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Title: *A phase I study of SGI-110 combined with irinotecan followed by a randomized phase II study of SGI-110 combined with irinotecan versus regorafenib or TAS-102 (lonsurf) in previously treated metastatic colorectal cancer patients*

Principal Investigator: *Nilofer Azad, MD*
Bunting-Blaustein Cancer Research Building I
1650 Orleans Street, Room 4M10
Baltimore, MD 21231
(p)410-614-9169; (f)410-614-9006,
Email: nazad2@jhmi.edu

Coordinating Center: *Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins*

USC Investigator: *Anthony el-Khoueiry, MD (site PI)*
University of Southern California

VUMC Investigator: *Henk Verheul, MD PhD (site PI)*
VU University Medical Center, Netherlands

MSKCC Investigator: *Andrea Cercek, MD (site PI)*
Memorial Sloan Kettering Cancer Center

Statistician: *Marianna L. Zahurak*
Division of Oncology Biostatistics and Bioinformatics
The Sidney Kimmel Comprehensive Cancer Center
550 N. Broadway, Suite 1103
Baltimore, MD 21287
Office: 410-955-4219
Fax: 410-955-0859
Email: zahurma@jhmi.edu

SKCCC Study Coordinator: *Tearra Miles*
Research Coordinator
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
201 North Broadway 8th floor Space 8120-43
Baltimore, MD 21205-2009
Phone: 410-502-5328
Fax: 410-502-0834
Email: tlawre18@jhmi.edu

SKCCC Research Nurse: *Ellen Lilly-Foreman*
Johns Hopkins Kimmel Cancer Center
Research Nurse-GI Oncology
201 N. Broadway,
Baltimore, MD 21205
Phone: 443-287-4961
Fax: 410-502-0834
Email: lillyel@jhmi.edu

Sponsor: *Nilofer Azad, MD*

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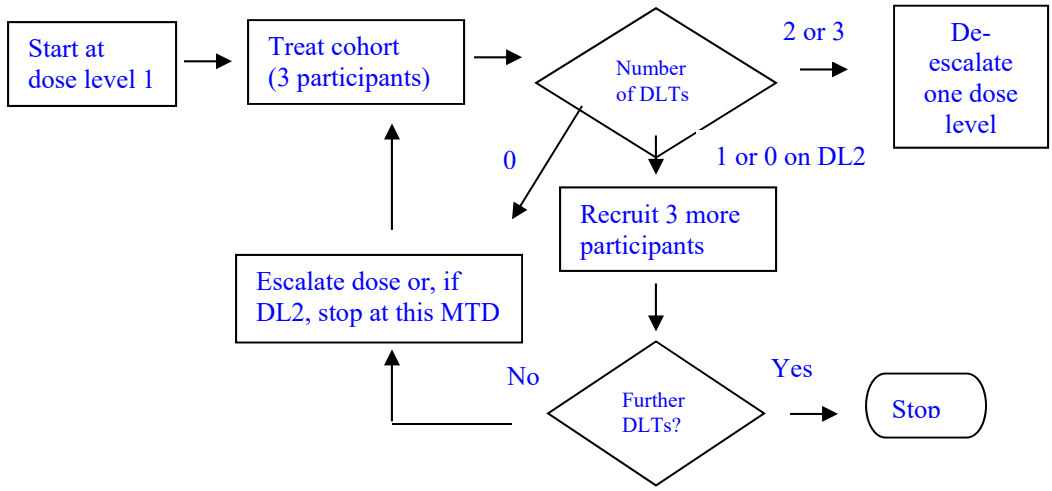
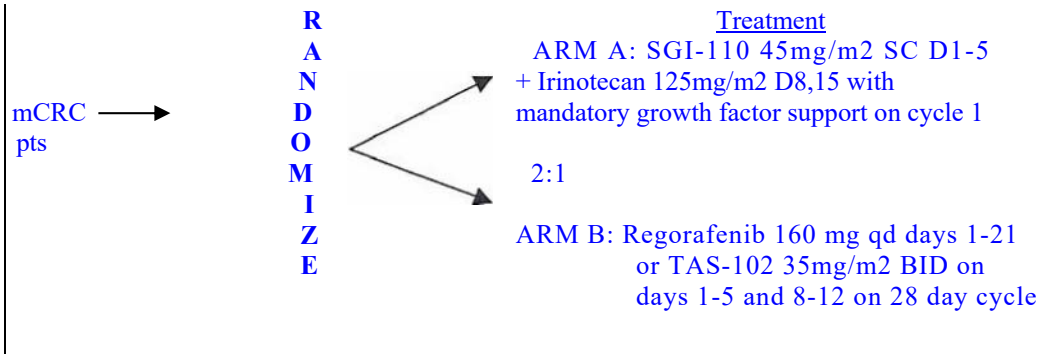
SCHEMA

Stage I



Stage II



1. OBJECTIVES

1.1 Study Design

This is a phase I/randomized phase II study of the combination of SGI-110 and irinotecan in previously treated metastatic colorectal cancer patients. This study will be conducted in two components. First, patients will be enrolled in a phase I study of SGI-110 combined with irinotecan in a standard 3+3 design. After the maximum tolerated dose (MTD) is determined, patients will subsequently be enrolled in a 2:1 randomized phase II study of SGI-110 and irinotecan versus the standard of care regorafenib or TAS-102 (lonsurf).

1.2 Primary Objectives

Phase I Component: Stage I
--To assess safety and tolerability of combination SGI-110 + irinotecan in colon cancer patients
--To determine the phase II dose of the combination of SGI-110 + irinotecan

Phase II Component: Stage II
--To improve median progression-free survival from that reported with regorafenib and TAS-102 to 4 months for SGI-110+irinotecan in previously treated metastatic colon cancer patients who have progressed on irinotecan.

1.3 Secondary Objectives

Phase I Component: Stage I
--To assess changes in global methylation and expression at the tumor level with SGI-110 and irinotecan treatment
--To assess for pharmacokinetic interactions of SGI-110 and irinotecan
--To assess for correlation between disease response and drug exposure

Phase II Component: Stage II
--To evaluate response rate as determined by RECIST criteria 1.1
--To evaluate concurrent SGI-110+irinotecan treatment versus regorafenib or TAS-102 alone
--To improve median overall survival from historical rate of 6.4 months
--To assess for potential predictive biomarkers of response and survival using baseline tissue

2. BACKGROUND

2.1 SGI-110

2.1.1 SGI-110 Background

For further information on SGI-110 please refer to the most up to date version of the Investigator's Brochure (IB).[1]

SGI-110 is a DNA methyltransferase inhibitor (a demethylating agent). The active metabolite of SGI-110 (2'-deoxy-5-azacytidylyl-(3'→5')-2'-deoxyguanosine sodium salt), a dinucleotide, is

decitabine. SGI-110 is resistant to modification by cytidine deaminase, a common pathway of decitabine metabolism and deactivation.[2] The molecular weight of SGI-110 and decitabine are 580 Da and 228 Da, respectively. Therefore, the molar equivalent dose of 1 mg of decitabine is approximately 2.5 mg of SGI-110. SGI-110's activity was demonstrated with the same preclinical pharmacodynamic assays used to demonstrate decitabine's efficacy e.g., re-expression of p15, p16, and MLH1 and induction of fetal hemoglobin, *in vivo*. In xenograft studies, SGI-110 demonstrates promising preclinical activity in both hematologic and solid tumors.

In vitro evidence suggests that SGI-110 has a longer half-life than decitabine in the presence of cytidine deaminase. These promising observations suggest that SGI-110 has improved pharmaceutical properties and biological activities that expand on decitabine's current clinical utility. SGI-110 has shown to be better tolerated in mice than decitabine and is as effective *in vivo* in inducing p16 expression, reducing DNA methylation at the p16 promotor region, and retarding EJ6 human bladder cancer tumor growth in athymic mice.[3]

SGI-110 has been developed for subcutaneous administration. SGI-110 is pharmacologically active both *in vitro* and *in vivo* in a variety of tumor cells and murine xenograft models when administered subcutaneously. Treatment is well tolerated via the subcutaneous route in murine xenografts. When administered subcutaneously to non-human primates, SGI-110 releases decitabine slowly compared to other species, possibly prolonging the effect over longer periods. SGI-110 has been developed as a non-aqueous formulation to ensure formulation stability.

2.1.2 Nonclinical pharmacokinetics

The overall pharmacokinetic characteristics of SGI-110 are summarized as follows. The relative bioavailability of SGI-110 dosed subcutaneously to the rat is close to 100%. Circulating SGI-110 levels were very low in the mouse, rat and rabbit. However, higher levels were observed in the monkey post subcutaneous dosing. Rapid decline in systemic exposure of SGI-110 with elimination plasma half-life (T1/2) in the range of 0.4-1 hours in rat and monkey was observed. High levels of decitabine were observed after a subcutaneous dose of SGI-110 in the mouse, rat, and rabbit (maximum in rat, 54 μg/mL). Levels in the monkey (maximum 463 ng/mL) were substantially lower. Rapid decline in systemic exposure of decitabine with elimination plasma T1/2 of 3.7 hour in rats and 1 hour in monkey was observed.

In monkey, pharmacokinetic parameters were similar on Day 1 and Day 15 of a study in which they were dosed once weekly for three consecutive weeks suggesting no significant accumulation of the parent or the active metabolite, decitabine.

The metabolic characteristics of SGI-110 are summarized as follows. SGI-110 was more stable in human, dog and mouse and was less stable in rat and rabbit plasma. Incubation of SGI-110 with liver microsomes from mouse, rat, rabbit, dog and human showed little apparent metabolism of the compound. Incubation of SGI-110 with human hepatocytes also showed little apparent metabolism of the compound, based on disappearance of the parent. SGI-110 does not significantly bind to human plasma proteins; *in vitro* unbound fraction was estimated to be 91%. SGI-110 has poor *in vitro* bidirectional permeability which correlates well with its poor oral

absorption *in vivo*. SGI-110 shows no appreciable induction of CYP1A1/2, CYP2C9 and CYP3A4 in human hepatocytes. SGI-110 does not have any CYP450 inhibitory effect on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.

2.1.3 Nonclinical safety of SGI-110

SGI-110 toxicity findings in rat and rabbit studies are similar to the non-clinical study findings of decitabine in New Drug Application (NDA) supporting Good Laboratory Practices (GLP) toxicology studies. Myelosuppression, decreases in thymus weight, and testicular atrophy, the main study findings of the SGI-110 studies, were also observed as the main study findings in repeat dose toxicity studies with decitabine in mice, rats, rabbits, and dogs. As with SGI-110, myelosuppression and thymus toxicities after decitabine administration were reversible during recovery periods while testicular atrophy persisted. Myelosuppression, particularly neutropenia, has been reported as a dose-limiting toxicity for decitabine in human clinical studies. Signs of testicular toxicity has not been observed in any of the published clinical studies of decitabine to date.[4]

No studies have been performed to evaluate the genotoxic, mutagenic, carcinogenic or reproductive and developmental toxicity of SGI-110. Decitabine may have genotoxic potential; decitabine is mutagenic and in preclinical studies in mice and rats, decitabine was teratogenic, fetotoxic, and embryotoxic.[4]

2.1.4 SGI-110 Clinical Data

SGI-110 is being studied in a first-in-human, single-agent study (SGI-110-01).[5] This study is a Phase 1/2, dose escalation, multicenter study of two subcutaneous regimens of SGI-110 in subjects with intermediate or high-risk myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML). This study has two parts, a Dose Escalation Segment and a Dose Expansion Segment. The study seeks to evaluate the biological activity, preliminary safety, and efficacy of SGI-110 with two dosing schedules in intermediate to high risk MDS or relapsed or refractory AML subjects, while the Dose Expansion Segment further evaluates safety and efficacy at the recommended dose. The study is based on a 3 + 3 design within each regimen. Eligible subjects are being randomized to receive 1 of 2 dosing regimens of SGI-110 with the following starting doses: Regimen 1: 3 mg/m2/day subcutaneously on Days 1-5 of a 28-day course, Regimen 2: 6 mg/m2 subcutaneously Weekly x 3 on Days 1, 8, 15 of a 28-day course. As of 23 April 2012, the study has enrolled 78 subjects into 7 dose cohorts in the Dose Escalation Segment, as shown below (Table 1). The minimum dose achieving maximal biological activity or biologically effective dose (BED) for the daily regimen has been reached at 60 mg/m2 daily on Days 1-5.

Table 1: Study SGI-110-01 Enrollment by Cohort

Cohort	SGI-110 Dose		Number of Subjects		
	Daily	Weekly	Daily	Weekly	Total
Cohort 1	3 mg/m^2/day	6 mg/m^2/week	4	5	9
Cohort 2	9 mg/m^2/day	18 mg/m^2/week	4	3	7
Cohort 3	18 mg/m^2/day	36 mg/m^2/week	5	6	11
Cohort 4	36 mg/m^2/day	60 mg/m^2/week	6	6	12
Cohort 5	60 mg/m^2/day	90 mg/m^2/week	7	8	15
Cohort 6	90 mg/m^2/day	125 mg/m^2/week	6	6	12
Cohort 7	125 mg/m^2/day	-	12	-	12
			Total		78

2.1.5 Human pharmacokinetics

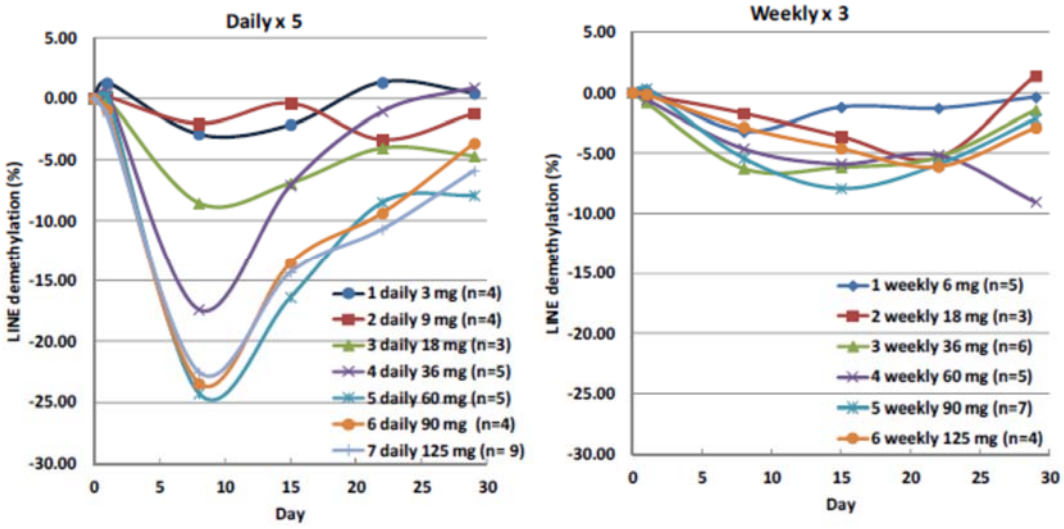
The PK of SGI-110 and decitabine are being evaluated in Study SGI-110-01. Decitabine forms from SGI-110 as it undergoes cleavage by phosphodiesterase (PDE) enzymes. SGI-110 after SC injection undergoes efficient conversion to decitabine and delivers decitabine exposures as measured by AUC that are equivalent or higher than those achieved by IV decitabine infusion at 20 mg/m2, while maintaining significantly lower decitabine Cmax. It is hypothesized that lower Cmax may be associated with less toxicity.

Due to slower release of decitabine from SGI-110, the effective half-life of decitabine after SC SGI-110 is prolonged and the observed decitabine exposure window is longer (8+ hrs) compared to IV (3-4 hrs). At Cohort 6, doses of 90 mg/m2 for the daily regimen and 125 mg/m2 for weekly, the observed decitabine AUCs were approximately 1.41 and 1.77 fold higher than with IV decitabine at the approved dose of 20 mg/m2 IV, whereas Cmax levels were only at 0.35 and 0.44-fold for the daily and weekly regimens, respectively. It is hypothesized that longer exposure to decitabine may allow more drug to be incorporated into the DNA thus resulting in better hypomethylation and better biological activity. Clinical drug-drug interaction studies have not been conducted with SGI-110. *In vitro*, SGI-110 does not inhibit the activity nor induce levels of major human CYP enzymes, hence, the likelihood of CYP-mediated drug-drug interactions with SGI-110 is remote.[5]

2.1.6 Clinical efficacy and biological activity of SGI-110

Based on PD assessment of global hypomethylation from the first 7 cohorts in Study SGI-110-01, the LINE-1 demethylation data show dose dependent hypomethylation induction in the daily schedule reaching a plateau at Cohort 5 (60 mg/m2 SC dailyx5). The hypomethylation of the weekly schedule was inferior to the daily schedule and plateaued early (Figure 1). The Biologically Effective Dose or BED was therefore established as 60 mg/m2 dailyx5.

Figure 1: LINE demethylation



There have been 5 documented major responses (2 CRs and 2 CRi, 1 CRp) all in heavily-pretreated refractory AML subjects regardless of prior hypomethylating agents (HMA) treatment : 1 CR and CRp with weekly (60 mg/m2 and 125mg/m2, respectively) and 2 CRi and 1 CR with daily (36/60 mg/m2 and 60 mg/m2 respectively). The major responses were observed in 5/19 refractory AML patients when adequate hypomethylation (>10%) was achieved. Five MDS patients who all received prior treatment with HMAs had hematological improvements or marrow CR.

As of 27 March 2012, there were 54 subjects who have information in the clinical database in the Phase 1/2 study with single-agent SGI-110. This data cutoff date includes all subjects in both cohorts up to Cohort 5.

Of the 54 subjects in the clinical database, 46 (85%) have reported at least 1 adverse event (AE). There were 26 (48%) subjects with an AE considered related by the Investigator. The most common related AEs, occurring in at least 3 subjects (6%) in decreasing incidence were injection site pain (n=12, 22%); fatigue (n=6, 11%); nausea (n=5, 9%); and thrombocytopenia, anaemia and diarrhea (n=3, 6%). All of these were Grade 1 or 2 AEs with the exception of 1 subject (1 in 60 mg/m2 dailyx5) with Grade 4 thrombocytopenia and 2 subjects (1 in 3 mg/m2 dailyx5 and 1 in 60 mg/m2 dailyx5) with Grade 3 anaemia.

As of 31 May 2012, there were 156 serious adverse events (SAEs) in 66 subjects in the safety database regardless of relationship to the study drug. The most common SAEs (≥5%) were febrile neutropenia (n=24, 36%), pneumonia (n=17, 26%), disease progression (n=7, 11%), sepsis (n=6, 8%), pyrexia (n=5, 9%), bacteraemia and skin infection (n=4, 6% each). Most events were considered not related to study drug as they were expected from disease progression of the study population (MDS and AML). Twelve (12) subjects had SAEs considered by the investigator as related to the use of SGI-110: 3 subjects with febrile neutropenia, 2 subjects with thrombocytopenia, and 1 subject each with pseudomonal sepsis, atrial fibrillation, chest pain, pleural effusion, bacteraemia, sepsis and dysphagia.

As of May 2012, there have been three expedited safety reports submitted to regulatory authorities by the Sponsor concerning suspected adverse reactions related to SGI-110: one event of pleural effusion for which no other cause was established and did not return upon cessation of therapy (at 36 mg/m2 in the weeklyx3 regimen) and 2 events of febrile neutropenia (both at 125 mg/m2 dailyx5), one of which was associated with sepsis that resulted in the subject's death. Both febrile neutropenia cases were the only 2 cases that were considered as Dose Limiting Toxicities (DLTs) and they both occurred in patients with MDS in Cohort 7 (125 mg/m2 dailyx5).The maximum administered dose (MAD) for the dailyx5 regimen is 125 mg/m2 and the MTD is considered to be 125 mg/m2 dailyx5 for AML patients and 90 mg/m2 dailyx5 for MDS based on 2 cases of febrile neutropenia that were considered as Dose Limiting Toxicities (DLTs) that occurred in patients with MDS in Cohort 7 (125 mg/m^2 dailyx5).The MTD for the weekly regimen was not established as no DLTs were observed up to the highest dose tested of 125 mg/m2 weeklyx3 regimen. The risks of SGI-110 in humans are described further in Section 6, Expected Toxicities. For more detailed information, please refer to the IB for SGI-110. The two main drug-related adverse reactions of SGI-110 are myelosuppression (neutropenia, febrile neutropenia, thrombocytopenia, and anemia) and its consequences (such as infection, fever, and sepsis), and injection-site events such as pain, irritation, and inflammation. At the SGI-110 proposed in this study (30-60 mg/m2 daily on Days 1-5), there have been no reports of drug-related myelosupression in MDS and AML subjects in study SGI-110-01. The recommended starting dose of 45 mg/m2 daily on Days 1-5 is 50% of the MTD for single-agent treatment with SGI-110 and about one-third of the MAD based on first-in-human study SGI-110-01.

Pain and burning at the injection site has been reported that are related to dose and volume of injection. Other than these events described above, SGI-110 has been well tolerated up to 90 mg/m2 dailyx5 and 125 mg/m2 weekly x3.

In summary, SGI-110 single agent treatment for MDS and AML patients was well tolerated up to 125 mg/m2 weeklyx3 and 90 mg/m2 dailyx5 given every 4 weeks. DLTs of febrile neutropenia with bacteremia or sepsis were only observed at 125 mg/m2 dailyx5. Daily dosing achieved much better hypomethylation than the weekly regimen and the BED of the daily regimen was established at 60 mg/m2 dailyx5. At that dose there were no DLTs observed in Study SGI-110-01.[5]

2.2 Irinotecan

2.2.1 Background

Irinotecan is an FDA approved, commercially available topoisomerase I inhibitor with activity against several solid tumor cell lines including colon, lung, and ovary. Irinotecan received an accelerated approval in June 1996 to treat metastatic cancer of the colon or rectum that has recurred or progressed after standard therapy with 5-FU. Irinotecan received full FDA approval in 1998 as a second-line treatment for metastatic colorectal cancer. In April, 2000, irinotecan received accelerated approval from the FDA as first-line treatment for metastatic colon cancer when combined with 5-FU and Lecovorin. The cytotoxic effect is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary

complex formed by topoisomerase I, DNA, and either irinotecan or SN-38 (its active metabolite).[6]

2.2.2 Dose and Toxicology.

The drug is given intravenously with the MTD for weekly dosing being 125 mg/m^2 as a single agent. The typical schedule of irinotecan administration in metastatic colon cancer is four consecutive weekly doses combined with 5-FU and leucovorin given every six weeks. Conversion of irinotecan to SN-38 (its active metabolite) occurs in the liver. Half-life for irinotecan is about 6 hours. Half-life for SN-38 is about 10 hours. SN-38 is 95% bound to plasma proteins.

Irinotecan is associated with both early (during or shortly after infusion) and late (more than 24 hours after infusion) forms of diarrhea that may be severe. Early diarrhea may be accompanied by symptoms such as sweating, flushing and abdominal cramping. Late diarrhea can be prolonged, life threatening, and should be treated promptly. In two Phase III trials in second line treatment of colorectal cancer, adverse events occurring more commonly in patients on irinotecan than best supportive care or 5-FU based therapy were diarrhea, nausea, vomiting and neutropenia. In these studies, 60 percent of patients receiving irinotecan were hospitalized at least once due to adverse events, compared with 63 percent receiving best supportive care alone and 39 percent receiving 5-FU. Patients also experienced temporary hair loss which usually returned after the completion of therapy.[7]

2.3 Regorafenib

2.3.1 Background of regorafenib

Various signaling pathways have been implicated in the development and progression of colorectal cancer, involving receptor tyrosine kinases (eg, EGFR, VEGF receptor, platelet-derived growth factor receptor [PDGFR], and fibroblast growth factor receptor [FGFR]) and downstream signalling cascades (RAS-RAF-MEK-ERK and PI3K-PTEN-AKT-mTOR).[8] Regorafenib is a novel oral multikinase inhibitor that blocks the activity of several protein kinases, including kinases involved in the regulation of tumour angiogenesis (VEGFR1 [also known as FLT1], VEGFR2 [KDR], VEGFR3 [FLT4], TIE2 [TEK]), oncogenesis (KIT, RET, RAF1, BRAF, and BRAF^{V600E}), and the tumour microenvironment (PDGFR and FGFR).[9] In preclinical studies, regorafenib has shown antitumour activity, including in colorectal cancer models.[9]

In a phase 1b study, oral regorafenib, given at a dose of 160 mg once daily for the first 3 weeks of each 4 week cycle, showed a tolerable toxicity profile and preliminary evidence of antitumour activity in 38 patients with progressive colorectal cancer who had received previous therapy for metastatic disease (median four lines).[10] The disease control rate (partial response plus stable disease) was 74% (20 of 27 assessable patients). On the basis of these results and the high unmet need in this population of patients, the decision was made to proceed to a randomized phase 3 trial.

2.3.2 CORRECT trial

From Lancet: "CORRECT was a randomized, placebo-controlled, phase 3 study involving 114 centers in 16 countries in North America, Europe, Asia, and Australia. Patients were eligible to participate when they had histological or cytological documentation of adenocarcinoma of the colon or rectum. They had to have received locally and currently approved standard therapies and to have disease progression during or within 3 months after the last administration of the last standard therapy or to have stopped standard therapy because of unacceptable toxic effects. Patients were randomly assigned in a 2:1 ratio to regorafenib or placebo.

753 patients initiated treatment (regorafenib n=500; placebo n=253; population for safety analyses). The primary endpoint of overall survival was met at a preplanned interim analysis. Median overall survival was 6·4 months in the regorafenib group versus 5·0 months in the placebo group (hazard ratio 0·77; 95% CI 0·64–0·94; one-sided p=0·0052). Treatment-related adverse events occurred in 465 (93%) patients assigned regorafenib and in 154 (61%) of those assigned placebo. The most common adverse events of grade three or higher related to regorafenib were hand-foot skin reaction (83 patients, 17%), fatigue (48, 10%), diarrhoea (36, 7%), hypertension (36, 7%), and rash or desquamation (29, 6%)."[11]

Based on these results, the FDA approved regorafenib for heavily pretreated colorectal cancer patients on September 27, 2012.[12]

2.4 TAS-102 (Lonsurf)

2.4.1 Background of TAS-102

For several decades, 5-FU was the only active chemotherapy in the treatment of metastatic colon cancer. Since then it, and other fluoropyrimidines, have remained the backbone to chemotherapy regimens for colorectal maligancies. Fluoropyrimidines act by inhibiting thymidylate synthase, which is necessary for the synthesis of pyrimidine nucleotides and DNA synthesis. TAS-102 is an oral combination of two agents: trifluridine (thymidine-based nucleic acid analog), which directly incorporates into DNA, and tipiracil hydrochloride (thymidine phosphorylase inhibitor), which delays the breakdown of trifluridine.[13]

In a Japanese double-blind, randomized, placebo-controlled, phase 2 trial of 169 patients with metastatic colorectal cancer who had been heavily pretreated (two or more prior standard chemotherapy regimens) and were refractory or intolerant to fluoropyrimidines, irinotecan, and oxaliplatin. Patients were randomized in a 2:1 ratio to receive TAS-102 (35mg/m2) orally twice a day for 2-week cycle of 5 days of treatment followed by a 2-day rest period, and then a 14-day rest period or placebo. 112 patients were assigned to receive TAS-102 and 57 to placebo, with median overall survival of 9.0 months in TAS-102 group versus 6.6 months in the placebo group (HR 0.56, 95% CI 0.39-0.81, p=0.0011).[14] A subsequent, international Phase III trial was undertaken to evaluate the benefit.

2.4.2 RECOURSE trial

RECOURSE was a randomized, placebo-controlled, phase 3 study involving 116 centers in 13 countries in North America, Europe, Asia and Australia. Patients were eligible to participate if

they had biopsy-proven metastatic colorectal adenocarcinoma who had received at least two prior regimens of standard chemotherapies with subsequent progression within 3 months after last administration of standard therapy or intolerable side effects; or if patients had received adjuvant therapy, developed progression within 6 months. Patients were randomly assigned in a 2:1 ratio to receive TAS-102 or placebo.

798 patients initiated treatment (TAS-102 n = 533; placebo n = 265; intention to treat). All patients had previously received fluoropyrimidine, oxaliplatin, and irinotecan; 17% of the TAS-102 group had previously received regorafenib. The primary endpoint of median overall survival was 7.1 months in the TAS-102 group and 5.3 months in the placebo group (HR 0.68, 95% CI 0.58-0.81; p<0.001). The median time to an ECOG performance status of 2 or higher was 5.7 months in the TAS-102 group versus 4.0 months in the placebo group. Treatment-related adverse events occurred in 524 (98%) of patients receiving TAS-102 and in 247 (93%) of those receiving placebo. 370 (69%) of TAS-102 patients had grade 3 or higher adverse events, in comparison to 137 (52%) of those receiving placebo. The most common adverse events of grade three or higher related to TAS-102 included neutropenia (38%), febrile neutropenia (4%), anemia (18%), thrombocytopenia (5%). Higher grade three adverse events for those treated with TAS-102 compared to placebo were noted for nausea (2% v 1%), vomiting (2% v <1%), and diarrhea (3% v <1%).[15] Based on these results, the FDA approved TAS-102 for refractory metastatic colon cancer on September 22, 2015.[16]

2.5 Study disease: colorectal cancer

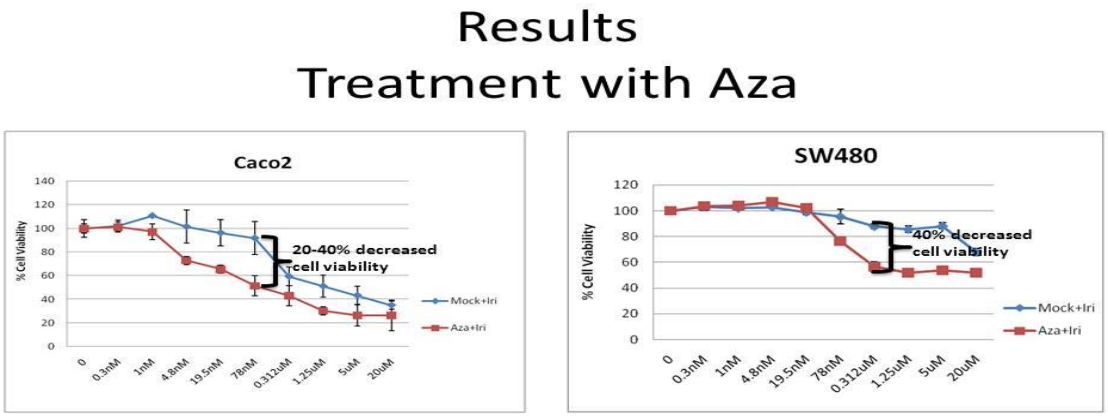
Metastatic colorectal cancer (mCRC) is the second leading cause of cancer death in the United States. In 2012, approximately 143,460 Americans will be diagnosed with CRC, and 51,690 will die from this disease.[17] The five-year survival for all patients with metastatic disease from CRC is only 8.1%.[18] Median survival with current generation of chemotherapies, including oxaliplatin or irinotecan as well as newer biologic therapies such as bevacizumab and cetuximab is only 20-24 months. The only predictive biomarker that is currently used in clinical colorectal cancer practice is *KRAS* gene status which has only been in clinical application since 2008 when mutant *KRAS* was found to predict a lack of benefit to anti-EGFR agents. Therefore, there are currently only three lines of therapy for the 40% of patients with KRAS mutation (oxaliplatin-based and irinotecan-based regimens) with a potential added option for KRAS wildtype patients.

Patients with mCRC have a 60% likelihood of responding to first-line chemotherapy with an oxaliplatin or irinotecan containing regimen and drops to 10% in the second line. Agents effective and or approved in the third line such as panitumumuab and recently reported regorafenib have a less than 5% response rate, and an expected median overall survival of 6.4 months. However, in patients with KRAS wildtype tumors who progressed on irinotecan, Cunningham et al. showed that retreatment with irinotecan with cetuximab resulted in a 22% response rate, and cetuximab is now FDA-approved for this indication. No such irinotecan retreatment option exists for KRAS mutated patients.

2.6 Rationale

Preliminary data from our group's phase I/II study of 5-azacitidine and entinostat in non-small cell lung cancer has shown that, in patients who received subsequent therapy with varied agents, approximately one third had at least a partial response by RECIST criteria to that subsequent therapy.[19] In CRC, as there are no subsequent therapy options for patients after 2nd or 3rd line therapy (based on KRAS mutational status), our group has been exploring the potential of DNMT inhibitor therapy to synergize subsequent therapy. Ishiguro et al. first demonstrated that treatment with DAC resulted in gene re-expression of known tumor suppressor genes in CRC; moreover, in both *in vitro* and *in vivo* models, DAC treatment substantially decreased tumor growth when combined with irinotecan or SN-38 (active irinotecan metabolite) compared to either agent alone.[20] In squamous cell cervical cancer, DAC restored SN-38 sensitivity in cells cultivated for SN38 resistance.[21] 5-aza has also been shown to increase irinotecan sensitivity in multiple CRC cell lines, with a synergistic effect seen in a p53-mutated cell line.[22] We have found that pretreatment of colorectal cancer cell lines with 5-aza or decitabine has sensitized CRC cell lines *in vitro* to subsequent treatment with SN38, the active metabolite of irinotecan, by both MTS and clonogenic assays (Figures 1-3).

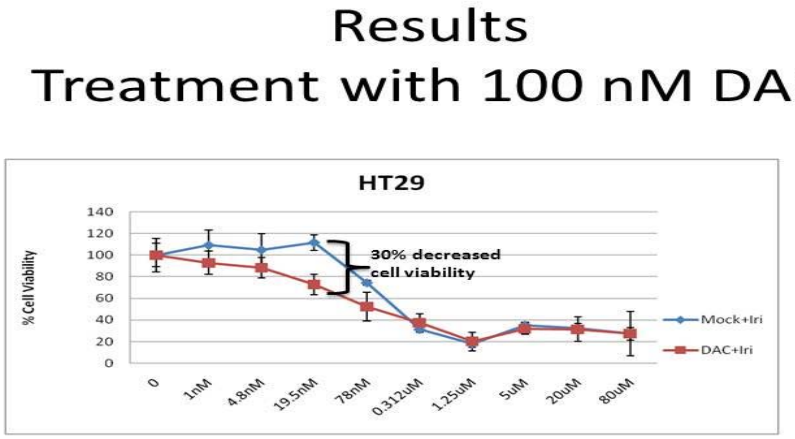
Figure 1



Cell line	IC50 of Mock+Iri treated cells	IC50 of Aza+Iri treated cells
CACO2	1.25 uM	78 nM
SW480	>20 uM	312 nM

Figure 1: CRC cell lines were treated in 96 well plates with azacitidine at 500 nM for 3 days followed by two day exposure to SN38 and then analyzed by MTS assay.

Figure 2



Cell line	IC50 of Mock+Iri treated cells	IC50 of DAC+Iri treated cells
HT29	< 312 nM	78 nM

Figure 2: CRC cell lines were treated in 96 well plates with decitabine at 100 nM for 3 days followed by two day exposure to SN38 and then analyzed by MTS assay.

Figure 3

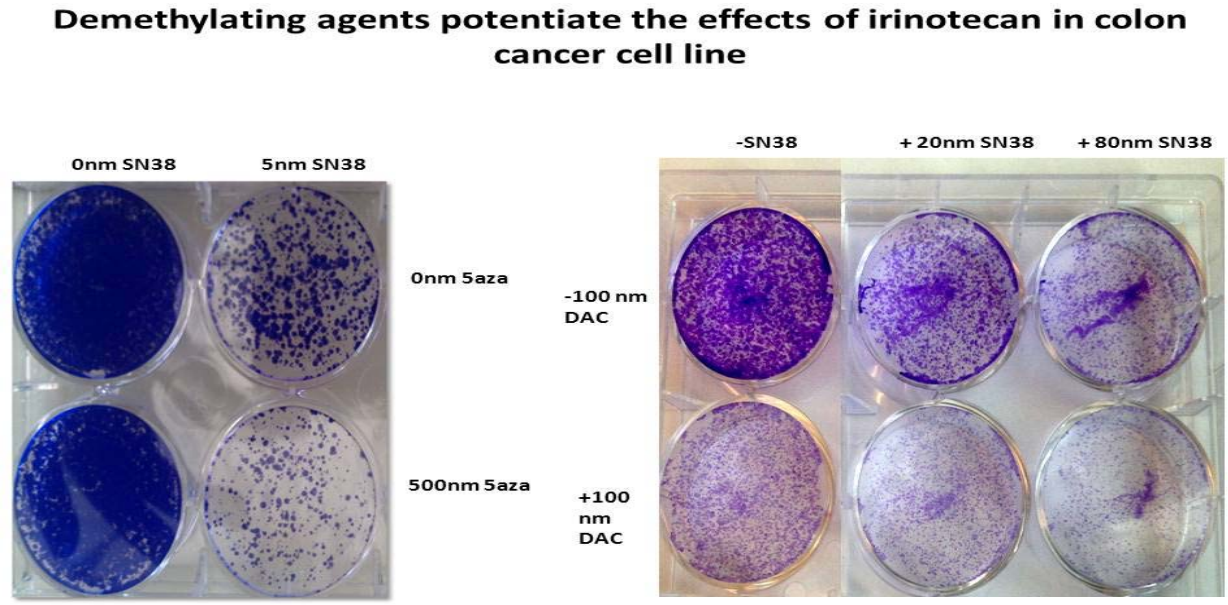
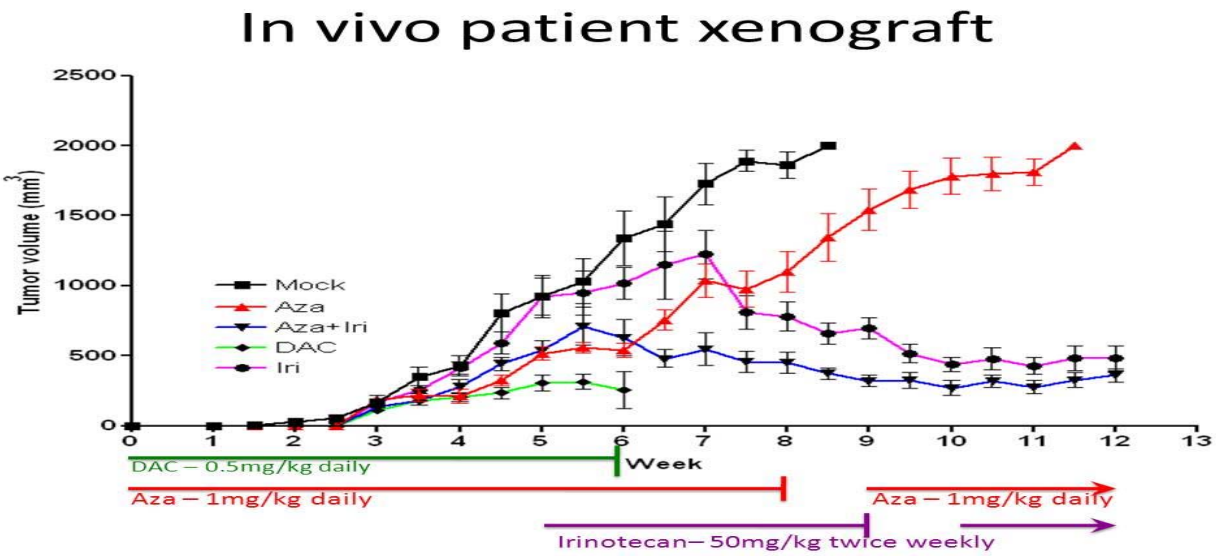


Figure 3: 10^5 CRC cell lines were treated with decitabine at 100 nM for 3 days and then +/- SN38

We have seen similar effects in a primary patient-derived xenograft *in vivo* (Figure 4).

Figure 4



Based on these data, we hypothesize that DNMTi therapy with SGI-110 will sensitize advanced colorectal cancer to treatment with Irinotecan. We propose a phase I study to assess the safety and tolerability of SGI-110 in combination with standard irinotecan therapy in 3rd line + metastatic colon cancer, followed by a randomized phase II study to evaluate efficacy of the combination.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.2 Participants must have histologically or cytologically confirmed adenocarcinoma of the colon or rectum

3.1.3 Patients in the phase I cohort must have biopsiable disease and be amenable to having two research biopsies

3.1.4 Archival tissue must be procured if available

3.1.5 Participants 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) as ≥ 20 mm with conventional techniques or as ≥

10 mm with spiral CT scan. See section 10 for the evaluation of measureable disease.

3.1.6 Patients in the phase II cohort must have progressed while receiving irinotecan therapy in the metastatic setting. There are no limitations on number of prior therapies in the metastatic setting.

3.1.7 Age minimum of 18 years.

Because no dosing or adverse event data are currently available on the use of SGI-110 in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials.

3.1.8 Life expectancy of greater than 12 weeks.

3.1.9 ECOG performance status ≤1

3.1.10 Participants 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 < 1.5X institutional upper limit of normal
- AST (SGOT)/ALT (SGPT) ≤ 3.0 X institutional upper limit of normal with or without liver metastases
- Creatinine < 1.5X institutional upper limit of normal or creatinine clearance ≥ 50 mL/min/1.73 m^2 for subjects with creatinine levels above institutional normal .

3.1.11
The effects of SGI-110 on the developing human fetus are unknown. For this reason and because oncological agents 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 participating in this study, she should inform her treating physician immediately.

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

3.2 Exclusion Criteria

3.2.1 Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.2 Participants who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to enrolling in the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

3.2.3 Participants may not be receiving any other study agents.

3.2.4 Participants 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.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to irinotecan, decitabine or SGI-110.

3.2.6 Subjects who have received prior therapy with any hypomethylating agents.

3.2.7 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.

3.2.8 Pregnant women are excluded from this study because SGI-110 is a/an hypomethylating agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with SGI-110, breastfeeding should be discontinued. These potential risks may also apply to other agents used in this study

3.2.9 Individuals with a history of a different malignancy are ineligible except for the following circumstances: individuals with a history of other malignancies who have been disease-free for at least 5 years; or individuals with another malignancy that are deemed by the investigator to be at low risk for clinically meaningful recurrence (ex. cervical cancer *in situ*, definitively treated early stage prostate cancer (confined to prostate with Gleason 6 or below), definitely treated breast ductal or lobular carcinoma *in situ*, basal cell or squamous cell carcinoma of the skin).

3.2.10 HIV-positive individuals on combination antiretroviral therapy are ineligible, as these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate

studies will be undertaken in participants receiving combination antiretroviral therapy when indicated

3.2.11 Previous treatment with regorafenib AND TAS-102 (This applies to phase II only. If patients have previously received either regorafenib OR TAS-102, they must be able to receive the alternate regimen if randomized to the standard of care arm)

3.2.12 Hospitalization for an acute medical issue within 4 weeks prior to screening visit that would otherwise not be managed in an infusion center or outpatient clinic setting (e.g., a patient admitted to complete a transfusion would not be ineligible.).

3.2.13 Symptomatic bowel obstruction within 6 months prior to enrollment. Patients who undergo surgical correction of obstructing lesion will be eligible within 6 months

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

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 the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (SKCCC) by the Lead Study Coordinator. A record of patients who fail to meet entry criteria (*i.e.* screen failures) will be maintained. Patient registration must be complete before beginning any treatment or study activities.

Following registration, patients should begin protocol treatment within10 business 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 Lead Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institutions will order SGI-110 agents directly from Astex pharmaceuticals. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded to and acknowledged by the Coordinating Center.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed to 410-502-0834, or emailed to the Lead Study Coordinator at tbrown55@jhmi.edu:

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form
- Facsimile Transmittal Page
- Completed Registration Patient Worksheet
- Eligibility Checklist

To complete the registration process, the Lead Study Coordinator will

- assign a patient study number
- assign the patient a dose
- register the patient on the study
- Fax or email the patient study number and dose to the participating site
- Call or email the research nurse or data manager at the participating site and verbally confirm registration

4.3 Patient assignment and randomization procedure for second cohort

Each subject who satisfies the eligibility criteria and is accepted for the study will be assigned a unique identification number. The subject number will be used to identify the subject throughout the study and will be entered on all study documents. Once the subject identification number has been assigned, a confirmation email will be sent to all site personnel.

For the Randomized Cohort, subjects will be randomized to each treatment arm (SGI-110+irinotecan or regorafenib/TAS-102) prior to treatment administration and should not exceed 1 week of the planned dose. The assignment will not be blinded. Randomization will be stratified by standard treatment (Regorafenib or TAS-102) and time from last irinotecan treatment (> 6 months or < 6 months) through a dynamic algorithm to maintain balance between the arms as much as possible. For those assigned to treatment arm with standard of care therapy will receive regorafenib (if previously received TAS-102) or TAS-102 (if previously received regorafenib). If they have never received either regorafenib or TAS-102, choice of therapy will be deferred to treating physician and patient.

This is an open-label study. There will be no blinding of treatment assignment.

5 TREATMENT PLAN

5.1 Overall Study Design

There will be two stages in this study: A phase I lead-in (Stage 1) followed by a randomized, controlled, open-label stage (Stage 2). Treatment will be administered on an outpatient basis

5.1.2 Stage 1

Stage 1 will be a traditional phase I study with a standard 3+3 design.[23] A maximum of twenty-one eligible subjects will be enrolled to the phase I study. Study drugs will be escalated per the schema to maximize the dose of drugs when given concurrently. Patients will be treated with combined therapy immediately. Each cycle will last 28 days. All patients enrolled at each dose level will be evaluated for DLT for the purpose of determining the MTD. The dose escalation scheme is shown below.

Table 2: Dose escalation schedule

Dose Level	SGI-110 (SC)	Irinotecan (IV)	Growth Factor Support with Filgrastim (or institutional equivalent) and/or Pegfilgrastim (SC)
-1G	30 mg/m2 days 1-5	125 mg/m2 days 8 and 15 each cycle	Filgrastim 5mcg/kg/day Cycle 1 Days 9-14 and peg-filgrastim 6mg D16, additional growth factor support during Cycle 1 and subsequent cycles per clinician judgment
-1	30 mg/m2 days 1-5	125 mg/m2 days 8 and 15 each cycle	Per clinician judgment
1 (starting dose level)	45 mg/m2 days 1-5	125 mg/m2 days 8 and 15 each cycle	Per clinician judgment
1G	45 mg/m2 days 1-5	125 mg/m2 days 8 and 15 each cycle	Filgrastim 5mcg/kg/day Cycle 1 Days 9-14 and peg-filgrastim 6mg D16, additional growth factor support during Cycle 1 and subsequent cycles per clinician judgment
2	60 mg/m2 days 1-5	125 mg/m2 days 8 and 15 each cycle	Per clinician judgment
3	60 mg/m2 days 1-5	125 mg/m2 days 1, 8 and 15 each cycle	Per clinician judgment

At least three patients will be treated at each dose level. If 0/3 patients exhibits a dose-limiting toxicity (DLT) after 4 weeks, the next patient will be treated at the next dose level. If one patient exhibits a DLT related to the study agent(s), the cohort will be expanded to up to 6 patients. If 1 of 6 patients exhibits a DLT related to the study agent(s), then the next patient will enroll at the next dose level. If 2 of 3-6 patients exhibit a DLT related to the study agent(s), the MTD is considered to have been exceeded and at least 6 patients will be entered at the dose level below the one at which DLT is defined. If this occurs on first dose level, the dose of SGI-110 will be reduced to 30 mg/m2 days 1-5 (Dose level -1). If no DLTs are encountered at Dose level -1, the next 3 patients will be entered at the next dose level, Dose level 1G. If one DLT is encountered at Dose level -1, this dose level will be expanded to 6 total patients at Dose level -1. And if two or more DLTs are encountered at Dose level

-1, at least an additional 3 patients will be enrolled at the lower dose level, Dose level -1G. If 0/3 or 1/6 DLTs occur on Dose level -1G, the next 3 patients will be dose escalated to Dose level 1G. If 2 or more DLTs out of 6 are experienced at Dose level -1G, the MTD will have been exceeded. Dose escalations will occur no sooner than 4 weeks after the last patient on the dose level has begun therapy to allow for full assessment of DLT.

Table 3: Dose escalations schema

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Toxicity will be evaluated using the NCI Common Terminology Criteria for Adverse Events Active Version. The frequency of toxicities per organ system will be tabulated using descriptive statistics. All patients who receive any amount of the study drug will be evaluable for toxicity.

5.1.3 Stage 2

The second stage of this study will be conducted as an open-label, randomized trial.[24] As of December 2015, the maximally tolerated dose from the phase I trial was determined at level 1G:

- SGI-110 45mg/m2 SC D 1-5
- Irinotecan 125mg/m2 IV D 8 and 15

- Mandatory growth factor support during cycle 1:
    - Filgrastim (or institutional equivalent) 5mcg/kg SC C1 D 9-14
    - Peg-filgrastim 6mg SC C1 D16
    - Additional growth factor support during Cycle 1 and subsequent cycles per clinician judgment

Ninety-six eligible subjects will be randomized in a 2:1 ratio to receive either (1) SGI-110+irinotecan combination treatment in 28-day cycles (n=64) or (2) regorafenib 160 mg QD days 1-21 in 28-day cycles or TAS-102 35mg/m2 BID days 1-5 and 8-12 in 28-day cycles as per physician choice (n=32). Safety data will be reviewed at least on a quarterly basis by the SRC.

As epigenetic agents may take many months to show their benefit as seen in MDS/AML studies,[25] in both Stage 1 and Stage 2, subjects receiving SGI-110 + irinotecan may continue receiving their assigned treatment if their disease progresses based on RECIST criteria, version 1.1 if 1) the patient is clinically stable, 2) the patient is informed of their scan results and agrees to continue therapy, and 3) the patient provider agrees that remaining on study would be appropriate for the patient.

Crossover to the SGI-110+irinotecan combination treatment arm of the study will be permitted for subjects in the regorafenib/TAS-102 arm after there is evidence of disease progression.

Response and PFS will be evaluated from images by the designated investigators at each site.

5.2 Screening and pre-treatment criteria

5.2.1 Screening Procedures

Screening procedures and tests will be performed within 14 days before treatment administration with the exception of informed consent, tumor biopsy and radiologic response assessment which may be performed within 28 days of first dose of study drug.

- Provision of written informed consent. The ICF must be signed and dated by the subjects before collection of any samples or performance of any study-specific evaluations.
- Complete medical history, including demographics (date of birth, sex, race). A disease history, including the date of initial diagnosis and list of prior treatments and responses to these treatments, including time from last Irinotecan exposure (< or > 6 months), also will be recorded. Concurrent medical signs and symptoms must be documented to establish baseline conditions.
- Record concomitant medications.

- Record all study-procedure related AEs from the time of informed consent and then treatment-emergent AEs after the start of study drug administration (Cycle 1, Day 1) through 30 days after the last dose of study drug.

- Investigator's confirmation of eligibility. Perform all necessary procedures and evaluations to document that the subject meets each eligibility criterion.

- Complete physical exam including weight and examination of the skin, eyes and fundi, ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, nervous system, and genitourinary system.

- Vital signs include resting systolic/diastolic blood pressure, resting heart rate, resting respiration rate, body temperature and pulse oximetry. Assess after the subject has rested in the sitting position for at least three minutes.

- ECOG performance status.

- 12-lead ECG (rhythm, atrial rate, ventricular rate, PR interval, QRS duration, and QT/QTc, morphology and overall interpretation).

- Height.

- Sample collection for clinical laboratory tests (hematology, chemistry and urinalysis).

- Serum or urine pregnancy test: for female subjects of child-bearing potential only. Results must be negative for the subject to be eligible for enrollment into the study.

- Tumor biopsies in all phase 1 subjects on study. Additionally, tumor biopsies will be collected on a select number of subjects in phase 2. The following sites: SKCCC, VUMC, MSKCCC, and USC-Norris, will obtain biopsies on a total of 36 patients (distributed roughly evenly between the sites) for the first patients enrolled to the phase 2 if they have both biopsiable disease and are randomized to SGI-110 + Irinotecan. Optional biopsies will also be requested of patients with baseline research biopsies who remain on trial through Cycle 6-9.

- Radiologic response assessment.

- CEA

- Quality of Life evaluation at start of study using the FACT-C questionnaire (Phase II only).

5.2.2 Cycle 1, Day 1

In order for patients to be treated, evaluations must meet laboratory guidelines per the eligibility criteria

5.2.3 Cycle 1 Day 8, 15 and Subsequent Cycles, Day 1, 8, 15

In order for patients to be treated, evaluations must meet the following criteria:

- ANC $\geq$ 1250 /mcL
- Platelet count $\geq$ 75,000 /cu mm
- Bilirubin $\leq$2 mg/dL
- Creatinine $\leq$ 2 mg/dL

5.3 Agent Administration

5.3.1 SGI-110

SGI-110 administered SC daily on Days 1-5. Dose level for Phase II is 45mg/m2 (modifications as per Section 6.3)

SGI-110 is administered by SC injection preferably in the abdominal area. The total amount (in mg) of SGI-110 to be administered will be determined based on the body surface area (BSA). In calculating the BSA, actual heights and weights should be used. There will be no adjustments to "ideal" body weight. The institutional standard for calculating BSA is acceptable.

The site(s) of SGI-110 SC injections will be captured on the dosing CRF. Additional guidelines regarding subcutaneous injection will be detailed in the SGI-110-02 Study Drug Reconstitution and Administration Manual.

Investigators are prohibited from supplying SGI-110 to any subjects not properly enrolled in this study or to any physicians or scientists except those designated as sub-investigators on Food and Drug Administration (FDA) Form 1572. The investigator must ensure that subjects receive SGI-110 only from personnel who fully understand the procedures for administering the study treatment.

5.3.2 Irinotecan

Irinotecan will be administered IV over 90 minutes at 125 mg/m^2 per Table 3 (dose modifications as per Section 6.3).[26] Cycles are 28 days long. In cycle 1 irinotecan is given on days 8 and 15 in dose levels -1G, -1, 1, 1G, and 2, and on days 1, 8, and 15 for dose level 3. Patients who develop acute diarrhea secondary to irinotecan may receive atropine and/or supportive measures per specific site's institutional standard.

Irinotecan will be diluted in 5% Dextrose injection (D5W), USP (preferred) or 0.9% Sodium Chloride Injection (NS), USP, to a final concentration range of 0.12

to 2.8 mg/mL (D5W, or NS). The drug will be administered intravenously over 90 minutes, or per institutional standards

5.3.3 Regorafenib

Patients randomized to non-experimental therapy arm who opt for regorafenib, will be administered the agent as per standard dosing.[27] Patients will take 160 mg once daily for days 1-21 of each 28 day cycle. Patients will be instructed to take the drug with a low-fat breakfast and to take the pills whole (not crushed). They will also be instructed to record drug intake on a patient pill diary (Appendix B).

5.3.4 TAS-102

Patients randomized to non-experimental therapy arm who opt for TAS-102, will be administered the agent as per standard dosing. Patients will take 35mg/m2 (based on trifluridine component, with maximum single dose 80mg) twice daily for days 1-5 and 8-12 of each 28 day cycle.[16] Patients will be instructed to take the drug after breakfast and dinner and to take the pills whole (not crushed). They will also be instructed to record drug intake on a patient pill diary (Appendix C).

5.4 Definition of Dose-Limiting Toxicity

MTD will be defined based on the incidence of DLTs at each dose level.

Dose limiting toxicity is defined as any of the following occurring through cycle 1 (Up to Cycle 2 day 1) of therapy related to SGI-110 or the combination of SGI-110 and irinotecan. The DLT is defined using the Common Terminology Criteria for Adverse Events (CTCAE).

1. Hematologic toxicities deemed a DLT are:

    a. Grade 4 thrombocytopenia or neutropenia lasting > 7 days.

    b. Any incidence of Grade 3 or 4 febrile neutropenia.

2. Non-hematologic toxicities deemed a DLT are:

    a. Grade ≥ 3 non-hematologic toxicity unless it could be appropriately managed by supportive treatment (e.g. hyperglycemia, nausea/vomiting, or diarrhea).

    b. Any other clinically significant adverse event which in the opinion of the Safety Review Committee (SRC) would place subjects at undue safety risk, or results in discontinuation of treatment.

Dose-limiting toxicity (DLT) is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE).[28]

Management and dose modifications associated with the above adverse events are outlined in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

5.5 General Concomitant Medication and Supportive Care Guidelines

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed, provided their use is documented in the subject records and on the appropriate case report form. If toxicity occurs, the appropriate treatment per specific site's institutional standard will be used to ameliorate signs and symptoms (including antiemetics for nausea and vomiting, anti-diarrheals for diarrhea and anti-pyretics and anti-histamines for drug fever). All supportive measures for optimal medical care will be given during the period of study.

5.5.1 **Antibiotics**

Antibiotics may be utilized to prevent or manage febrile neutropenia based on institutional standard practice. Febrile neutropenia is defined as temperature at least 38.5ºC when the ANC is < 1000 µL. Febrile subjects should be evaluated by physical examination, complete blood count (CBC) with differential, and blood culture. Subjects with febrile neutropenia or suspected sepsis on the basis of the physical examination are to be hospitalized for appropriate broad spectrum antibiotic coverage, consistent with local pathogen sensitivities.

5.5.2 Hematopoietic Growth Factors

Growth factor support is mandatory in cycle 1 for Phase II patients (Section 5.1.3). Additional granulocyte-colony stimulating factor (GCSF) may be administered per ASCO guidelines from Cycle 1 onwards.[29] Use of other white blood cell stimulating factors after Cycle 1 can be employed according to accepted practice or institutional guidelines, at the discretion of the treating physician. RBC transfusions can be administered at the discretion of the treating physician.

5.5.3 Prohibited Medications

The administration of any other anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed.

5.6 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, patients may continue on treatment until one of the following criteria applies:

- Disease progression (for regorafenib or TAS-102 arm in Phase II), see below * regarding SGI-110 + irinotecan cohort.
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),

- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

*In both Stage 1 and Stage 2, subjects receiving SGI-110 + irinotecan may continue receiving their assigned treatment if their disease progresses based on RECIST criteria if 1) the patient is clinically stable, 2) the patient is informed of their scan results and agrees to continue therapy, and 3) the patient's provider agrees that remaining on study would be appropriate for the patient.

5.7 Duration of Follow Up

After removal from study, participants will be followed every three months or sooner until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Post-study case report forms must be completed at least every three months until death and will capture information including subsequent treatments, response to subsequent therapy, and survival.

5.8 Cross Over in Phase II

As the primary endpoint of the Phase II portion of this study is PFS, patients who progress on standard of care treatment in phase II may crossover to the study arm A. The minimum washout required period is 14 days from last dose. Subjects will be followed for PFS, response, and survival as well as monitored for toxicity.

5.9 Criteria for Removal from Study Treatment

Participants will be removed from study treatment when any of the criteria listed in Section 5.6 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In addition, a patient may be withdrawn by the investigator if he/she violates the study plan or for administrative and/or other safety reason after discussion with the sponsor. When a subject discontinues/withdraws prior to study completion, all applicable activities for the final study visit should be performed at the time of discontinuation, including completion of the quality of life questionnaire FACT-C for subjects enrolled in the phase II portion. Any adverse experiences which are present at the time of discontinuation/withdrawal should be followed. A follow-up off-study visit will occur within 30 days after last treatment.

If a patient withdraws for non-medical reasons the following procedures will be followed:

- Physical examination will be performed.
- Safety assessment (physical examination, vital signs, hematology and serum chemistry), performed within 30 days after last treatment

6 EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.[28]

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, throughout the study, and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made and/or whether the event requires expedited reporting **in addition** to routine reporting. Refer to the most recent versions of the full Prescribing Information for irinotecan (Camptosar®), regorafenib (Stivarga®), TAS-102 (Lonsurf®) and the Investigator's Brochure for SGI-110 for further information.[1,7,27]

6.1.1 Adverse Event Lists(s) for regorafenib

The most common adverse reactions (≥40%) with regorafenib are fatigue, hypocalcemia, hypophosphatemia, palmar-plantar erythrodysesthesia, anorexia, diarrhea, transaminitis, hyperbilirubinemia, anemia, lymphopenia thrombocytopena, and proteinuria. Other adverse experiences (>10%) are hypertension, dysphonia, fever, pin, rash, hyponatremia, hypokalemia, and infection.

6.1.2 Adverse Event List(s) for irinotecan

The most common adverse reactions (≥50%) with irinotecan are alopecia, anemia, thrombocytopenia, leukopenia, hyperbilirubinemia, weakness, anorexia, vomiting, and abdominal pain and cramping. Other adverse experiences (>10%) are cholinergic toxicity (47%), fever, dizziness, headache, chills, constipation,

mucositis, weight loss, stomatitis, cough, dyspnea, rhinitis, and diaphoresis. Prior studies with irinotecan have reported neutropenia and/or late diarrhea (diarrhea occurring more than 24 hours after irinotecan administration) as the dose-limiting toxicities. The aggressive use of loperamide can significantly decrease the incidence of severe delayed diarrhea. Other side effects include nausea, vomiting, anorexia, abdominal cramping, alopecia, anemia, and asthenia. An immediate onset diarrhea may occur in the context of a cholinergic-like syndrome which is characterized by diaphoresis, abdominal cramps, lacrimation, salivation, and miosis or other visual disturbances. Cases of colitis complicated by ulceration, bleeding, ileus, and infection have been observed. Additionally, isolated cases of pulmonary toxicity have been encountered. Finally, infrequent abnormalities of serum creatinine or hepatic enzymes, thrombocytopenia, mucositis, or local irritation at infusion sites has been noted in some patients. The use of irinotecan in the setting of liver dysfunction is currently under investigation.

6.1.3 Adverse Event List(s) for TAS-102

As per the RECOURSE trial, the most common adverse reactions (≥40%) are nausea, neutropenia, leukopenia, anemia, and thrombocytopenia. Other adverse experiences (>10%) include vomiting, diminished appetite, fatigue, diarrhea, abdominal pain, fever, and asthenia.

6.1.4 Adverse Event List(s) for SGI-110

The most common AEs suspected by the investigators in Study SGI-110-01 to be related to the drug and observed to date in the MDS/AML population are: injection site pain, fatigue, nausea, thrombocytopenia, anemia and diarrhea. Based on the mechanism of action of the drug and its active form decitabine, myelosuppression (neutropenia, thrombocytopenia, and anemia) and the related consequences such as infection (e.g. pneumonia), sepsis, and bleeding are the most likely risks for the drug. Mucositis has also been reported. The doses of SGI-110 being studied in this protocol (up to 60 mg/m^2 dailyx5) have not been shown to cause drug-related myelosuppression when the drug is used as a single agent. However, in MDS/AML population it is difficult to assess the relationship to the study drug since most patients present with myelosuppression as part of their disease condition. Preliminary safety data from an ongoing ovarian cancer trial in combination with carboplatin showed significant neutropenia and thrombocytopenia when 45mg/m2 SGI-110 days 1-5 is combined with full dose of carboplatin (AUC 5).

Pain and burning at the injection site has been reported that are related to dose and volume of injection as well as the speed of injection. Care must be taken to avoid intradermal injection. If injection site pain is reported upon injection, the practice of a slow injection (over 30-60 seconds), and the application of ice packs to the injection site both before and after injection (a duration of 5-10 minutes each is recommended) have been reported to attenuate the injection site events. If the

injection site events are reported at subsequent injections despite slow injection and the use of ice packs, pre-treatment with topical or systemic analgesics can be considered.

6.2 Toxicity Management

6.2.1 Irinotecan

Patients who develop acute diarrhea secondary to irinotecan may receive atropine and/or supportive measures per specific site's institutional standard. Patients will be monitored until symptoms abate. *Patients who experience new or worsening loose stools and/or diarrhea will be recommended loperamide (or other equivalent anti-motility agent(s)) to take as directed following each loose bowel movement. Patients will be regularly evaluated by the research nurse until resolution of the diarrhea*. Either acute or chronic diarrhea could require hospitalization if the patient is volume depleted or if other clinical symptoms warrant admission.

If grade 2 or greater abdominal cramping occurs, further chemotherapy will not be given until the cramping has fully resolved. The patient will also be evaluated by the research nurse to determine the possible etiology of the cramping. If cramping secondary to irinotecan is suspected, the treatment for abdominal cramping will be equivalent to diarrhea (as stated above).

6.3 Dose Modifications/Delays

The Investigator should try to the best of his/her ability to assess whether an adverse event is possibly related to study treatment, and if so, attribute it to SGI-110 only, irinotecan only, or both SGI-110 and irinotecan, and treat the subject accordingly by only reducing the study drug that has most likely contributed to the individual toxicity necessitating dose reduction. This section provides suggested guidelines for the management of various study drug-related toxicities in subjects receiving both SGI-110 and irinotecan. Preferably, only one study drug should be reduced at any one time even if both drugs are suspected to have contributed to the toxicity (e.g. myelosuppression). SGI-110 should be reduced first unless an adverse event can most likely be attributed to irinotecan only (e.g., acute diarrhea or hyperbilirubinemia as they are not expected from SGI-110 treatment). Lymphopenia of any grade that is asymptomatic will not require dose modification.

During Stage 2 of the study, subjects may be randomized to receive either regorafenib or TAS-102 versus SGI-110 and irinotecan. Dose modifications for regorafenib or TAS-102 will be at the Investigator's discretion and judgment per standard clinical practice and per most current FDA-approved package inserts.

During Phase 2, treatment delays not related to adverse events (i.e. vacations) will be allowed beginning after cycle 4 for up to a period of 2 weeks at the discretion of the Investigator, except if due to a holiday that cannot be avoided.

6.3.1 SGI-110 dose modifications

In general, SGI-110 dosing should be withheld for Grade 4 myelosuppression or ≥ Grade 3 SGI-110-related non-hematologic toxicities until toxicity has resolved to ≤ Grade 1 or baseline levels. Treatment with SGI-110 may then be resumed at one dose level below the administered dose levels (i.e., 45 mg/m^2 if the patient received 60 mg/m2; or 30 mg/m2 if the patient received 45 mg/m2. One exception is neutropenia and anemia that can be managed by growth factors, and transfusion in subsequent cycles. In that case dosing may be resumed at the same dose level. Dose reduction to less than 30 mg/m2 will not be allowed, and SGI-110 may not be omitted.

If the subject has already received treatment with SGI-110 on Day 1 or beyond, subsequent treatment days (Day 2-5 in the 5-day regimen) will be withheld if the subject develops any DLT and the skipped doses will be missed. Dosing will also be withheld on Day 1 of any subsequent treatment cycles if the subject still has a DLT or other drug-related clinically significant toxicity that has not resolved to ≤ Grade 1 or baseline. Dosing may restart at a lower dose level once the subject becomes eligible again and the DLT or other drug-related toxicity has resolved to ≤ Grade 1 or baseline. If dosing is delayed by more than 28 days because of drug-related toxicity, then the subject will have SGI-110 permanently discontinued and will be taken off of Arm A.

6.3.2 Irinotecan dose modifications

Irinotecan dosing should be withheld for ≥ Grade 3 irinotecan-related toxicities, and resumed depending on the timing of recovery and number of episodes occurred (see Table 4 and 5). If irinotecan dosing is delayed due to irinotecan-related toxicities for > 2 consecutive weeks despite supportive treatment per standard clinical practice and/or more than 2 dose reductions of irinotecan is required, the subject should discontinue irinotecan treatment but may be continued on SGI-110 alone. If both drugs cannot be restarted, the subject should be withdrawn from the study and enter the 30-day follow up period.

Table 4 below provides suggested guidelines for the Investigator to use along with his/her best judgment for SGI-110 and irinotecan dose delay and/or reduction based on the AEs observed. If SGI-110 dosing is delayed due to SGI-110-related toxicities for > 28 consecutive days despite supportive treatment per standard clinical practice and/or more than 1 dose reduction of SGI-110 and 2 dose reductions of irinotecan are required, stop SGI-110 and irinotecan therapy, discontinue the subject from the study, and complete the follow-up visit within 30 days of the last administration of SGI-110 or irinotecan, whichever is discontinued last.

Table 4: Dose Modification Guidance for SGI-110 and Irinotecan for Selected Study Drug-Related Toxicities (Phase I)

		Neutropenia or febrile neutropenia or anemia
Occurrence	**Grade**	**Dosing Guideline After Recovery to Grade ≤ 1 or baseline**
1st	G 4	Treat anemia with transfusion; Start GCSF prophylactically in Cycle 2. No change in SGI-110 dose or irinotecan dose.
2nd	G 4	Despite GSCF prophylaxis at ≥ Cycle 2, decrease SGI-110 dose to 45 mg/m^2 if previous SGI-110 dose was 60 mg/m^2 or to 30 mg/m2 if previous dose was at 45 mg/m2.
3rd	G 4	Continue SGI-110 at 30 mg/m2 but reduce irinotecan dose to 75% of previous dose. and continue GCSF prophylaxis
4th	G 4	Discontinue subject from study
Thrombocytopenia		
Occurrence	**Grade**	**Suggested Action**
1st	G 3 G 4	No change in SGI-110 or irinotecan dose Reduce SGI-110 by one dose level
2nd	G 4	Despite SGI-110 dose reduction, decrease irinotecan dose to 75%
3rd	G 4	Despite SGI-110 dose reduction, decrease irinotecan dose to 50%
4th	G 4	Discontinue subject
Transaminitis or Increased Creatinine		
Occurrence	**Grade**	**Suggested Action**
1st	≥ G 3	No change in SGI-110 dose; reduce irinotecan by 25%
2nd	≥ G 3	Reduce irinotecan to 50%
3rd	≥ G 3	Discontinue patient from study treatment

Table 5: Dose Modification Guidance for SGI-110 and Irinotecan with mandatory growth factor support for Selected Study Drug-Related Toxicities (Phase II)

SGI-110 Dose Level 1G = 45mg/m2 D1-5, Irinotecan 125mg/m2 D8 and 15, filgrastim 5mcg/kg C1D9-14 and peg-filgrastim 6mg C1D16 (further growth factor support in subsequent cycles at physician discretion)

		Neutropenia or febrile neutropenia or anemia
Occurrence	**Grade**	**Dosing Guideline After Recovery to Grade ≤ 1 or baseline**
1st	G 4	Treat anemia with transfusion; Decrease SGI-110 dose to 30mg/m2, or if already reduced*, decrease irinotecan to 75%
2nd	G 4	Decrease irinotecan dose to 75%, or to 50% (if previously reduced to 75%)
3rd	G 4	Decrease irinotecan dose to 50% from 75% or discontinue if already reduced to 50%
4th	G 4	Discontinue subject from study
Thrombocytopenia		
Occurrence	**Grade**	**Suggested Action**

1st	G 3 G 4	No change in SGI-110 or irinotecan dose Decrease irinotecan dose to 75%
2nd	G 4	Despite irinotecan dose reduction, decrease SGI-110 to 30mg/m2
3rd	G 4	Despite SGI-110 dose reduction, decrease irinotecan dose to 50%
4th	G 4	Discontinue subject
Transaminitis or Increased Creatinine		
Occurrence	**Grade**	**Suggested Action**
1st	≥ G 3	No change in SGI-110 dose; decrease irinotecan to 75%
2nd	≥ G 3	Decrease irinotecan dose to 50%
3rd	≥ G 3	Discontinue patient from study treatment

*SGI-110 cannot be dose reduced beyond 30mg/m2

6.3.3 Dose modification for regorafenib or TAS-102 toxicities

Patients randomized to regorafenib or TAS-102 treatment in phase II of the study will have dose modifications at the Investigator's discretion and judgment per standard clinical practice and per most current FDA-approved package insert.

7 DRUG FORMULATION AND ADMINISTRATION

7.1 SGI-110

7.1.1 Description

Sodium (2*R*,3*S*,5*R*)-5-(4-amino-2-oxo-1,3,5-triazin-1(2*H*)-yl)-2 (hydroxymethyl) tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methyl phosphate

7.1.2 Form

SGI-110 product is supplied in a two-vial configuration.

SGI-110 for Injection, 100 mg is a 5 mL glass vial containing lyophilized SGI-110 drug powder for reconstitution and subcutaneous injection using the custom diluent supplied in a separate vial. Each vial is stoppered with fluoropolymer coated butyl rubber closure and sealed with a blue flip-off cap. *SGI-110 for Injection, 100 mg* vial is individually packaged in a heat-sealed aluminum foil pouch with a single desiccant bag to protect from moisture.

SGI-110 Diluent for Reconstitution, 3 mL or 1.2mL is a 5 mL glass vial with 3 mL or 1.2mL of custom diluent. Each vial is stoppered with fluoropolymer coated butyl rubber closure and sealed with a white flip-off cap.

7.1.3 Storage and Stability

SGI-110 for Injection, 100 mg vial is stored at refrigerated condition of 2–8ºC in the original packaging until use. *SGI-110 Diluent for Reconstitution, 3 mL* can be stored at 2–30°C, and *1.2mL* can be stored at is stored at 2–8°C in upright position until use. Both vials are preservative-free and for single use only. SGI-110 must be stored in a secure, locked facility accessible only to authorized study personnel. OSHA Guidelines for handling cytotoxic drugs outlined in the American Journal of Hospital Pharmacy must be followed.[26] As with other potentially toxic anti-cancer agents, care should be exercised in the handling and preparation of SGI-110. The use of gloves and protective garments is recommended. Preparation should occur in a vertical laminar flow biological hood using proper aseptic technique. If SGI-110 contacts the skin, it should be immediately be treated with borax buffer solution pH 10 followed by washing immediately and thoroughly with soap and water. If SGI-110 contacts the mucous membranes, flush thoroughly with water.

Drug spilling can be inactivated by 2 N sodium hydroxide solution.

7.1.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.5 Availability

SGI-110 is an investigational agent and will be supplied free-of-charge from Astex Pharmaceuticals.

7.1.6 Ordering and accountability

An initial supply of SGI-110 will be shipped to each study center's pharmacy when all the initiation documents, including IRB approvals and IRB approved ICF, have been received and reviewed by Astex Pharmaceuticals and upon activation of the study center by the Coordinating Center JH SKCCC. Thereafter, it is the responsibility of the trial pharmacist to order a resupply.

SGI-110 must be kept in a locked limited access room. The study drug must not be used outside the context of the protocol. Under no circumstances should the Investigator or other study center personnel supply SGI-110 to other Investigators, subjects, or clinics or allow supplies to be used other than as directed by this protocol.

An accurate accounting of the study drugs and investigational devices must be maintained. These records must show dates, lot numbers, quantities received, and dispensed. SGI-110 accountability records must be maintained and readily available for inspection by regulatory authorities at any time.

7.1.7 Destruction and Return

At the end of the study, unused supplies of SGI-110 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 Irinotecan

7.2.1 Description

This is an FDA approved chemotherapy drug for the treatment of metastatic colon cancer. It is also active in other diseases, namely other GI cancers, lung and gynecologic malignancies. Irinotecan (125 mg/m^2/w) will be administered IV. It is commercially available.

7.2.2 Form

Irinotecan is commercially available. Vials contained 100 mg of irinotecan as a pale yellow transparent solution in 5 mL total volume per vial. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, =3.0 to 3.8) with sodium hydroxide or hydrochloric acid.

7.2.3 Storage and Stability

Intact vials are to be stored at controlled room temperature, protected from light until use. Once diluted, the solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2 to 8C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixture using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates.

7.2.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.2.5 Availability

Irinotecan is a standard of care, commercially available agent that is FDA – approved for metastatic colorectal cancer. It is readily available in oncology pharmacies at SKCCC and USC Cancer Center and will be billed as standard of care.

7.3 Regorafenib

7.3.1 Description

The chemical name for regorafenib is 4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino)-3fluorophenoxy]-N-methylpyridine-2-carboxamide monohydrate.

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

Regorafenib is highly protein bound (99.5%) and is metabolized by CYP3A4 and UGT1A9. The mean (range) elimination half-lives for a single 160 mg dose of regorafenib and the M-2 metabolite in plasma are 28 hours (14 to 58 hours) and 25 hours (14 to 32 hours), respectively. The M-5 metabolite has a longer mean (range) elimination half-life of 51 hours (32 to 70 hours). Approximately 71% of a radiolabeled dose was excreted in feces and 19% of the dose was excreted in urine (17% as glucuronides) within 12 days after administration of a radiolabeled oral solution at a dose of 120 mg.

In vitro studies with human hepatic microsomes or recombinant enzymes showed that regorafenib and its metabolites competitively inhibit CYP2C8, CYP2C9, CYP2B6, CYP3A4, CYP2D6, CYP2C19, UGT1A1, UGT1A9, BCRP, and P-glycoprotein.

Regorafenib is a substrate of CYP3A4 and UGT1A9. In pharmacokinetic studies conducted in humans, mean regorafenib AUC concentrations were increased and decreased in the setting of concomitant administration with strong CYP3A4 inhibitors and inducers, respectively.

7.3.2 Form

Regorafenib 40 mg tablet is the only commercially available formulation. It is pink and oval in appearance.

7.3.3 Storage and Stability

Regorafenib tablets are supplied in packages containing three bottles, with each bottle containing 28 tablets for a total of 84 tablets per package. Tablets must be stored in their original bottle. The desiccant should not be removed. Unused tablets should be discarded 28 days after opening the bottle in accordance our chemotherapy waste policies.

Regorafenib should be stored at 25°C (77°F) with excursions permitted from 15 to 30°C (59 to 86°F).

7.3.4 Availability

Regorafenib is a FDA-approved, standard of care agent for metastatic, pretreated colorectal cancer. It will be provided through standard Special Order Pharmacies and billed as standard of care.

7.3.5 Ordering

Regorafenib will be ordered through standard of care prescription and Specialty Order Pharmacies.

7.4 TAS-102 (Lonsurf)

7.4.1 Description

TAS-102 (Lonsurf) is an antitumor nucleoside drug that combines FTD and TPI at a molar ratio of 1:0.5. The chemical name for TAS-102 (Lonsurf) is trifluridine (α,α,α-trifluorothymidine) and tipiracil hydrochloride (5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]pyrimidine-2,4-(*1H,3H*)-dione monohydrochloride

As per the manufacturer's guide: "After twice daily dosing of LONSURF, systemic exposure AUC of trifluridine increased more than dose-proportionally over the dose range of 15 to 35 mg/m2. After administration of LONSURF 35 mg/m2 twice daily, the mean elimination half-life (t1/2) of trifluridine was 1.4 hours and of tipiracil was 2.1 hours after a single dose. The mean elimination half-life at steady state of trifluridine was 2.1 hours and of tipiracil was 2.4 hours. The accumulation of trifluridine was 3-fold for AUC0-last and 2-fold for peak plasma concentration (Cmax) at steady state while no accumulation was observed for tipiracil. Administration of a single dose of LONSURF containing tipiracil and trifluridine 35 mg/m2 increased the mean AUC0-last of trifluridine by 37-fold and Cmax by 22-fold with reduced variability compared to trifluridine 35 mg/m2 alone.

Following a single oral administration of LONSURF at 35 mg/m2 in patients with cancer, the mean time to peak plasma concentration (Tmax) of trifluridine was around 2 hours.

A standardized high-fat, high-calorie meal decreased trifluridine Cmax, tipiracil Cmax and AUC by approximately 40%, but did not change trifluridine AUC compared to those in a fasting state in patients with cancer following administration of a single dose of LONSURF 35 mg/m2. It is recommended to take LONSURF within 1 hour after completion of the morning and evening meals based on the observed correlation between the increase in the Cmaxof trifluridine and the decrease in neutrophil counts.

Trifluridine mainly binds to human serum albumin. The in vitro protein binding of trifluridine in human plasma is greater than 96%, independent of drug concentration and presence of tipiracil. Plasma protein binding of tipiracil is below 8%

Trifluridine and tipiracil are not metabolized by cytochrome P450 (CYP) enzymes. Trifluridine is mainly eliminated by metabolism via thymidine phosphorylase to form an inactive metabolite, 5-(trifluoromethyl) uracil (FTY). No other major metabolites were detected in plasma or urine.

Following a single dose of LONSURF at 60 mg, the mean 48-hour cumulative urinary excretion was 1.5% for unchanged trifluridine, 19.2% for FTY, and 29.3% for unchanged tipiracil."

7.4.2 Form

TAS-102 is formulated as an immediate-release film-coated tablet, which is supplied in strengths (expressed as FTD content):

- The 15-mg white, round tablet contains 15 mg FTD and 7.065 mg TPI as active ingredients.
- The 20-mg pale-red, round tablet contains 20 mg FTD and 9.42 mg TPI as activeingredients.

Both tablet strengths contain lactose monohydrate, pregelatinized starch, stearic acid,hypromellose, titanium dioxide, polyethylene glycol, and magnesium stearate. The 20-mg tablet for TAS-102 also contains red ferric oxide.

i. Storage and Stability

TAS-102 cards must be stored at room temperature between 59°F and 86°F (15°C and 30°C).

ii. Availability

TAS-102 is a FDA-approved, standard of care agent for metastatic, pretreated colorectal cancer. It will be provided through standard Special Order Pharmacies and billed as standard of care.

iii. Ordering

TAS-102 will be ordered through standard of care prescription and Specialty Order Pharmacies.

8 CORRELATIVE/SPECIAL STUDIES

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.

Research bloods and biopsies will be batched and sent to Dr. Ahuja's laboratory at the conclusion on enrollment for the study as described in this section.

After shipping, samples and associated data will be stored at Johns Hopkins unless the patient withdraws consent.

Up to two sources of tumor DNA (blood and tumor biopsies) will be evaluated for DNA methylation status in each patient. Blood will be drawn from all patients in the study prior to starting treatment, as well as on days 8, 15, and 22 of the study for Cycle 1. For the subsequent cycles, blood will be collected prior to reconvening treatment on Day 1 and 15 (as well as on day 8 of cycle 2), for patients on Arm A. On Arm B, cycles 1 and 2, samples will be collected on days 1 and 15. Subsequent cycles (Cycle 3 and beyond) correlative samples will be collected on day 1 of a cycle. The blood samples will be used to assess the methylation status of free tumor DNA circulating in the blood. Tumor biopsies will be performed on all patients prior to starting treatment and post-treatment biopsies will be obtained prior to treatment (Cycle 1 Day1), and on ~ Cycle 1 Day 8 in Phase I and cycle 2 Day 8-15 for patients selected for biopsy in Phase II, and at the conclusion of the trial (optional).Methylation status will be assessed pre- and post-treatment. In those patients who have evidence of methylation changes comparing pre and post treatment analyses, further confirmatory studies will be done to assess if these changes in methylation correlate with gene re-expression.

Methylation-specific PCR (MSP) will be used to detect the presence of methylated alleles for specific genes described below.[30-34] Genes found to be methylated in pre-treatment specimens will be monitored for post-treatment changes in methylation using MSP. Methylation analysis may then be performed by using a nested MSP approach. The nested MSP approach has a sensitivity of 1 in 50,000 methylated allele to determine the genes that are methylated amongst the panel for each patient. A non-nested real-time MSP approach will then be used to follow the biologic response across various time points in the study for the genes of interest. This strategy of nested MSP followed by non-nested MSP has been validated in the ongoing study in the MDS/AML patients using bone marrow specimens as a

useful monitoring tool for biologic response.

Patients will have plasma samples analyzed for DNA methylation patterns pre-treatment as well as on Days 8, and 15 and 22 for Cycle 1. For the subsequent cycles, blood will be collected prior to reconvening treatment on Day 1 and 15 (as well as on day 8 of cycle 2), for patients on Arm A. On Arm B, cycles 1 and 2, samples will be collected on days 1 and 15. Subsequent cycle (Cycle 3 and beyond) correlative samples will be collected on day 1 of a cycle. Candidate genes that will be analyzed for promoter methylation include potentially (but not limited to): *p16, SFRP 1, TFPI-2, IGFBP3, O6-MGMT, APC, CHFR, RASSF1A, WRN, DR1,* and *hMLH1*. Genes found to be methylated in pre-treatment specimens will be monitored for post-treatment changes in methylation using quantitative MSP. DNA will first be extracted from plasma using the Methylation-On-Beads (MOB) technology, a novel nano-based methodology designed to capture extremely low quantities of blood found in blood.[34-35] Serum-based methylation analysis has been performed and reported previously using a variety of approaches.[36-38] In previous studies, this nano-enabled assay demonstrated high sensitivity for screening of common cancers such as lung and colorectal and points to the enormous potential of using this platform for surveillance of responses to epigenetic therapy. The utility of the nano-enabled platform will be explored in the ongoing correlative studies to develop a sensitive assay for detection of response to epigenetic therapy. Methylation analysis will then be performed by using a quantitative MSP approach.

8.1 Archival tissue

We will consent patients to obtain paraffin-embedded blocks of prior tissues including their primary cancer, if possible. This will allow us to perform methylation analysis on primary tumors, and determine if the primary and metastatic lesions have a similar profile. DNA will be extracted and analyzed similar to the treatment for tumor biopsies above. Expression analysis will also be performed in selected cases, as feasible.

Archival tissues will be requested for each patient from all prior surgeries. Paraffin blocks **containing tumor sections** will be requested. Paraffin blocks must be confirmed by the pathologist to contain primary tumor. These blocks must be sectioned to generate a total of 10 curls of 10 micron thickness. These curls should be placed in two 2ml eppendorf tubes with 5 curls in each. The eppedorf tubes must be clearly labeled with the Patient ID, date from which the primary tumor was collected (date of surgery), and appropriate pathology report for the block will be requested. Additionally, one stained hematoxylin and eosin slide (H&E) and two unstained plus slides should be created from the same blocks. These slides must be labeled with the Patient ID, and the date of surgery as above.

8.2 Pharmacokinetic Studies

Pharmacokinetic evaluation will be conducted during Phase 1 only, in all subjects in order to evaluate exposures of SGI-110 and decitabine in the disease population. SGI-110 does not inhibit nor induce CYP3A4, which is the major contributing enzyme to irinotecan metabolism. As such,

irinotecan pharmacokinetics will not be evaluated in this study as its exposures are not expected to be affected by SGI-110.

Pharmacokinetics of SGI-110 and decitabine will be assessed from plasma concentrations.
Plasma samples will be prepared from blood drawn at the following time-points:
Cycle 1, Day 1: pre-dose, 15 min, 30 min, 60 min, 90 min, 2 hr, 4 hr, 6 hr and 8 hr post-dose.
Sampling windows are up to 10% of protocol specified time point but no more than 1 hour.
Details for procedure for PK sample collection are included below.
Plasma samples will be appropriately labeled and shipped for analysis to:

Nabeela Aleem, Sample Coordinator
Frontage Laboratories, Inc.
700 Pennsylvania Drive
Exton, PA 19341
P: 484-348-4790
Email : <naleem@frontagelab.com>

Procedures:
A. Preparation of K2EDTA Vacutainer tubes containing THU (tetrahydrouridine)
Materials—
THU: Calbiochem 584222, EMD Chemicals, Inc.

Notes and Preparation of THU:

1. The BioVision, Inc datasheets indicate that the 10 mg vial of THU must be stored frozen and protected from light at –20°C. The material is hygroscopic and difficult to weigh, and reconstitution of the entire contents at the intended concentration is strongly suggested.
2. Following reconstitution in water (do not use methanol), aliquots can be purged with nitrogen and frozen at –20°C. The datasheets indicate that these aliquots are stable for up to 3 months at –20°C.
3. Prepare a 0.4 mg/mL solution of THU. Since the datasheet purity of THU is listed as >80% (by TLC), assume that there are 8 mg of THU per vial of THU. Diluting this to a volume of 20 mL in a graduated cylinder or graduated centrifuge tube gives a 0.4 mg/mL (400 ug/mL) solution of THU. Keep this solution on ice.
    - Measure a volume of 10 ml of sterile water into a graduated cylinder or a 50 mL graduated centrifuge tube
    - Use a serological pipette to add approximately 1 ml of water to the vial of THU.
    - Mix/shake the THU vial as best as possible, until the powder seems to be dissolved in the water.
    - Transfer the liquid from the THU vial into the graduated cylinder or graduated centrifuge tube
    - Bring the total volume of the container to 20 ml.

Preparation of Vacutainer tubes:

1. Pop the top of the vacutainer tube to break the vacuum seal.
2. Using a pipettor, immediately deliver at least 20 uL (20-30 uL) of the THU solution into each of the labeled K2EDTA 6 mL Vacutainer tubes.

3. When all K2EDTA Vacutainer tubes have been prepared with the THU and labeled, store the tubes refrigerated for up to 30 days.
4. Toss the THU aliquot remainder when done.

B. Plasma Sample Collection:

Note: Blood samples for PK studies need to be processed immediately. It is imperative that the following procedures be followed after collection of the sample to stabilize the drug and its various metabolites.

1. Using a syringe, collect a 6 mL peripheral blood sample and transfer to the 6 mL K2EDTA tube preloaded with THU and stored at 4°C.
2. Immediately upon collection, mix the blood sample by gently inverting the tube 8-10 times and then immediately place the tube in a container of wet ice. Samples must be kept on the wet ice until centrifuged. Use wet ice to avoid sample hemolysis.
3. Centrifuge the EDTA tube, pre-loaded with THU, at either 1800 g x 10 min at 4°C or 1500 g x15 min at 4°C. The tube should be centrifuged within 1- 2 hours of collection.
4. Immediately after centrifugation, use the pipettes provided to transfer at least 1.0 mL of plasma into each of 2 labeled screw top tubes and store in a –20°C freezer until shipment.
5. Store the samples for a minimum of 8 hours at –20°C until packaging for shipment to Frontage Labs. Samples should be shipped to Frontage for analysis within 90 days of collection, if possible.
6. Samples should be packaged for shipment in triple packaging, sealed and cushioned and containing sufficient amount of crushed dry ice to last the duration of transport.

PK Specimen Collection guide

<table>
<tr><td rowspan="2">PK Decitabine/SGI-110</td><td rowspan="2">PK Deci/SGI

(Per Timepoint)</td><td>PK Deci/SGI A (Per Timepoint)</td><td rowspan="2">6mL Lavender K2 EDTA Vacutainer Tube
  </td><td>1.8mL NUNC Cryovial w/ White Insert
  </td><td>Frozen</td></tr>
<tr><td>PK Deci/SGI B

(Per Timepoint)</td><td>1.8mL NUNC Cryovial w/ White Insert
  </td><td>Frozen</td></tr>
</table>

8.3 Pharmacodynamic Studies

8.3.1 Tumor Biopsies

Mandatory tumor biopsies will be required at baseline and on Cycle 1 Day 8 in the phase I cohort. Tumor biopsies at baseline and during Cycle 2 (between C2D8 and C2D15) will also be collected from the first 36 patients enrolled in the phase II cohort with biopsiable disease randomized to SGI-110 + Irinotecan (Arm A) approximately evenly spread between the following study sites: SKCCC, VUMC, MSKCCC, and USC-Norris. Core biopsies are required; 4-6 passes must be obtained. Fine needle aspiration biopsies may be allowed only after PI approval. Pre- and post- biopsy should be performed from same organ site. For example: if

pre- biopsy is liver, then post- biopsy should also be liver and preferably the same lesion. Biopsy sites (liver, colon, intra-abdominal implants) in metastatic colorectal cancer are generally amenable to biopsy, and pre- and post-treatment tissue sampling is an important strength of this study. Post-treatment biopsies will be done ~cycle 1 day 8 (and between C2D8 and C2D15 during the phase II portion of the study).The timing of the post-treatment biopsy will be performed based on prior experience in the leukemic patients where the majority of demethylation events have been seen at between 8-15 days of each cycle. Patients with baseline biopsies who remain on the trial beyond 6 cycles will be asked to participate in another research biopsy between cycles 6-9.

There was no comparable data in solid tumors. All biopsy specimens will be stored as fresh tissue at least -70°C for extraction of DNA and RNA. Biopsy specimens will be studied for tumor methylation profiles, as described below. Evaluation of expression of genes that become demethylated post-treatment will be performed using quantitative real-time PCR and/or expression arrays. If an attempt at biopsy is unsuccessful, the patient will still be eligible for treatment and the subsequent biopsies will be foregone. Tumor biopsies and plasma from the phase I portion of the trial will be analyzed prior to moving onto phase II for demethylation and the timing of the biopsies in the phase II component of the trial may be adjusted based on these results. As of December 2015, our Phase I data showed no clear pattern in demethylation at C1D8, while serum demethylation appeared to have a delayed and dose-dependent nadir in the middle of cycle 2, therefore timing of the second biopsy was moved to C2D8-15.

Tumor Biopsy Processing

Prior to initiation of therapy, tumor samples will be collected by direct or ultrasound-guided core biopsy from accessible tumor tissue. A maximum of 6 core tissue samples will be obtained from each patient. Core biopsy is preferred and FNAs are only to be done when core biopsy is not safe (such as with lung lesions). There are 4 parts to the procedure: preparation, slide creation, dry protocol, and RNAlater protocol. **Biopsies must be flash frozen within minutes of biopsy collection, and immediately stored at -70C or below. Biopsies may be batched and shipped at the end of this study to Dr. Nita Ahuja's laboratory as detailed in this protocol. DO NOT ship biopsies out on Thursday or Friday to prevent weekend arrival; shipment should occur the following Monday. All samples, blood and biopsies MUST BE OVERNIGHT EXPRESSED to ensure next day delivery. When preparing for shipment, fill an insulated styrofoam box with DRY ICE (NOT ICE), enough to fill at least ¾ of the box. This will ensure that plenty of dry ice remains during the duration of the delivery process to protect the quality of the specimens. Fill half the box with DRY ICE, place the specimens in the middle, and fill up to top with DRY ICE.**

a. CORE BIOPSY PROTOCOL

i. Preparation:

1.Prepare a 50:50 mixture of dry ice and isopropanol mixture in a styrofoam bucket to a depth of 1 inch. A liquid nitrogen bath is also acceptable.
2. Label 5 cryovials with Patient ID, Cycle/ Day, Specimen Type (biopsy, location), and Date of collection.
3.Fill one of the vials with 1mL RNAlater and add RNAlater designation on the tube.

ii. Slide creation:

1. The first biopsy passage should be used for the creation of an H&E or cryoslide which an onsite pathologist will use to confirm the biopsy is tumor tissue.
   a. If it is not possible to obtain an onsite pathologist, please create an H&E slide and send to Dr. Nita Ahuja's laboratory for analysis
   b. After the pathologist's confirmation, keep and store slides at room temperature and send to Dr. Nita Ahuja's laboratory.

iii. Dry protocol:

1.During each of the subsequent passes, transfer each biopsy to an enclosed empty 1.8mL cryovial (Use sterile techniques)
2.Flash freeze each vial immediately by adding the cryovial to the 50:50 mixture in the bucket or in the liquid nitrogen.

iv. RNAlater protocol:

1. On the last passage, add a core biopsy to the cryovial filled with RNAlater and flash freeze by immersing the tube in the 50:50 mixture or liquid nitrogen.
   a.NOTE: **RNAlater is NOT sterile and needle dipped in this solution should not be reused on the patient**
2. Samples will be stored at or below -70C until shipped to Dr. Nita Ahuja via overnight express in DRY ICE. ADD ENOUGH DRY ICE TO FILL AT LEAST ¾ OF THE INSULATED SHIPPING BOX. Biopsies may be batched and shipped at the end of this study to Dr. Nita Ahuja's laboratory. Avoid shipping on Thursdays and Fridays to prevent weekend arrival. Store in -70C or below until following Monday to ship for Tuesday arrival.

b. FNA PROTOCOL

There are 3 steps to this protocol: preparation, slide creation, and saline protocol.

i.Preparation

1.Prepare a 50:50 mixture of dry ice and isopropanol mixture in a Styrofoam bucket to a depth of 1 inch. A liquid nitrogen bath is also acceptable.

2.Label 5 cryovials with patient identification, date of collection, Cycle/Day, and specimen type (biopsy location.)
3.Add 1mL of saline to each tube.

ii. Slide Creation
1.Perform the first FNA biopsy pass
2.Create an H&E slide or Cryoslide
3.Have an onsite pathologist confirm the sample is of tumor.
a. If an onsite pathologist is not available, please send the slides to Hopkins for analysis
b. Keep the slides at room temperature

iii. Saline protocol:
1. Transfer subsequent FNA aspirate to separate cryovials filled with saline.
a.Inject and swish the cells into the saline
2.Flash freeze the cells immediately by immersing cryovials to the 50:50 mixture in the bucket.
3.Samples are to be stored at -70C.
4.**When shipped, the samples should be shipped overnight express in enough DRY ICE to fill at least ¾ of the box.**

8.3.1.1 Methylation analysis

Genome-wide methylation analysis will be performed using the Infinium Illumina based assay that allows methylation analysis of >450,000 CpG sites covering greater than 14,000 genes. Beta values indicating methylated CpG islands will be isolated. Pre- and post-treatment biopsies will be compared to determine genes that demethylated following treatment. Cannonical tumor suppressor genes will be closely scrutinized for CpG island methylation. These results will then be correlated with expression values.

Methylation patterns in the promoter regions of selected tumor suppressor genes will be determined using quantitative real time nested Methylation Specific PCR (MSP). This technology is ideally suited for increasing the throughput of PCR analysis and gaining a level of quantification not possible with the standard MSP analysis. DNA will be isolated from blood, and tumor biopsies and treated with sodium bisulfite under denaturing conditions (123). Bisulfite modified DNA from each post treatment specimen will be analyzed for changes in DNA methylation at specific gene promoters (to include p16, SFRP1, IGFBP3, TFPI2, RASSF1A, and mlh1). PCR primers for these have been described and are in frequent use in the Ahuja lab.

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. A separate PCR reaction

will utilize primers specific for the unmethylated promoter region of the beta-actin gene, a gene necessary for normal cell structure and function, which has never been observed to be methylated in any cancer. Real-time curves for the MSP reaction will be compared to the unmethylated reaction and to the beta-actin control. 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

8.3.1.2 Expression analysis

The goal of DNA methyltransferase inhibition is reversal of transcriptional inhibition due to repressive chromatin conformation related to hypermethylation and changes in histone tails along with changes in miRNA. Given the heterogeneity of metastatic colorectal cancer the selection of specific genes to be studied across patient samples is challenging. Evaluation of expression of genes that become demethylated post-treatment will be performed using quantitative real-time PCR, whenever possible. Gene re-expression will be studied also using genome wide strategies using quantitative PCR using the Ampliflour if additional funds are available. Universal amplification and detection system (Intergen). This system has the advantage of the use of multiple fluorophores for each gene (up to four fluorophores can currently be analyzed). RT-PCR used semi-quantitatively will be combined with real-time quantification using the ABI 7700 system within the Johns Hopkins Oncology Center. Beta-actin will be used as a control. Expression will be reported as a ratio of gene specific to control gene expression levels.

8.3.2 Research Blood

Blood samples for methylation analysis will be collected from all participants into the enclosed purple top tubes (10 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. For each collection day, total of 50 mL of blood will be drawn from the patient (5 tubes of 10 mL each). Each purple tube holds approximately 10 of blood, yielding close to 5ml of plasma.

Blood tubes must be kept in ice, and processed within 30 minutes of collection. If collected at the Johns Hopkins Hospital, the blood samples will be processed in Dr. Ahuja's laboratory. For other participating sites, blood must be kept on ice, processed within 30 minutes, and the plasmas and WBC collected must be stored at or below -70C

until they can be shipped to Dr. Ahuja's laboratory. Overnight express shipping and use of adequate amount of dry ice will be practiced to preserve the quality of specimen in the shipping process. All collected specimens will be stored at or below -70C in Dr. Ahuja's laboratory.

Processing of Blood: (For Plasma and White blood cells)

1. Take 1 empty 15 mL conical tube for each K2-EDTA tube collected
2. First add 3 mL of Ficoll-Paque PLUS buffer to each conical tube.
   (NOTE: Ficoll-Paque PLUS buffer MUST BE KEPT AT 4ºC and out of light for storage.)
3. Using one conical tube for each purple top blood tube, Carefully layer the blood on to Ficoll-Paque PLUS buffer 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 **(Figure A)**. Repeat this step for each of the tubes.
4. Next, centrifuge tubes for 10 minutes at 3000 rpm at 4ºC. Be sure that the centrifuge is balanced. The layers will now separate as shown below **(Figure B).**
5. Carefully pipet the plasma layer (the clear yellow layer on top) from each conical tube into a new 15 mL conical tube (1 tube for each).
   Pay careful attention to not draw from the white buffy coat (the second layer below plasma in Figure B). To ensure this, stop pipetting plasma at a level 3.5 mL above the RBC layer (Red layer at the bottom). For example, if the top of the RBC is at 3mL, then pipet plasma out down to 6.5 mL and stop.
6. Pipet the buffy coat (WBCs) from each conical tube pooling them into a single new 15 mL conical tube.
   NOTE: Gently navigate your pipette tip to collect clumps of WBC around the buffy coat. Be careful not to draw the Ficoll-Buffer sitting below the white buffy coat (the third layer).
7. Once plasma and the white buffy coats are collected from each conical tube you should have, depending on the patient sample, 5 conical tubes filled with 4-5 mL plasma each and one, conical tube filled with 2-4 mL WBCs. You may now discard the conical tubes filled with ficoll and RBCs.
8. Take the single conical tube filled with WBCs and add an equal volume of 1x PBS to the volume of WBCs present, mix by inverting 5 times.
9. Take the single tube with pooled WBCs from step 11 and the 5 tubes with plasma from step 8. Spin all five sample tubes in the centrifuge for 5 minutes at 1500 rpm at 4ºC. Be sure that the centrifuge is balanced.
10. After centrifugation you may have a small white pellet at the bottom of each of the plasma tubes (5 tubes) and you will have a larger white pellet in the single WBC tube.
    Be careful not to disturb the pellets in the plasma tubes or the WBC tubes (Although you will not be collecting the pellets from the 4 tubes with plasma you do not want them to contaminate the plasma)
11. Take the plasma from the 5 conical plasma tubes. Into 6 Eppendorf (1.5 mL) tubes Add 0.5 mL plasma.
    Do not disturb any pellet that may have formed.
12. Add the remaining plasma in 1.0 mL aliquots into additional 1.5 mL Eppendorf tubes (between 12 and 16 tubes).
    Do not disturb any pellet that may have formed.

13. Now take the conical tube with the WBCs. There should be a white pellet at the bottom **You want this pellet.**
14. **Being careful not to disturb the pellet** Remove by pipetting **(Do not decant)** and discard any liquid from the conical tube **(leave a little liquid ~200 uL behind to make sure the pellet is not disturbed)**.
15. Add 3 mL 1x PBS to the pellet and mix by flicking the tube gently to re-suspend the cells. **(If the cells do not re-suspend by flicking the tube you may carefully pipet the cells up and down to re-suspend them in the PBS)**
16. Once the cells have been re-suspended add 6 aliquots of 0.5 mL of the WBCs in step 18 into 1.5 mL Eppendorf tubes.
17. When finished you should have 6 tubes with 0.5 mL of plasma from step 14; 16-20 tubes with 1.0 mL of plasma from step 15; and 6 tubes with 0.5 mL of WBCs from step 19.
18. Label samples as **PLASMA** OR **WBC**, as applicable, including study number (J1369), unique patient ID (assigned by the consortium), date of collection, and time point in treatment cycle.
19. Freeze plasma and WBC samples in their Eppendorf tubes at or below -70ºC until shipment or transfer to Dr. Nita Ahuja's Laboratory.

Figure A :

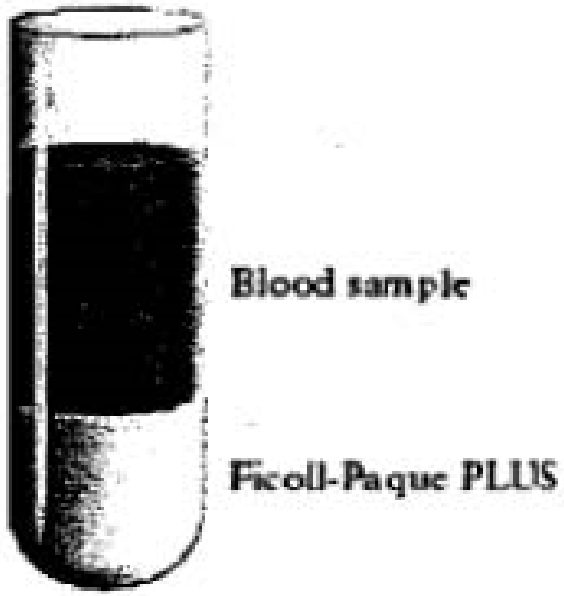
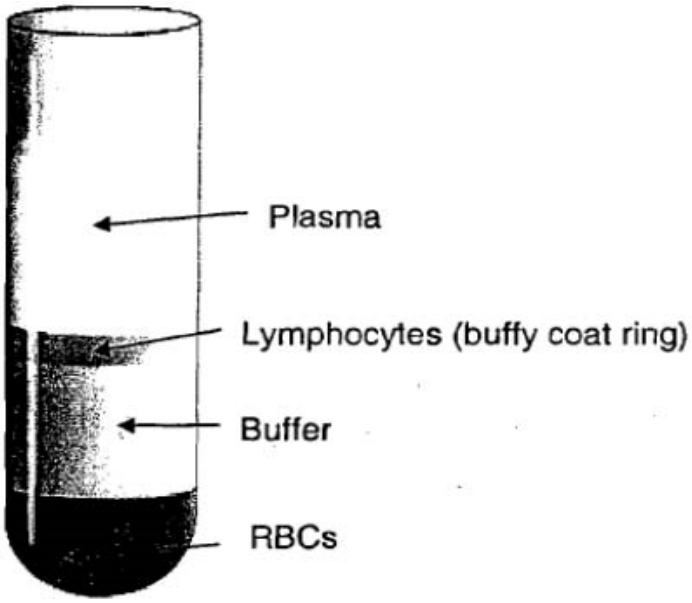


Figure B:



CRITICAL POINT: At time of shipping, fill an insulated styrofoam box with **DRY ICE (NOT ICE)**, enough to fill at least ¾ of the box. This will ensure that plenty of dry ice remains during the duration of the delivery process to protect the quality of the specimens. Fill half the box with DRY ICE, place the specimens in the middle, and fill up to top with DRY ICE.

a. Shipping of processed Plasma/WBC can be bundled and sent at the end of the trial. They must be sent via overnight express. Please avoid shipping out on Fridays to prevent arrival of items over the weekend.
b. Shipping of the biopsies may be sent to Dr. Nita Ahuja's laboratory as a batched collection as long as they were prepared and stored according to the instructions outlined above **(must be stored at or below -70C until shipment). To avoid weekend arrivals, biopsies must not be shipped out on a Thursday or Friday.**

All tubes must be clearly labeled with the unique patient ID (assigned by the consortium), Cycle and Day, specimen type (Plasma, WBC, or biopsy) and Date of collection.

During the course of the study, blood and tumor biopsies will be shipped from the centers to the coordinating center lab at Johns Hopkins University School of Medicine. After conclusion of the study, samples will be sent for analysis to Dr. Nita Ahuja's laboratory at the Yale School of Medicine.

9 STUDY CALENDARS

Baseline evaluations are to be conducted within 2-week prior to start of protocol therapy. Scans must be done ≤4 weeks prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy

All assessments must be performed prior to administration of any study medication. All study assessments and medications should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

Phase I Portion – Dose levels -1G, -1, 1, 1G, and 2

	Pre-Study	C1 d1	C1 d8	C1 d9-14	C1 d15	C1 d22	C2 d1	C2 d8	C2 d15	C2 d22	C3 d1[a]	C3 d8[a]	C3 d15[a]	C3 d22[a]	Off Study[c]
SGI-110 Days 1-5		X					X				X				
Irinotecan			X		X			X	X			X	X		
Growth Factor Support				X[f]											
Informed consent	X														
History	X														
Concurrent meds	X	X	X		X	X	X	X	X		X	X	X		X
Physical exam (Ht, Wt, BSA, VS)[e]	X	X					X				X				X
Performance Status	X	X					X				X				X
CBC w/diff, plts	X	X	X		X	X	X	X	X		X	X	X		X
Serum chemistry[b]	X	X	X		X	X	X	X	X		X	X	X		X
CEA	X	X					X				X				X
Urinalysis	X	X					X				X				X
ECG	X														
Adverse event evaluation		X----------X													X
Tumor measurements	X		Repeated every 8 weeks. Documentation (radiologic) must be provided for subjects removed from study for progressive disease.												X[c]
Radiologic evaluation	X		Radiologic measurements should be performed every 8 weeks												X[c]
B-HCG	X[d]														
Tumor biopsy	X		X												
Archival Tissue	X														
Research Bloods		X	X		X	X	X	X	X		X				X
PK Study		X													

a: All study calendar evaluations for cycle 3 are representative of cycle 3 and beyond.
b: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
c: Off-study evaluation should be performed within 30 days of last dose.
d: Serum pregnancy test (women of childbearing potential).
e: Baseline height will be measured and recorded once during pre-study, and this value for height will be used through the patient's duration of study.
f: Growth factor support will be initiated with filgrastim 5mcg/kg/day on cycle 1 day 9-14 for patients enrolled on Dose levels -1G and 1G. Additional growth factor support during Cycle 1 and on subsequent cycles will be provided per clinician judgment.

Phase I Portion – Dose level 3

	Pre-Study	C1 d1	C1 d8	C1 d9-14	C1 d15	C1 d22	C2 d1	C2 d8	C2 d15	C2 d22	C3 d1[a]	C3 d8[a]	C3 d15[a]	C3 d22[a]	Off Study[c]
SGI-110 Days 1-5		X					X				X				
Irinotecan		X	X		X		X	X	X		X	X	X		
Informed consent	X														
History	X														
Concurrent meds	X	X	X		X	X	X	X	X		X	X	X		X
Physical exam (Ht, Wt, BSA, VS)[e]	X	X					X				X				X
Performance Status	X	X					X				X				X
CBC w/diff, plts	X	X	X		X	X	X	X	X		X	X	X		X
Serum chemistry[b]	X	X	X		X	X	X	X	X		X	X	X		X
CEA	X	X					X				X				X
Urinalysis	X	X					X				X				X
ECG	X														
Adverse event evaluation		X----------													----------X
Tumor measurements	X		Repeated every 8 weeks. Documentation (radiologic) must be provided for subjects removed from study for progressive disease.												X[c]
Radiologic evaluation	X		Radiologic measurements should be performed every 8 weeks												X[c]
B-HCG	X[d]														
Tumor biopsy	X		X												
Archival Tissue	X														
Research Bloods		X	X		X	X	X	X	X		X				X
PK Study		X													

a: All study calendar evaluations for cycle 3 are representative of cycle 3 and beyond.
b: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
c: Off-study evaluation should be performed within 30 days of last dose.
d: Serum pregnancy test (women of childbearing potential).
e: Baseline height will be measured and recorded once pre-study, and this value for height will be used through the patient's duration of study.

Phase II Portion
Arm A – SGI-110 + Irinotecan

	Pre-Study	C1 d1	C1 d8	C1 d9-14	C1 d15	C1 d22	C2 d1	C2 d8	C2 d15	C2 d22	C3 d1[b]	C3 d8[b]	C3 d15[b]	C3 d22[b]	Off Study[c]
SGI-110 Days 1-5[a]		X					X				X				
Irinotecan[a]			X		X			X	X			X	X		
Growth factor support				X---[a]											
Informed consent	X														
History	X														
QoL questionnaire		X									X[i]				X
Concurrent meds	X	X	X		X	X	X	X	X		X	X	X		X
Physical exam (Ht, Wt, BSA, VS)[g]	X	X					X				X				X
Performance Status	X	X					X				X				X
CBC w/diff, plts	X	X	X		X	X	X	X	X		X	X	X		X
Serum chemistry[d]	X	X	X		X	X	X	X	X		X	X	X		X
CEA	X	X					X				X				X
Urinalysis	X	X					X				X				X
ECG	X														
Adverse event evaluation			X---------- -------X												X
Tumor measurements	X		Repeated every 8 weeks. Documentation (radiologic) must be provided for subjects removed from study for progressive disease.												X[e]
Radiologic evaluation	X		Radiologic measurements should be performed every 8 weeks												X[e]
Archival Tissue	X														
B-HCG	X[f]														
Tumor Biopsy	X[h]							X[h] -------X[h]							
Research Bloods		X	X		X	X	X	X	X		X		X		X

	a: Arm A -Dose Level 1G from Phase I, SGI-110 45mg/m2 D1-5, Irinotecan 125mg/m2 D8 and 15. Treatment cycle is 28 days. Growth factor support of filgrastim (or institutional equivalent) 5mcg/kg/day on days 9-14 and peg-filgrastim 6mg on day 16 of cycle 1 is mandatory. Additional growth factor support during Cycle 1 and on subsequent cycles will be provided per clinician judgment. b: All study calendar evaluations for cycle 3 are representative of cycle 3 and beyond. c: Off-study evaluation should be performed within 30 days of last dose. d: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium. f: Serum pregnancy test (women of childbearing potential). g: Baseline height will be measured and recorded once pre-study, and this value for height will be used through the patient's duration of study h: SKCCC, VUMC, MSKCCC, and USC-Norris, will obtain biopsies on a total of 36 patients (distributed roughly evenly between the sites) for the first patients enrolled to the phase 2 if they have both biopsiable disease and are randomized to SGI-110 + Irinotecan. Additional optional biopsy will be requested of patients with baseline research biopsy who remain on trial through Cycle 6-9. i: QoL surveys will be done on C3D1 and every other cycle subsequently (i.e. Cycle 5 Day 1, Cycle 7 Day 1, etc)

Arm B - Regorafenib

	Pre-Study	C1 d1	C1 d8	C1 d15	C1 d22	C2 d1	C2 d8	C2 d15	C2 d22	C3 d1[a]	C3 d8 [a]	C3 d15 [a]	C3 d22 [a]	Off Study[b]
Regorafenib[c]		X ----------				X ----------				X ----------				
Informed consent	X													
History	X													
QoL questionnaire		X								X[d]				X
Concurrent meds	X	X		X		X		X		X				X
Physical exam (Ht, Wt, BSA, VS)[e]	X	X				X				X				X
Performance Status	X	X				X				X				X
Blood Pressure[f]		X	X	X	X	X	X							
CBC w/diff, plts	X	X		X		X		X		X				X
Serum chemistry[g]	X	X		X		X		X		X				X
Lipase		X												
CEA	X	X				X				X				X
Urinalysis	X													X
ECG	X													
Adverse event evaluation		X----------												----------X X
Tumor measurements	X	Repeated every 8 weeks. Documentation (radiologic) must be provided for subjects removed from study for progressive disease.												X[b]
Radiologic evaluation	X	Radiologic measurements should be performed every 8 weeks												X[b]
Archival Tissue	X													
B-HCG	X[h]													
Research Bloods		X		X		X		X		X				X

a: All study calendar evaluations for cycle 3 are representative of cycle 3 and beyond.
b: Off-study evaluation should be performed within 30 days of last dose.
c: Arm B (regorafenib 160 mg/day days 1-21). Treatment cycle is 28 days
d: QoL surveys will be done on C3D1 and every other cycle subsequently (i.e. Cycle 5 Day 1, Cycle 7 Day 1, etc)
e: Baseline height will be measured and recorded once pre-study, and this value for height will be used through the patient's duration of study
f: Weekly blood pressures for first 6 weeks (can be checked with primary care physician or at patient's home), further blood pressure monitoring as determined by history of abnormality
g: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
h: Serum pregnancy test (women of childbearing potential).

Arm B – TAS-102

	Pre-Stud	C1 d1	C1 d8	C1 d15	C1 d22	C2 d1	C2 d8	C2 d15	C2 d22	C3 d1[a]	C3 d8 [a]	C3 d15 [a]	C3 d22 [a]	Off Study[b]
TAS-102[c]		X----------				X----------				X----------				
Informed consent	X													
History	X													
QoL questionnaire		X								X[d]				X
Concurrent meds	X	X		X		X		X		X				X
Physical exam (Ht, Wt, BSA, VS)[e]	X	X				X				X				X
Performance Status	X	X				X				X				X
CBC w/diff, plts	X	X		X		X		X		X				X
Serum chemistry[f]	X	X		X		X		X		X				X
CEA	X	X				X				X				X
Urinalysis	X													X
ECG	X													
Adverse event evaluation		X----------X												X
Tumor measurements	X	Repeated every 8 weeks. Documentation (radiologic) must be provided for subjects removed from study for progressive disease.												X[b]
Radiologic evaluation	X	Radiologic measurements should be performed every 8 weeks												X[b]
Archival Tissue	X													
B-HCG	X[g]													
Research Bloods		X		X		X		X		X				X

a: All study calendar evaluations for cycle 3 are representative of cycle 3 and beyond.
b: Off-study evaluation should be performed within 30 days of last dose.
c: Arm B (TAS-102 35mg/m2 twice a day on Days 1-5 and 8-12). Treatment cycle is 28 days
d: QoL surveys will be done on C3D1 and every other cycle subsequently (i.e. Cycle 5 Day 1, Cycle 7 Day 1, etc)
e: Baseline height will be measured and recorded once pre-study, and this value for height will be used through the patient's duration of study
f: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
g: Serum pregnancy test (women of childbearing potential).

10 MEASUREMENT OF EFFECT

As epigenetic agents may take many months to show their benefit as seen in MDS/AML studies,[25] in both Phase 1 and Phase 2, subjects receiving SGI-110 + irinotecan may continue receiving their assigned treatment if their disease progresses based on RECIST 1.1 criteria if 1) the patient is clinically stable, 2) the patient is informed of their scan results and agrees to continue therapy, and 3) the patient's provider agrees that remaining on study would be appropriate for the patient.[39]

Although response is not the primary endpoint of this trial, participants with measurable and/or non-measurable disease will be assessed by RECIST 1.1 criteria.[39] For the purposes of this study, participants should be reevaluated approximately every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of an objective response.

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 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 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guideline.[39] Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

10.1.1 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants 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 participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable).

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at

least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Reminder: A lesion in a previously irradiated area is not eligible for measurable disease unless there is objective evidence of progression of the lesion prior to study enrollment. Lesions in previously irradiated areas must be clearly identified as such.

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 < 15mm short axis, are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

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. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions.

All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

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

FDG PET and PET/CT. FDG PET will not be used for response assessment

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

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

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

Complete Response (CR):

Disappearance of all target lesions. Any pathological lymph node 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 with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

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.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.2 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 subject to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions.

Overall level of substantial worsening that merits discontinuation of therapy. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

10.1.4.3 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 participant'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 for 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	

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

Note: Participants 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	NonCR/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 an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.		

10.1.5 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 recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

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

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

10.1.6 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time to progression event, including objective disease progression, clinical deterioration attributed to disease, or death.

10.2 Other Response Parameters

CEA will be followed on study monthly for exploratory purposes but will not be used for response assessment.

Additionally in the phase II portion of the study, all subjects will be asked to complete a quality of life assessment using the Functional Assessment of Cancer Therapy (FACT-C) questionnaire at the start of study Cycle 1 Day 1, Cycle 3 Day 1, every other cycle

subsequently (i.e. Cycle 5 Day 1, Cycle 7 Day 1, etc), and at end of study/study withdrawal (optional).

11 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive

treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6 for a listing of expected adverse events associated with the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Related – There is reasonable evidence that the AE is related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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 website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

Each participating investigator is required to abide by the reporting requirements. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the PI of this study, Dr. Azad, and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the Principal Investigator, Dr. Azad, on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and related with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the Principal Investigator Dr. Azad within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by, email to:

Email: GISafetyReporting@jhmi.edu

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Coordinating center will fax or email serious adverse events (initial and follow-up reports) to Astex Drug Safety within 24 hours of receiving SAE report to:

Astex Drug Safety
North America Local Fax: 925.551.3226
North America Toll-Free Fax: 1.800.576.6568
Email: drugsafety@astx.com

Astex Pharmaceuticals will provide a quarterly listing of all SAEs received to Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins for comparison with the Sponsor's listing. Any missing SAEs or any discrepancies will be reported at that time.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the Principal Investigator Dr. Azad on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

Dr. Nilofer Azad

Email: GISafetyreporting@jhmi.edu

The Principal Investigator Dr. Azad will submit SAE reports from outside institutions to the SKCCC IRB per their policies and procedures in reporting adverse events.

11.6 Reporting to the Food and Drug Administration (FDA)

The Principal Investigator Dr. Azad, as holder of the IND, will be responsible for all communication with the FDA. Dr. Azad will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and related to the study treatment (Suspected Unexpected Serious Adverse Reactions or SUSARs) according the regulatory-defined timelines (7-day or 15-day reports). Dr. Azad will copy Astex pharmaceuticals (SGI-110 manufacturer) on any such reports.

In accordance with the regulation 21 CFR § 312.33, the IND Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. All IND annual reports will be submitted to the FDA by the IND Sponsor.

Events will be reported to the FDA using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

7 Calendar-Day Telephone or Fax Report:
The IND Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the investigational agent. Such reports are to be telephoned or faxed (301-796-9845) to the FDA within 7 calendar days of first learning of the event.

15 Calendar-Day Written Report:
The IND Sponsor is required to notify the FDA of any serious adverse event that is unexpected and possibly related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports. Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event.

11.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.8 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12 DATA AND SAFETY MONITORING

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 11.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 This is a Level II study under the SKCCC Data Safety Monitoring Plan. Data monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by Dr. Nilofer Azad and externally by the SKCCC CRO QA Office and the Coordinating Center in accordance with the JHU SKCCC Data Safety Monitoring Plan. Outside sites, USC, VUmc, and MSKCCC will use their internal clinical research offices to monitor their study patients and regulatory documents. Outside sites will provide copies of monitoring reports to the coordinating center. Eligibility for both sites will be monitored by the SKCCC CRO as well.

All trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

Authorized representatives of the Coordinating Center may visit any satellite sites connected to the outside sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

12.1.2 Dr. Azad will be holding the IND for this study. She will comply with all regulated reporting requirements to the FDA.

13 REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

    - Title 21 Part 11 – Electronic Records; Electronic Signatures
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

    - Title 21 Part 312 – Investigational New Drug Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Data Collection

Data will be collected and reported on either paper case report forms (CRF) or through an internal data monitoring system, which will be determined prior to the start of the study.

13.7 Multi-center Guidelines

This protocol will adhere to the policies and requirements of the SKCCC.

- The Overall 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.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.
- Except in very unusual circumstances, each participating institution will order the agent(s) directly from the supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

Multicenter Guidelines

Protocol Chair (Dr. Azad)
The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites.

Coordinating Center
The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

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.

14 STATISTICAL CONSIDERATIONS

The hypothesis of this study is that SGI-110, a demethylating agent, can sensitize pretreated metastatic colorectal cancer patients to retreatment with irinotecan.

14.1 Study Design/Endpoints

This is a Phase I/II study of Irinotecan and SGI-110 in patients with locally advanced or metastatic colorectal cancer. The dose escalation portion of the study will be used to determine the preferred dose of SGI-110, 30mg/m^2, 45 mg/m^2 or 60 mg/m^2, in combination with Irinotecan. The second portion of this study will be an open-label randomized phase II study with one arm receiving combination SGI-110 plus Irinotecan at the recommended dose from phase 1 (determined to be SGI-110 45mg/m2, Irinotecan 125mg/m2, with mandatory growth factor support) and the other arm receiving Regorafenib or TAS-102 at the standard commercial dose. The primary objective of the study is based on a comparison of PFS in the SGI-110 arm to that in the Regorafenib or TAS-102 arm, the two approved standard of care drugs in this setting. The primary endpoint for the Phase II study will be progression free survival (PFS). The study will randomize patients to the SGI-110 versus Regorafenib or TAS-102 at a ratio of 2:1. The randomized Regorafenib or TAS-102 group will provide information about PFS with the standard of care drugs to verify assumptions made in the design of the study.

Phase I primary objective: To assess safety and tolerability of Irinotecan in combination with SGI-110 in patients with advanced colon cancer. Determine the maximum tolerated dose (MTD) of Irinotecan in combination with SGI-110 and recommend a dose for phase II study.

Phase I secondary objectives:

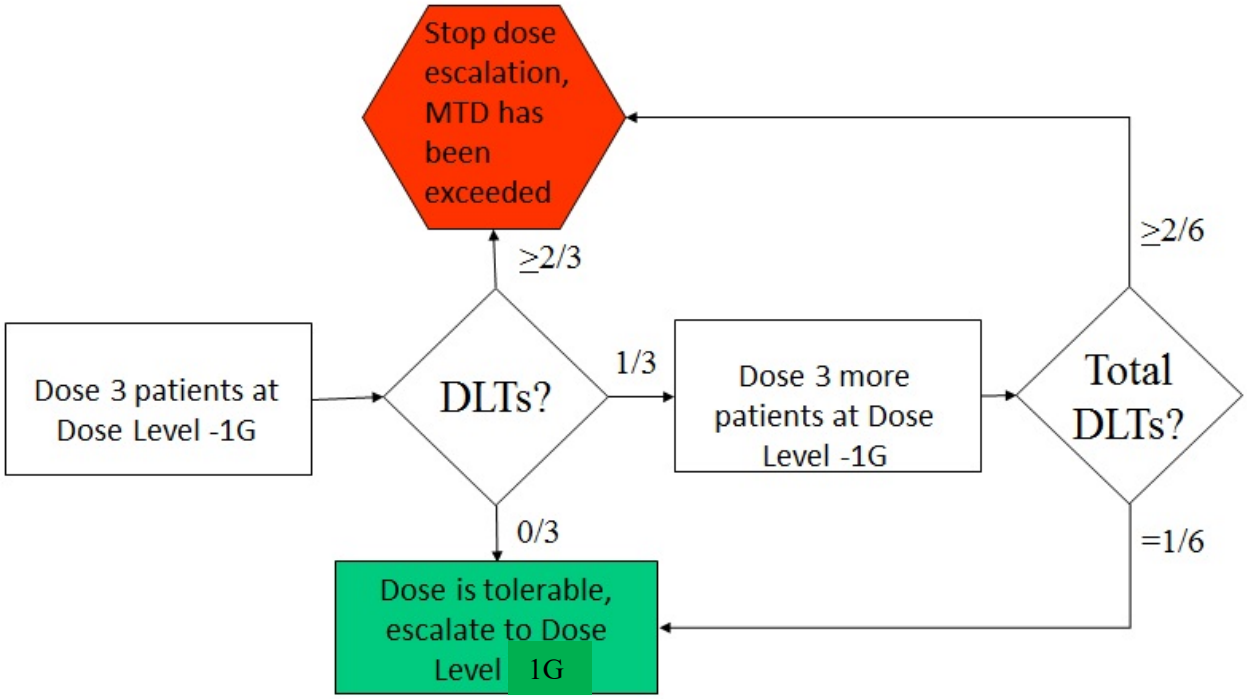
1. To assess changes in global methylation and expression at the tumor level with SGI-110 and Irinotecan treatment.
2. To assess the pharmacokinetics of SGI-110 in metastatic colon cancer patients and correlate with changes in global methylation.

Dose Escalation: Three dose levels of SGI-110, 30mg/m^2, 45 mg/m^2 and 60 mg/m^2, will be tested in combination with Irinotecan in this portion of the study. Dose level 1 will be IRI 125 mg/m^2

days 8 and 15 and SGI-110 45 mg/m^2 days 1-5 and dose level 2 will be IRI 125 mg/m^2 days 8 and 15 and SGI-110 60 mg/m^2 days 1-5; dose level 3 would be SGI-110 at 60 mg/m2 days 1-5 with IRI at 125 mg/m2 days 1, 8, and 15. Starting with dose level 1, three patients will be treated in a standard 3+3 dose escalation design. If none of the first three patients experiences a DLT then the next three will be treated using the next higher dose. If one of the first three has a DLT, an additional three patients will be enrolled at that level. If only one of six develops a DLT, then the dose will be escalated for the next three patients. If two or more of the six have a DLT, then the dose will be de-escalated to the previous dose where an additional three patients will be treated. The MTD is defined as the highest dose at which 0 or 1 dose-limiting toxicities are observed in six patients. A total of 6 patients will be treated at the MTD. The target toxicity with this type of design is less than 30%.

If there are two DLTs at the first dose level, a decreased dose schedule of SGI-110, which is less myelosupressive, will be considered with a similar 3+3 escalation of SGI-110. The next dose level below dose level 1 (dose level -1) would consist of IRI 125 mg m^2 days 8 and 15 and SGI-110 30 mg/m^2 days 1-5. If no DLTs are encountered with dose level -1, the next three patients will be entered at dose level 1G, or IRI 125mg/m2 days 8, and 15 and SGI-110 45mg/m^2 days 1-5. If one of the three patients has a DLT at dose level -1, an additional three patients will be enrolled at that level. If only one of six develops a DLT, then the dose will be escalated for the next three patients to dose level 1G. If two or more DLTs out of 6 are encountered at Dose level -1, at least an additional 3 patients will be enrolled at the lower dose level, Dose level -1G. If 0/3 or 1/6 DLTs occur on Dose level -1G, the next 3 patients will be dose escalated to Dose level 1G. If 2 or more DLTs out of 6 are experienced at Dose level -1G, the MTD will have been exceeded.

Figure 14.1.1. De-escalation Schema.



Sample size/accrual rate for dose escalation: The accrual rate for the dose escalation portion of this study is expected to be 3 patients per month. The maximum sample size in the dose escalation portion of the study could be 12 patients. If no patients experience a DLT, 9 patients will be enrolled.

Analysis of primary objective in dose escalation cohort:
The number of dose-limiting toxicities at each dose level will be reported. Additional toxicity frequencies by type and grade will be summarized by dose level for all doses.

Analysis of secondary objectives in dose escalation:

1) The design of this study will allow assessment of global and candidate gene methylation differences at the tumor level. We hypothesize that Irinotecan resistance may be reversed with the use of the demethylating SGI-110 therapy. Changes in global methylation by LINE-1 will be assessed in post-treatment biopsy specimens in the dose escalation cohort for proof of the effect of SGI-110. Changes will be plotted by dose level and overall using boxplots of the log transformed raw data. A paired t-test, or the nonparametric Wilcoxon signed rank test, will be used to test if the changes are significantly different from zero. Similar analyses will be used to assess gene expression changes in WRN, DR1, TPAF2E, DEXI, BNIP3, and MED1.
2) Pharmacokinetic (PK) sampling studies are proposed for all participating patients who undergo pharmacodynamic endpoints. Single dose PK samples will be collected on Cycle1, Day 1, at the following time points: pre-dose 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hr. The following PK parameters describing the concentration time profile of SGI-110 will be calculated: total exposure will be calculated as area under the plasma concentration-time curve (AUC) using the linear trapezoidal rule by using noncompartmental methods (Winonlin, version 5.3) and/or compartmental modeling (Adapt II, release 4.0). Other parameters such as maximum concentration (C_{max}), time to maximal concentration (T_{max}) and half-life ($T_{1/2}$) will also be calculated. C_{max} and T_{max} will be obtained from the data, while the half-life will be calculated as 0.693/k, where k is the slope of terminal elimination phase. Associations between SGI-110 exposure parameters (C_{max} and AUC) and pharmacodynamic endpoints (i.e., global methylation changes and toxicity) will be assessed using appropriate non-parametric statistical tests.

Moving to Phase II Component: Our hypothesis is that SGI-110 treatment will sensitize colon cancer patients to retreatment with irinotecan. Accordingly, it will be important to see that the dose of SGI-110 that is at the MTD does have pharmacodynamics effects at the tumor level. Patient tumor biopsies post-treatment will be analyzed by LINE assay for demethylation to assess for at least 10% demethylation from baseline in the tumor biopsies. We aim to see this level of demethylation in at least 60% of post-treatment biopsies as previously described in phase O studies.

Phase II primary objective: Evaluate the progression free survival (PFS) in patients receiving combination SGI-110 plus Irinotecan compared to standard of care treatment with regorafenib or TAS-102. Events are defined as disease progression or death from any cause. All patients treated

on protocol will be included in the determination of PFS, regardless of treatment modification or discontinuation.

Epigenetic therapy may take several cycles to exert its maximal benefit, and preclinical data has demonstrated initial tumor growth is possible prior to tumor shrinkage. Patients on treatment with SGI-110 and IRI will be allowed to remain on study if their tumor progresses on RECIST 1.1 criteria if they are 1) clinically stable, 2) their provider feels it would be in their best interest, and 3) the patient is informed of scan results and consents to remain on treatment. In this circumstance, they will be reimaged on schedule after 2 further cycles. If this subsequent imaging confirms progressive disease, the date of the progression even will be dated to the first (initial) scan date that progression was first documented.

Phase II secondary objectives:

1. Evaluate and compare response rates between arms of the study as determined by RECIST criteria 1.1.
2. Evaluate and compare overall survival (OS) between arms of the study.
3. Assess for potential predictive markers of survival using baseline tissue.
4. To assess the pharmacokinetics of SGI-110 in metastatic colon cancer patients and correlate with changes in global methylation.

Sample Size/Accrual Rate: The median PFS of patients on the phase III CORRECT trial of Regorafenib in metastatic colorectal cancer reported by Grothey and Van Cutsem was 1.9 months.[11] The median PFS of patients on the phase III RECOURSE trial of TAS-102 in metastatic colorectal adenocarcinoma reported by Mayer[15] was 2.0 months. The PFS curves of these two trials were superimposable and therefore hazard rates over four time intervals were estimated based on the published PFS curve (figure 3.) for Regorafenib from the CORRECT trial. Simple linear regressions were used to fit the natural logarithm of the Kaplan-Meier product limit estimate of the PFS function as a function of time in four intervals: from 1 to 6 weeks, 6 to 12, 12 to 18, and 18 to 56 weeks. These estimated hazard rates: 0.0113, 0.0558, 0.3238, and 0.0748 per person week were used in simulations to evaluate power for this study. We hypothesize that the SGI-110 combination treatment will improve the 4 month PFS from 23% to 46.5%. Patients will be randomized to the SGI-110 + irinotecan (Arm A) and the Regorafenib or TAS-102 arm (Arm B) in a 2:1 ratio. Using a one sided 5% type I error allowance, accrual of 48 patients per year for 2 years (Arm A and B target sample sizes of 64 and 32 respectively) with an additional 2 years of follow up, this study will provide sufficient events to have 83% power to detect an improvement of the median PFS from 1.9 months to 3.6 months, i.e. a hazard ratio of 0.526.

Quality of Life Assessment: Quality of life will be assessed with the Functional Assessment of Cancer Therapy (FACT-C) quality of life tool. An overall quality of life score for the domains: physical well-being, social/family well-being, emotional well-being, and functional well-being, as well as the colon cancer specific subscale score will be obtained at the start of the study (Cycle 1 Day 1), at the beginning of cycle three (Cycle 3 Day 1), every other cycle subsequently (i.e. Cycle 5 Day 1, Cycle 7 Day 1, etc), and at the off study visit for every patient. Scoring will follow guidelines provided by FACIT administration guidelines manual. The change in total score between pre-treatment and the start of cycle three will be compared between arms of the study with a t-test. Two subscale sums of the total score: the sum of physical and functional well-being,

and the sum of social/family and emotional well-being, will be similarly analyzed. The change in the colon cancer specific domain will also be compared between arms of the study. We anticipate that we will have minimal missing data at the start of cycle 3 as this is the first time a patient could come off study for progression. At this time point, any missing data would be due to DLTs, SAE events, or voluntary withdrawal. In these cases, documentation of the reason for missing data and the QoL at the time of withdrawal will be obtained with the off study evaluation.

Futility monitoring plan: We will monitor the study for futility using a non-parametric Bayesian predictive probability approach.[40-42] This monitoring plan is based on calculations of the posterior distribution of the survival distribution and simulations to determine the probability that the PFS at 4 months on the treatment arm will be greater than that on the control arm at the end of the study. When the trial is monitored, Gibbs sampling is used to generate samples from the posterior distribution of the survival distribution via Monte Carlo simulation, given the data and a Dirichlet process prior. Censored observations are treated as random quantities and PFS event times are simulated for each censored observation. This is repeated many times and ultimately converges to a realization of the parameters drawn from the posterior distribution. The algorithm is that reported in Kuo and Smith.[42] If the probability that the treatment 4 month PFS is greater than the control PFS at 4 months is very low, less than 33.3%, the study will be stopped for futility.

The reference hazard rates were estimated based on the published PFS curve (figure 3.) for Regorafenib.[11] Simple linear regressions were used to fit the natural logarithm of the Kaplan-Meier product limit estimate of the survival function as a function of time in four intervals: from 1 to 6 weeks, 6 to 12, 12 to 18, and 18 to 56 weeks. Survival times were then generated using a piecewise exponential distribution with these estimated hazard rates: 0.0113, 0.0558, 0.3238, and 0.0748 per person week. For simulation purposes, the effect of treatment was assumed to multiply these hazard rates by factors of 1.0 (no difference), 0.80, 0.60, 0.526, 0.50, 0.40 and 0.35. The randomization ratio for these simulated studies was 2:1 and a single interim analysis, evaluating 4 month PFS, was performed after 50% of the patients were enrolled, i.e. n=32 in Arm A (SGI-110) and n=16 in Arm B (Regorafenib or TAS-102). A one-sided 0.05 alpha level log-rank test was used for the final analysis. Plots of the reference curve (black) and six scenarios of treatment effects (red) are shown in figure 1. In scenario one, the reference and treatment hazard rates are equal. Table 1 gives operating characteristics of this monitoring plan for these scenarios. Column 4 in table 1 is the proportion of studies rejecting the null hypothesis out of the number of studies that did not stop for futility. Column 5 is the overall proportion of studies with a positive result.

Table 1. Operating characteristics of monitoring rule from 500 simulated studies with interim analysis on 4 month PFS and final analysis based on the log-rank test.

Scenario	Control hazard rate (λ) multiplier	Stop for Futility %	Rejected at final analysis %*	Overall studies rejecting Ho %
1	1.00	26.4%	5.4%	4.0%
2	0.80	12.8%	22.0%	19.2%

3	0.60	5.2%	68.8%	65.2%
4	0.526	2.0%	84.7%	83.0%
5	0.50	1.2%	89.1%	88.0%
6	0.40	0.6%	98.4%	97.8%
7	0.35	0.0%	99.8%	99.8%

*Denominator is the number of simulations that do not stop early for futility.

Analysis of primary objective:
Standard life table methods will be used to analyze PFS. We will report the 4-month, 1-year and median PFS with 95% confidence intervals for both arms of the study.

Analysis of secondary objectives:

1. The 4-month PFS will be compared between arms of the study using a one-sided, 0.05 alpha level, log-rank test.
2. All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. 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).
   All subjects in the study will 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. Response rate will be estimated overall and by treatment arm. A Fisher's Exact or chi square test will be use to compare response rates across treatment arms.
3. Standard life table methods will be used to analyze OS. Four month, 1-year and median survival will be reported, overall and by treatment arm. We acknowledge that OS assessment will be compromised with the cross-over design and will report as such.
4. Baseline global methylation will be categorized as high or low and OS Kaplan Meier curves plotted by category. The change in global methylation will be categorized as positive or negative and OS Kaplan Meier curves plotted by category. Similar analyses will be used for individual expression markers.
5. Pharmacokinetic (PK) sampling studies are proposed for all participating patients who undergo pharmacodynamic endpoints. Single dose PK samples will be collected on Cycle1, Day 1, at the following time points: pre-dose 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hr. The following PK parameters describing the concentration time profile of SGI-110 and Irinotecan will be calculated: total exposure will be calculated as area under the plasma concentration-time curve (AUC) using the linear trapezoidal rule by using noncompartmental methods (Winonlin, version 5.3) and/or compartmental modeling (Adapt II, release 4.0). Other parameters such as maximum concentration (C_{max}), time to maximal concentration (T_{max}) and half-life ($T_{1/2}$) will also be calculated. C_{max} and T_{max} will be obtained from the data, while the half-life will be calculated as 0.693/k, where k is the slope of terminal elimination phase. Associations between SGI-110 exposure parameters

(C_{max} and AUC) and pharmacodynamic endpoints (i.e., global methylation changes toxicity and toxicity) will be assessed using appropriate non-parametric statistical tests.

Stratification Factors

Patients will be stratified by two factors: assignment to Regorafenib or TAS-102, and time from last irinotecan treatment (>6 months or < 6 months) through an algorithm to maintain balance between the arms as much as possible.

14.2 Reporting and Exclusions

14.2.1 **Evaluation of toxicity.** All participants will be evaluable for toxicity from the time of their first treatment.

14.2.2 **Evaluation of response.** All participants included in the study must be assessed for response to treatment. Each participant should 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). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

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Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	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: **PATIENT DIARY**
Today's date: _____

Agent: Regorafenib _____ mg daily

Patient instructions: Regorafenib should be taken with a low-fat meal once a day.

Study Day	**Date**	**Time of Dose**	**# Tablets taken**	**Blood Pressure** (Cycle 1, Days 1, 8, 15, and 22 & Cycle 2, Days 1 and 8)	**Comments**
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					

_____
Patient Signature

_____
Date

APPENDIX C: PATIENT DIARY
Today's date _____

Agent: **TAS-102 _____ mg twice daily**

Patient instructions: TAS-102 should be taken twice daily within one hour after a meal.

Study Day	Date	Time of AM Dose	Time of PM Dose	# Tablets taken AM / PM	Comments
1				/	
2				/	
3				/	
4				/	
5				/	
6					
7					
8				/	
9				/	
10				/	
11				/	
12				/	
13					
14					
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21					
22					
23					
24					
25					
26					
27					
28					

_____
Patient Signature

_____
Date

APPENDIX D: FACT-C (Version 4)

Study #: _____ Subject ID: _____

Subject initials: _____ Subject date of birth: _____

Today's date: _____

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

	<u>PHYSICAL WELL-BEING</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	<u>SOCIAL/FAMILY WELL-BEING</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**

GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	<u>EMOTIONAL WELL-BEING</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4

GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4

GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	<u>ADDITIONAL CONCERNS</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
C1	I have swelling or cramps in my stomach area	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
C3	I have control of my bowels	0	1	2	3	4
C4	I can digest my food well	0	1	2	3	4
C5	I have diarrhea (diarrhoea)	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
Q2	Do you have an ostomy appliance? (Mark one box)	☐ No	or	☐ Yes		
	If yes, please answer the next two items:					

C8	I am embarrassed by my ostomy appliance	0	1	2	3	4
C9	Caring for my ostomy appliance is difficult	0	1	2	3	4