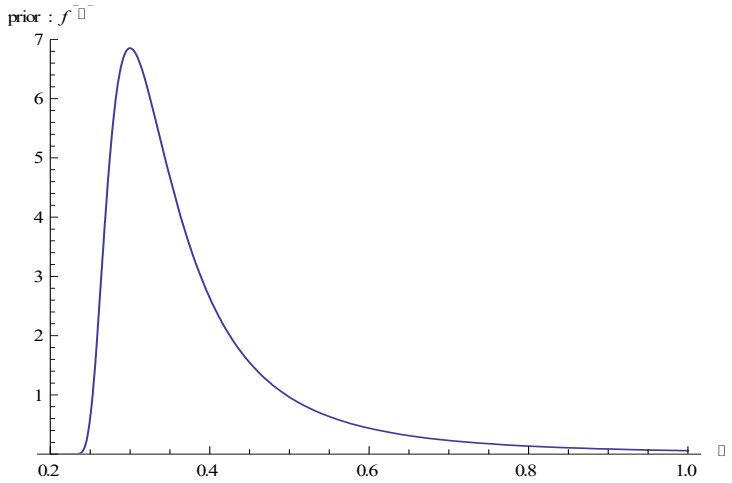
1) Progression-free survival (PFS) will be defined from the date of study entry to the date of progression or death. The median PFS will be compared to a historical median, estimated to be 8 weeks.

Study design: Considerations for the design of this study included efficiency with a small number of patients, aggressive early stopping if the treatment is ineffective, and an improvement of the response rate from a historical rate of 20% to a rate of 30% with the targeted combined therapy. The Bayesian hypothesis testing approach by Johnson and Cook[1] was chosen to accommodate these requirements. This design has increased efficiency compared to Bayesian designs defined from posterior credible intervals. The increase in efficiency results from using non-local prior densities to define the alternative hypothesis. Unlike vague prior specification or local prior densities, non-local prior densities are not positive at values of the parameter that are consistent with the null hypothesis, i.e. they are restricted to an interval consistent with the alternative. This increases the rate at which a trial can accumulate evidence in favor of a true null hypothesis. The class of non-local prior densities used in this design is called inverse moment (iMOM) densities[2]. For this trial, the null hypothesis of interest is a point null hypothesis that the response rate is 20% (i.e. H_o: $\theta = \theta_0$). With this null hypothesis, the form of an iMOM prior for the alternative hypothesis of 30% is given below with parameters: θ_0=0.20, τ=.015, k=1 and ν=2, for $0.20<\theta<1.0$. This prior assigns a value of zero outside the interval (0.20, 1.0) since, under the alternative hypothesis, θ is greater than 0.20.

$$\pi_1[\theta; \theta_0, k, \nu, \tau] = \frac{k\tau^{\frac{\nu}{2}}}{\text{Gamma}[\frac{\nu}{2k}]}((\theta-\theta_0)^2)^{-\frac{\nu+1}{2}}\text{Exp}[-(\frac{(\theta-\theta_0)^2}{\tau})^{-k}]$$

When plotted over the range of 0 to 1 this prior takes the form shown in Figure 4. This density exponentially approaches zero as θ approaches the null, 0.20. τ represents a parameter that determines the dispersion of the prior around θ_0 and it is set so that the mode of the prior density occurs at values deemed most likely under the alternative, 0.30. k and ν have been set to recommended default values of 1 and 2 respectively.

Figure 4. Normalized iMOM prior density with parameters: θ_0=0.20, τ=.015, k=1 and ν=2



Sample size: For this trial a maximum of 40 patients are available for enrollment. The design is based on a hypothesis test using the iMOM prior with the null and alternative hypotheses:

$$\text{Ho: } \theta=0.20$$
$$\text{Ha: } \pi_1(\theta) \propto \pi_1(\theta; \theta_0=0.20, k=1, \nu=2, \tau=0.015)I_{(0.20,1)}(\theta)$$

This will test the null hypothesis that the combination treatment has a 20% response rate compared to an alternative hypothesis of greater than or equal to 20% with the specified iMOM prior (Figure 1.). It will be stopped for inferiority if the posterior probability for the alternative model is less than 0.15, and the trial will not be stopped for superiority. The stopping boundaries for this design call for accrual to stop if there are 0 responses out of 10 patients, 1 response in 14 patients, 2 responses in 18 patients, 3 responses in 21 patients, 4 responses in 25 patients, 5 responses in 29 patients, 6 responses in 33 patients, or 7 responses in 37 patients.

Operating characteristics: The exact operating characteristics[3] of this stopping boundary, given hypothetical true response rates of 0.05 to 0.40 by 0.05, are shown in Figure 2. If the study response rate is 10%, the probability the trial would be terminated early is 98% and the average sample size would be 16.1 patients. If the response rate is 20%, the trial would terminate early 64% of the time and have an average of 27.6 patients. A study with a 30% response rate would only terminate early 18% of the time and the average sample size would be 36.6.

Analysis of primary objective: If the trial is terminated early, the gemcitabine/docetaxel combination therapy for this group of patients will not be pursued further. If the trial is not terminated before the 40th patient accrues, the treatment may be worthy of further investigation. At the end of the trial the response rate and the 95% exact binomial confidence interval will be calculated. The logarithm of the Bayes Factor (BF), considered the weight of evidence, against the null hypothesis will also be calculated. This will be negative if the null hypothesis is more supported by the data and positive if the alternative is more supported. For this study, with a sample size of 40 and the specified null and alternative hypotheses, the expected (average) weight of evidence against the null when the null hypothesis is true is -1.56. Weights of evidence, positive or negative, in the range of 0-1 are considered inconclusive. The trial is stopped early for futility if the BF is less than -1.74 indicating moderate evidence for the null hypothesis. The treatment may not be considered further if the BF is between -1.74 and 1.6 and we would continue study of this treatment combination if the BF is greater than 1.6, suggesting moderate evidence against the null hypothesis. For this study, a response rate of 13/40 (32.5%) would correspond to a log(BF) of 1.3 and a response rate of 14/49 (35%) would yield a log(BF) of 2.0.

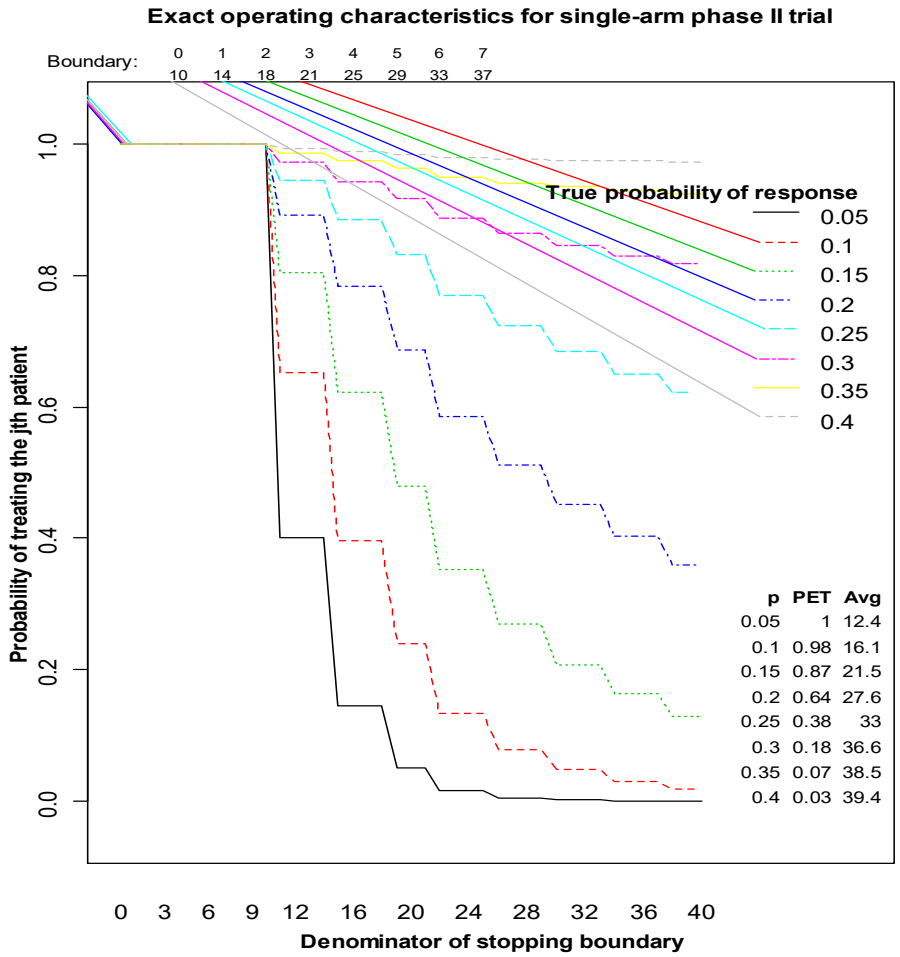
Analysis of secondary objectives:

1) **Progression free survival**: Standard life table methods will be used to analyze PFS. We will report the one and two-month PFS with 95% confidence intervals. The one-month PFS will be compared to the null PFS of x% with a Z-score and a one-sided alpha level of 0.05.
2) **Quality of life**: An overall quality of life score will be obtained from the QLQ-C30 and QLQ-CR29 standardized questionnaire pre-treatment and at the time of the 2nd radiologic assessment or time of progression, whichever comes first. The change in score will be evaluated with a paired t-test. Subscale scores of the quality of life questionnaire will be similarly analyzed
3) **Definitions and Analyses of Translational Endpoints**:
   All analyses described in this section are based on the availability of the data. We recognize that sample sizes are not sufficient to carry out formal hypothesis testing and the analyses will be of an exploratory fashion, to generate hypotheses for validation in subsequent studies.

   The statistical analyses described in this paragraph will be applied all the biomarkers listed in sections 'a' through 'c.' Associations between biomarkers listed below and endpoints of interest (confirmed response and PFS) will be analyzed using variety of methods. Biomarker measures will be analyzed as continuous variables (suitable transformation to assure normality may be applied), and as categorical variables by splitting into quantiles of the distribution. The Wilcoxon Rank Sum test (or Kruskal-Wallis test) and Fisher's exact test will be used to compare continuous and categorical variables between groups if applicable, respectively. Logistic regression and Cox proportional hazard regression techniques will be applied for dichotomous and time-to-event outcomes, respectively. These models may include baseline factors to adjust for confounding effect, when applicable. Additional exploratory analyses including, but not limited to, alternative approaches are expected and may be performed.
   a) **Gene expression by qRT-PCR.** Associations between measures of Gene expression and outcome of interest (tumor response and PFS) will be analyzed according the statistical methods described above.
   b) **Gene methylation status for selected genes (tumor).** Associations between measures of Gene methylation status in tumors and outcome of interest (tumor response and PFS) will be analyzed according the statistical methods described above. In addition, the associations between measures of gene methylation status in tumor and gene expression measures assessed in section 13.331 will be summarized by Trellis scatterplot matrix, along with the estimated Spearman correlation coefficients.
   c) **Gene methylation status for selected genes (circulating).** Associations between measures of Gene methylation status in circulating DNA and outcome of interest (tumor response and PFS) will be analyzed according the statistical methods described

above. In order to explore whether circulating DNA can be used as a surrogate for tissue base methylation measurements, the associations between measures of gene methylation in circulation DNA and those in tumor will be assessed using variety of methods, including to, not limit to, simple correlation method (Spearman correlation), linear regression.

Figure 5.



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[2]Johnson, V. and Rossell, D. On the use of non-local prior densitites in Bayesian hypothesis tests. J. R. Statist. Soc. B (2010) 72, Part 2, 143–170.

[3]Cook, J. Exact operating characteristics for single-arm Phase II trials (2008). *UT MD Anderson Cancer Center Department of Biostatistics Working Paper Series.* Working Paper 45.

13.2 **Sample Size/Accrual Rate:** The target sample size is 40 patients. We would expect to accrue approximately 2 patients per month with target completion within 20 months of study opening.

13.3 **Analysis of Secondary Endpoints:** Secondary endpoints for this study are standard progression free survival and overall survival. Progression free survival is the time from the start of study enrollment to the first evidence of radiographic progression or death. Overall survival is the time from the start of study enrollment to death.

13.4 **Reporting and Exclusions**

13.4.1 Evaluation of toxicity – All patients will be evaluable for toxicity from the time of their first treatment.

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

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

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

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*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 B

MULTICENTER GUIDELINES

Responsibility of the Protocol Chair

- The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- The Coordinating Center is responsible for assuring that each participating institution has an IRB approval and must maintain copies of IRB approvals from each participating site.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center will maintain documentation of AE reports. Participating institutions report to the Coordinating Center. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Selected patient records may be audited on-site at participating sites. If the Coordinating Center chooses to have an audit at the participating institution, then the Coordinating Center is responsible giving notice to the site to have all source documents, research records, all IRB approval documents, patient registration lists, response assessments scans, x-rays, etc. available for the audit. Though the Coordinating Center may schedule an audit, the sites will be responsible for their own internal monitoring and auditing on a regular basis. The Coordinating Center may request documentation of these internal processes.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.

- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

Appendix C

Cytochrome 450 3A4 interations

Inducers

Aminoglutethimide	Nevirapine
Bexarotene	Oxcarbazepine
Bosentan	Phenobarbital
Carbamazepine	Phenytoin
Dexamethasone	Primidone
Efavirenz	Rifabutin
Fosphenytoin	Rifampin
Griseofulvin	Rifapentine
Modafinil	St. John's wort
Nafcillin	

Inhibitors

Amiodarone	Imatinib
Amprenavir	Indinavir
Aprepitant	Isoniazid
Atazanavir	Itraconazole
Chloramphenicol	Ketoconazole
Clarithromycin	Lapatinib
Conivaptan	Miconazole
Cyclosporine	Nefazodone
Darunavir	Nelfinavir
Dasatinib	Posaconazole
Delavirdine	Ritonavir
Diltiazem	Quinupristin
Erythromycin	Saquinavir
Fluconazole	Tamoxifen
Fluoxetine	Telithromycin
Fluvoxamine	Troleandomycin
Fosamprenavir	Verapamil
Grapefruit juice	Voriconazole

APPENDIX D

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

Your birthdate (Day, Month, Year):

Today's date (Day, Month, Year): 31

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ – CR29

Patients sometimes report that they have the following symptoms or problems. Please indicate the e to which you have experienced these symptoms or problems during the past week. Please answ circling the number that best applies to you.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Did you urinate frequently during the day?	1	2	3	4
32. Did you urinate frequently during the night?	1	2	3	4
33. Have you had any unintentional release (leakage) of urine?	1	2	3	4
34. Did you have pain when you urinated?	1	2	3	4
35. Did you have abdominal pain?	1	2	3	4
36. Did you have pain in your buttocks/anal area/rectum?	1	2	3	4
37. Did you have a bloated feeling in your abdomen?	1	2	3	4
38. Have you had blood in your stools?	1	2	3	4
39. Have you had mucus in your stools?	1	2	3	4
40. Did you have a dry mouth?	1	2	3	4
41. Have you lost hair as a result of your treatment?	1	2	3	4
42. Have you had problems with your sense of taste?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
43. Were you worried about your health in the future?	1	2	3	4
44. Have you worried about your weight?	1	2	3	4
45. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
46. Have you been feeling less feminine/masculine as a result of your disease or treatment?	1	2	3	4
47. Have you been dissatisfied with your body?	1	2	3	4
48. Do you have a stoma bag (colostomy/ileostomy)? (please circle the correct answer)	Yes		No	

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
Answer these questions ONLY IF YOU HAVE A STOMA BAG, if not please continue below:				
49. Have you had unintentional release of gas/flatulence from your stoma bag?	1	2	3	4
50. Have you had leakage of stools from your stoma bag?	1	2	3	4
51. Have you had sore skin around your stoma?	1	2	3	4
52. Did frequent bag changes occur during the day?	1	2	3	4
53. Did frequent bag changes occur during the night?	1	2	3	4
54. Did you feel embarrassed because of your stoma?	1	2	3	4
55. Did you have problems caring for your stoma?	1	2	3	4

Answer these questions ONLY IF YOU DO NOT HAVE A STOMA BAG:				
49. Have you had unintentional release of gas/flatulence from your back passage?	1	2	3	4
50. Have you had leakage of stools from your back passage?	1	2	3	4
51. Have you had sore skin around your anal area?	1	2	3	4
52. Did frequent bowel movements occur during the day?	1	2	3	4
53. Did frequent bowel movements occur during the night?	1	2	3	4
54. Did you feel embarrassed because of your bowel movement?	1	2	3	4

During the past 4 weeks:	Not at All	A Little	Quite a Bit	Very Much
For men only:				
56. To what extent were you interested in sex?	1	2	3	4
57. Did you have difficulty getting or maintaining an erection?	1	2	3	4

For women only:				
58. To what extent were you interested in sex?	1	2	3	4
59. Did you have pain or discomfort during intercourse?	1	2	3	4