A phase I trial of a guadecitabine (SGI-110) and irinotecan in metastatic colorectal cancer patients previously exposed to irinotecan

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Running head: Guadecitabine+irinotecan in irinotecan-exposed mCRC patients

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Cancer Center Amsterdam, VU University Medical Center

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ABSTRACT

Purpose: Chemotherapeutic resistance eventually develops in all patients with metastatic colorectal cancer (mCRC). Gene silencing through promoter demethylation is one potential reversible mechanism of resistance with administration of hypomethylating agents. We evaluated the safety and tolerability of guadecitabine and irinotecan in mCRC patients previously treated with irinotecan.

Experimental Design: In this 3+3 dose-escalation study, mCRC patients previously exposed to irinotecan received guadecitabine days 1-5 of a 28 day cycle and irinotecan 125mg/m2 days 8 and 15 [dose level (DL) 1, guadecitabine 45mg/m2; DL -1: guadecitabine 30mg/m2; DL -1G: guadecitabine 30mg/m2 with growth factor support (GFS); DL 1G: guadecitabine 45mg/m2 with GFS.

Results: Twenty-two patients were treated across four DLs. Dose-limiting toxicities were neutropenic fever (DL 1 and -1G), biliary drain infection (DL -1), colonic obstruction (DL -1), and severe dehydration (DL 1G). Most common toxicities were neutropenia (82% any grade, 77% Grade 3/4), neutropenic fever (23%), leukopenia (73% any grade, 50% Grade 3/4), and injection site reactions (64% total, 0% Grade 3/4). Patients received a median of 4.5 cycles of treatment; 12/17 evaluable patients had stable disease as best response, with one having initial disease progression but subsequently durable partial response. Circulating tumor DNA showed decrease in global demethylation by LINE-1 after treatment.

Conclusion: We report the first study of chemo-priming with epigenetic therapy in GI cancers. Guadecitabine 45 mg/m2 and irinotecan 125mg/m2 with GFS was safe and tolerable in mCRC patients, with early indication of benefit. These data have provided the basis for an ongoing phase II randomized, multicenter trial.
Statement of translational relevance

Acquired resistance to cytotoxic chemotherapy invariably develops in all patients with metastatic colorectal cancer, and tracks with poor survival. Epigenetic changes in the methylation of tumor DNA have been implicated in acquired chemoresistance and may be reversible with DNT methyltransferase inhibitors. We tested this hypothesis in a phase I clinical trial of guadecitabine and irinotecan in patients with colorectal cancer who had previously been treated with irinotecan. We showed the combination to be tolerable and that a majority of the patients had disease benefit. We demonstrated pharmacodynamic effect with circulating tumor DNA showing decreased global demethylation. Our data supports further development of this combination in mCRC and a randomized phase II trial is ongoing. Future directions include applying this strategy of using DNMT inhibitors to reverse chemoresistance with other classes of agents and in other tumor histologies.
INTRODUCTION

Overall survival (OS) of metastatic colorectal cancer (mCRC) patients has improved to median 2.5 years over the past two decades with addition of irinotecan, oxaliplatin, bevacizumab, and epidermal growth factor receptor (EGFR) inhibitors to fluorouracil based therapy.\(^1\) While primary chemoresistance is a concern, even among patients with excellent response to therapies, resistance and intolerance to chemotherapeutic agents inevitably develops. In RAS wildtype mCRC, anti-EGFR antibodies combined with irinotecan results in increased response rate and progression free survival (PFS) compared to anti-EGFR therapy alone; no such option exists for RAS mutated disease.\(^2\) The remaining FDA approved third-line treatment options (regorafenib and TAS-102), provide less than a two-month OS benefit over placebos, highlighting the need for additional therapeutic options.\(^3,4\)

Epigenetic therapy with DNA methyltransferase inhibitors (DNMTi) show success in hematologic malignancies, but little benefit in solid tumors, including non-small cell lung cancer (NSCLC), mCRC, and breast cancer.\(^5-7\) However, follow-up of NSCLC patients treated in a phase I/II study of 5-azacitidine and entinostat, showed that 30% of these heavily pre-treated patients had Response Evaluation Criteria in Solid Tumors (RECIST) responses to subsequent therapies, with another half with stable disease. Similar preclinical and early phase clinical studies in ovarian cancer have shown DNMTi reversing resistance to platinum agents.\(^8\) In preclinical CRC models, DNMTis can induce re-expression of tumor suppressor genes.\(^9\) Our group has reported DNMTi can both induce sensitivity and reverse chemoresistance to irinotecan in \textit{in vitro} and \textit{in vivo} models.\(^10\)
Guadecitabine (SGI-110) is a subcutaneously administered dinucleotide of decitabine and deoxyguanosine. Decitabine is an FDA approved DNMTi for treatment of myelodysplastic syndromes. Pharmacokinetic studies of guadecitabine demonstrate a prolonged half-life of the metabolite decitabine, compared with intravenous decitabine, with resultant higher exposures to decitabine and more potent global hypomethylation. When administered to patients with acute myeloid leukemia and myelodysplastic syndrome in a phase 1 study, dosing of guadecitabine monotherapy at 60mg/m2 daily x 5 in a 28-day cycle was tolerable and led to potent dose-related DNA demethylation.\textsuperscript{11} This dosing was intolerable in sorafenib-refractory hepatocellular carcinoma (HCC) patients due to myelosuppression. However, while guadecitabine dosed at 45mg/m2 in HCC patients had 67% Grade 3/4 neutropenia and 11% febrile neutropenia, treatment maintained demethylation in both intratumoral and circulating DNA.\textsuperscript{12}

The primary objectives of this phase I dose-escalation study were to evaluate the safety and tolerability of guadecitabine in combination with irinotecan in patients with mCRC, and to determine the maximum tolerated dose (MTD). Secondary objectives were to assess changes in global methylation in the tumor and circulating cells after administration of combination therapy, assess pharmacokinetics (PK) of guadecitabine, and assess for disease response.
METHODS

Study Population

Patients were ≥ 18 years old, with histologically/cytologically confirmed metastatic colorectal adenocarcinoma, with prior exposure to irinotecan. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, intact organ and marrow function (leukocytes ≥ 3000/mcL, absolute neutrophil count ≥ 1500/cL, platelets ≥ 100,000/mcL, total bilirubin < 1.5 times the upper limit of normal (ULN), AST/ALT ≤ 3 times ULN, creatinine < 1.5 times ULN or creatinine clearance ≥ 50mL/min/1.73 m²), measurable disease according to the RECIST (version 1.1), and biopsiable disease. Major exclusion criteria included patients with known brain metastases and prior therapy, hypomethylating agents, and recent (< 6 month) small bowel obstruction.

Study Design

This phase I, open-label, nonrandomized, dose-escalation study was conducted at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center in the USA and the Cancer Center Amsterdam, VU University Medical Center in the Netherlands (NCT01896856). Patients were escalated per the 3+3 design, and at each dose level (DL) (Table 1) patients were administered guadecitabine subcutaneously (SQ) on Days 1-5 and irinotecan (IV) on days 8 and 15 of a 28-day cycle.

The study protocol and all amendments were approved by the independent institutional review boards or ethics committees for each study site and conducted in accordance with the Declaration
of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients provided informed consent.

Evaluations

All patients who received at least one dose of guadecitabine were considered evaluable for toxicity. Safety evaluation included laboratory assessments, physical examinations, and adverse events (AEs) were graded according to the NCI Common Terminology Criteria for Adverse events (CTCAE) version 4.0. Follow-up examinations and laboratory studies were done within 30 days of study completion. Dose limiting toxicities (DLT) were defined as cycle 1 adverse events attributable to study treatment defined as grade 4 thrombocytopenia or neutropenia last >7 days, any incidence of Grade 3 or 4 febrile neutropenia, Grade ≥ 3 non-hematologic toxicity unless it could be appropriately managed by supportive treatment, or any other clinically significant adverse event which would place the subjects at undue safety risk or resulted in discontinuation of treatment.

Patients who completed at least one cycle of treatment and underwent repeat assessment of disease were considered evaluable for response. Responses were determined according to RECIST 1.1, include complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

The maximum-tolerated dose (MTD) was the highest dose level at which less than 33% of patients experienced a DLT during cycle 1.
PK Analyses

Pharmacokinetics of guadecitabine were assessed from plasma concentrations on cycle 1, day 1, at nine time points: pre-dose, and 15 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 4 hours, 6 hours, and 8 hours post-dose. Plasma concentrations were determined using a validated LCMS/MS method with LLOW of 1 and 0.5 ng/mL for guadecitabine and decitabine, respectively. Calculations were made of total exposure as determined by area under the plasma concentration-time curve, maximum concentration (Cmax), time to maximal concentration (Tmax), and half-life (T1/2) by noncompartmental analysis using Phoenix software (v. 7.0).

LINE-1 Analysis

Tumor biopsies were obtained prior to first dosing of guadecitabine on Cycle 1 day 1 and prior to irinotecan dosing on cycle 1 day 8. Research serum/plasma samples were collected on days 1, 8, and 15 of cycles 1 and 2, and then on day 1 of subsequent cycles.

Previously published Methylation on Beads protocol was used for circulating DNA extraction. Bisulfite converted DNA from tissue, PBMCs and plasma was amplified using primers specific for Exon 1 of the L1H retrotransposon as previously described. Specific amplification was checked via Gel electrophoresis and LINE1 methylation levels were identified via bisulfite pyrosequencing on a Q24 (Qiagen).

To analyze LINE1 methylation, the first three CpG dinucleotides from the amplified sequence were used from each time-point and averaged to provide an average % methylation for each sample. A positive control consisting of in-vitro methylated DNA, and a negative control
consisting of DNA from a cell line (HCT116) with double knockout for DNMT1 and DNMT3a, was run with each batch of samples (Zymo).

**Statistical Methods**

The event time distribution for overall survival (OS) is estimated with the method of Kaplan and Meier. LINE1 methylation data are plotted over time by dose level and sample type: tissue or plasma. Paired t-tests were used to compare LINE1 methylation at cycle 1 day 1 to cycle 2 days 8 and 15.
RESULTS

Twenty-two patients were recruited between October 2013 and October 2015. Toxicity attributable to treatment was censored as of April 8, 2016, and follow-up data for OS was last censored as of January 1, 2017, at which time one patient was still on study. Patients were enrolled across four dose levels (six at DL1, three at DL-1, seven at DL-1G, and six at DL1G). An amendment was made due to the high rates of neutropenia to add DL -1G and 1G (which specified mandatory growth factor support for the first cycle that could be discontinued for subsequent cycles based on provider choice). Patients were heavily pretreated, receiving a median of 2.5 prior lines of treatments, 14 of 22 (64%) with prior progression on irinotecan as determined by treating physician (Table 2).

Patients received a median of 4.5 cycles of therapy (range 0 to 18), 16 patients received more than one cycle, of whom seven required dose reductions of guadecitabine, irinotecan, or both. Of the twenty-one patients who had discontinued treatment at data-lock, twelve were due to disease progression (57%), six were due to toxicity (29%), and two patients withdrew consent, one after receiving two doses of guadecitabine, the other after 12.5 cycles (9%), and a patient was taken off trial after developing renal insufficiency from ureteral stones unrelated to treatment. At a median follow-up of 20 months, the median OS for the 22 patients on study is 10.7 months. Six, 12 and 18 month OS is 68% (95% CI: 51, 91%), 47% (95% CI: 30, 75%), and 35% (95% CI: 19, 65%) respectively.

Safety

No DLTs were noted in the first dose level, but due to prolonged neutropenia, the investigative team decided to expand the cohort to six patients total; one of these six patients developed...
neutropenic fever and was removed from the study. Dose was de-escalated to DL-1 with guadecitabine at 30mg/m2, during which two patients suffered DLTs (biliary drain infection in setting of neutropenia and colonic obstruction secondary to disease progression). Among the nine patients on dose level 1G and -1G, six received growth factor support (two who suffered from neutropenic fever recovered prior to administration of growth factor support). At this time an amendment was made to add mandatory growth factor support and re-enroll the dose levels starting with DL-1G. Seven patients were enrolled at DL -1G (one patient withdrew consent after two doses of guadecitabine), with one DLT of neutropenic fever. After recovering, the patient was resumed on therapy and remained on trial (requiring growth factor support) at the time of data lock. The study was escalated to DL 1G, with enrollment of six additional patients, of whom one suffered severe dehydration and renal failure leading to death after receiving cycle 1 day 8 irinotecan; review of the case suggested non-compliance with supportive care medication. However, after review of the toxicities observed across the study as well as the pharmacodynamics data described below, the investigative team agreed to conclude dose escalation at DL 1G even though the trial had not exceeded the MTD as defined in the protocol. The phase 2 dose was defined as DL 1G, guadecitabine 45 mg/m2 days 1-5 and irinotecan 125 mg/m2 days 8 and 15 of a 28-day cycle with mandatory growth factor support during cycle 1.

Most common attributable toxicities were neutropenia (18 (82%)), nausea (16 (73%)), injection site reactions (14 (64%), fatigue (14(64%), and anemia (13 (59%)). The most common attributable Grade 3/4 toxicities were neutropenia (17 (77%)), including five episodes neutropenic fever (23%), anemia (3 (14%)), diarrhea (4 (18%)), and thrombocytopenia (2 (9%)) (Table 3).
Pharmacokinetics

Pharmacokinetics was assessed in 15 subjects (6 from 30 and 9 from 45 mg/m² dose) who contributed evaluable data. After guadecitabine SC injection, both guadecitabine and its active metabolite decitabine were detected in plasma from subjects at both dose levels. Plasma concentrations of guadecitabine peaked rapidly (within 1.5 h post dose) followed by a rapid decline in systemic exposure. Decitabine forming from guadecitabine also peaked within 1.5 h post-dose. Both guadecitabine and decitabine were still present in circulation at 8 hours in most subjects suggesting an exposure window for active metabolite decitabine of over 8 hours (Figure 1). The exposure of guadecitabine and the extent of formation of decitabine appeared to increase in a roughly dose proportional manner for the dose levels studied (Supplemental Table 1). Based on the ratio of mean decitabine to mean guadecitabine systemic AUC values on molar basis, conversion to the active metabolite was efficient.

Clinical Response

Seventeen patients were evaluable for response, twelve patients [70.6% (95% CI: 44.04, 89.69%)] had stable disease as best response, and five had progressive disease [29.4% (95% CI: 10.31, 55.96%)] at first re-evaluation (Figure 2). Patients received a median of 4.5 cycles. Fourteen patients were considered resistant to irinotecan upon enrollment; of the nine who were evaluable for response, five had stable disease after two cycles. Of those with irinotecan sensitive disease, 7 of 8 patients had stable disease at first evaluation (87.5%), and received a median of 7.5 cycles (range 2-12). One patient on DL -1G was noted to have a partial response in index lesions at the first follow-up scan along with a clinically notable decrease in CEA,
however also developed new sub-centimeter liver lesions. The patient had previously progressed on three lines of therapy, including hyperthermic intraperitoneal chemotherapy (HIPEC), with recurrent primary liver disease. Trial treatment was initiated four weeks after baseline scan, and the concern was that progression may have occurred prior to treatment initiation. Treatment was maintained and subsequent scans showed partial response by cycle four, with resolution of the initial liver lesions. After eight cycles, growth in a single hepatic lesion was noted and treated with radiation; the patient remained on trial for over 18 cycles by the time of data lock.

**LINE-1 Demethylation**

No consistent changes were seen in early methylation in the tissue samples or plasma methylation at day 8 compared to day 1 of therapy at either guadecitabine dose level (Supplemental figure 1 and 2). However, paired plasma methylation of circulating tumor DNA showed significant decreases from cycle 1 day 1 by cycle 2 day 15 of -6.6% (95% CI: -12.3, -0.83%) and -13% (95% CI: -22.1, -3.87%), p = 0.03 and p = 0.01 for guadecitabine dosing at 30mg/m2 and 45mg/m2 respectively (Figure 3). Patients with progressive disease and stable disease responses had similar mean and median changes in circulating LINE-1 methylation by cycle 2 day 15 (Mann-Whitney p = 1.0).
DISCUSSION

The role of epigenetic agents in solid tumors is still being defined, but shows promise for reversing chemo-resistance. Recent studies of combination DNMTi and HDACi in solid tumors has revealed little clinical response in mCRC patients (no responses out of 47 enrolled) or in triple negative breast cancer (no responses in thirteen enrolled); a single response was observed in hormone receptor positive breast cancer. Similar results were seen in the phase I/II trial of combination DNMTi and HDACi in lung cancer, where only two of 19 patients treated showed objective responses. However, long term follow-up showed possible prolonged median OS compared to historical controls, and further assessments revealed that multiple patients among this heavily pre-treated cohort had major objective responses to subsequent systemic treatments, suggestive of a priming mechanism for chemotherapies. The reversal of treatment resistance has been best demonstrated in ovarian cancer patients. In early phase trials, treatment with low-dose decitabine leads to global demethylation in tumor cells, alongside changes in specific cancer pathways, including mismatch repair, WNT, and apoptotic pathways. When epigenetic agents were combined with platinum-based therapy in Phase I/II studies, both decitabine and belinostat led to PR/stable disease in 46% and 44% in platinum-resistant ovarian cancer patients, recommending studies on a larger scale to better understand the clinical effect. A randomized phase II study of guadecitabine with carboplatin in ovarian cancer compared to physician choice showed a nonsignificant trend to improvement in 6 month PFS (37% vs 15%), median PFS (16.3 vs 10.9 months) and OS (11 vs 7.4 months). In advanced, pretreated colorectal cancer, Overman and colleagues reported 65% stable disease rate for a hypermethylator-phenotype enriched population with azacitidine and oxaliplatin and fluorouracil therapy. A second study of decitabine and the anti-EGFR targeted agent panitumumab in
KRAS wildtype patients showed similar benefit rate in 20 patients, but no differences based on prior anti-EGFR exposure.\textsuperscript{21}

For this trial we targeted patients with mCRC based upon work in our group demonstrating the ability of DNMTi to reverse irinotecan resistance in both resistant colon cancer cell lines and mouse models.\textsuperscript{10,22} There is no well-agreed upon mechanism that has explained irinotecan resistance.\textsuperscript{23,24} It has been hypothesized that that methylation of DCR1 and WRN, the latter being a DNA helicase, may affect sensitivity to irinotecan, but confirmatory studies to validate these findings in clinical data sets have not been successful.\textsuperscript{9,25-27} The multifocal effects of epigenetic therapies, such as guadecitabine, that result in widespread changes in gene expression across hundreds of cellular pathways, may well be responsible for reversing resistance to cytotoxic chemotherapies, but also create great barriers in understanding the mechanisms behind these effects.\textsuperscript{28-30}

Expected hematologic and gastrointestinal toxicities were seen throughout the trial, but treatments were better tolerated when combined with mandatory growth factor support. Notably for this refractory group of patients with prior oxaliplatin and irinotecan exposure, subjects remained on trial for a median of 4.5 cycles and had a median OS 10.7 months. Based upon this information, the decision was made between participating investigators, with the support of the Stand Up 2 Cancer Van Andel Research Institution and Astex Pharmaceuticals to move to a randomized phase II study comparing this regimen to regorafenib or TAS-102 (NCT01896856).
Phase I trials also allow for preliminary biomarker evaluations to assess molecularly targeted effects. While allowing for proof-of-concept studies, the role of biomarker studies in early oncology trials is difficult in part due to the hardship of obtaining tissue samples and the high cost of analysis.\textsuperscript{31} For epigenetic agents the global impact upon multiple targets makes it particularly difficult to identify which pathways are most relevant. Additionally, as seen in our trial, appropriate timing of sampling biopsies can potentially alter the apparent effectiveness of biomarker evaluation. Although there was no specific demethylation patterns in tumor samples by day 8 of treatment, the circulating DNA showed delayed and dose-dependent demethylation by the middle of cycle 2. These data recommended amendments to our phase II protocol for scheduling later biopsies, which will potentially allow for more useful comparisons of on-target epigenetic mechanisms of action with clinical response.\textsuperscript{32-34} Our experience reflects the importance of preliminary biomarker studies for helping to inform studies in subsequent trials.

The future of epigenetic therapy is wide ranging and a number of trials evaluating their efficacy in combination with systemic chemotherapy in other disease types are now underway. In particular, the impact of epigenetic therapy upon a wide number of gene targets may make it a strong companion treatment to checkpoint blockade and other immunotherapies.\textsuperscript{35-37} Multiple groups including ours have reported on the potential of DNMTi, HDAC inhibitors, and emerging epigenetic modulating agents to modulate the tumor microenvironment, including increased co-stimulatory molecule expression, MHC class I/II and neoantigen expression, and affecting polarity of immune cells to a pro-inflammatory phenotype.\textsuperscript{16,35,36,38,39} Clinical trials using epigenetic drugs to prime for immunotherapy agents are ongoing as a major research area for this class of agents, including in mCRC. In breast cancer, the epigenetic modulator entinostat, a
histone deacetylase inhibitor, has been shown to significantly improve survival in combination with hormonal therapy in hormone-resistant disease in a pivotal randomized phase II study, now being validated in a large, randomized phase III study (NCT02115282). However, epigenetic therapy is unlikely to play a role in all disease groups, and it will be important to identify molecular and clinical characteristics of patients likely to respond in order to better personalize future therapies.

We report the results of a phase I study evaluating safety, pharmacokinetics, and activity of the combination guadecitabine and irinotecan in mCRC. We determined that the phase 2 dose was guadecitabine at 45mg/m2 on days 1-5 and irinotecan at 125mg/m2 on day 8 and 15 with mandatory growth factor. A phase II study of guadecitabine and irinotecan versus standard of care (TAS-102 or regorafenib) supported by the Van Andel Research Institute-SU2C Dream Team is currently underway.

ACKNOWLEDGMENTS

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REFERENCES

1. Venook, A. P. et al. CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). *Journal of Clinical Oncology* **32**, LBA3-LBA3, doi:10.1200/jco.2014.32.18_suppl.lba3 (2014).


FIGURE LEGENDS

Figure 1 Pharmacokinetic changes on cycle 1 day 1 of guadecitabine by different dosing levels

Figure 2 Percent change in RECIST lesions as compared to baseline.
Tumor responses were measured every eight weeks. The values shown are the percentage change in the sum of the longest diameters from baseline measurements of each measurable tumor. One patient (who remained on trial for 18 cycles by the time of data-lock) developed multiple new liver lesions on first scan, which were thought to have developed between scan and initiation of trial due to profound decrease in CEA. The patient continued to have a partial response but developed growth of a single liver lesion that was subsequently radiated. One patient had an increase of RECIST target lesions from 12mm to 24mm upon first reevaluation, however, the burden of disease was in a nontarget lesion (omental caking) and so decision was to proceed for two further cycles until disease progressed.

Figure 3 Mean percent change in LINE-1 methylation in circulating DNA at CpG1-3 as compared to baseline levels on C1D1.
LINE-1 methylation is measured at CpG1-3 in circulating tumor DNA on days 1, 8, and 15 of cycles 1 and 2, and on the first day of cycle 3 for twenty patient specimens. Average methylation changes as compared to baseline were calculated according to dose of guadecitabine (30mg/m2 or 45mg/m2, whereby dose levels -1 and -1G, and 1 and 1G have been combined). Standard deviation for 30mg/m2 was 3.7, and for 45mg/m2 was 4.6.

Supplemental Figure 1: LINE1 changes in circulating DNA CpG 1-3 as compared to baseline.
LINE1 changes in circulating DNA as compared to baseline plotted over time per patient.

Supplemental Figure 2: LINE1 changes in tumor CpG 1-3 on cycle 1 day 8 from baseline.

LINE1 changes in tumor DNA in individual patient biopsy samples.
### Table 1: Dosing levels of guadecitabine and irinotecan

<table>
<thead>
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<th>Dose Level</th>
<th>Guadecitabine (mg/m²) D1-5</th>
<th>Irinotecan (mg/m²) D8 and 15</th>
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<tr>
<td>-1G</td>
<td>30</td>
<td>125</td>
<td>Filgrastim 5mcg/kg/day C1D9-14 C1D16 pegfilgastrim 6mg C2 and beyond per clinical judgement</td>
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<tr>
<td>-1</td>
<td>30</td>
<td>125</td>
<td>Per clinician judgment, not allowed cycle 1</td>
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<tr>
<td>1 (starting dose)</td>
<td>45</td>
<td>125</td>
<td>Per clinician judgment, not allowed cycle 1</td>
</tr>
<tr>
<td>1G</td>
<td>45</td>
<td>125</td>
<td>Filgrastim 5mcg/kg/day C1D9-14 not allowed cycle 1</td>
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<tr>
<td>2</td>
<td>60</td>
<td>125</td>
<td>Per clinician judgment</td>
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Table 2: Patient Characteristics

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<td>Median age at enrollment (Range) – years</td>
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<td>Left side/rectal</td>
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<tr>
<td>Male</td>
<td>12 (55)</td>
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<td>Non-hispanic caucasian</td>
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<tr>
<td>African American</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Fever</td>
<td>1(17)</td>
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<tr>
<td><strong>Renal and Hepatobiliary</strong></td>
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<tr>
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<tr>
<td>Increased Alk phosphatase</td>
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</tr>
<tr>
<td>Increased AST</td>
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<tr>
<td><strong>Skin</strong></td>
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Table 3: Toxicities
*Note: one patient in DL - 1 G withdrew consent after receiving only two doses of guadecitabine, and was considered evaluable for toxicity (and thus included in the analysis for toxicity), but not evaluable for response.

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<th></th>
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<th>3(50)</th>
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</tbody>
</table>
Figure 1A

![Graph showing the concentration of guadecitabine over time for two different doses: 30 mg/m² and 45 mg/m². The y-axis represents concentration (ng/mL), and the x-axis represents time (h). Error bars are included for each data point. The graph indicates a decrease in concentration over time for both doses.]
Figure 1B

A line graph showing the concentration of Guadecitabine over time. The x-axis represents time in hours (0-8), and the y-axis represents Guadecitabine concentration in ng/mL. Two lines are shown:
- Guadecitabine 30 mg/m² (dashed line)
- Guadecitabine 45 mg/m² (solid line)

Error bars indicate variability in the concentration measurements.
**Figure 2**

**RECIST - Sum of longest diameter of target lesions**

- **% Change from Baseline**
- **Number of Cycles**

**Legend**:
- **New/Enlarged non-target lesion PD**
  - 45mg/m²
  - 30 mg/m²
- **Irinotecan Resistant**
- **Irinotecan Sensitive**
Figure 3

% Methylation change from Day 1

- 30 mg/m2
- 45 mg/m2
Clinical Cancer Research

A phase I trial of a guadecitabine (SGI-110) and irinotecan in metastatic colorectal cancer patients previously exposed to irinotecan

Valerie Lee, Judy S. Wang, Marianna L Zahurak, et al.

Clin Cancer Res Published OnlineFirst August 10, 2018.

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