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ORIGINAL ARTICLE

A Phase I and pharmacokinetic study of selenomethionine in combination with a fixed dose of irinotecan in solid tumors

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Abstract
Purpose We conducted a phase I study to determine the recommended dose of selenomethionine (SLM) in combination with irinotecan that consistently results in a protective plasma selenium (Se) concentrations > 15 μM after 1 week of SLM loading.
Experimental Design A 3-3 standard escalation design was followed. SLM was given orally twice daily (BID) for one week (loading) followed by continuous once daily (QD) dosing (maintenance). Seven dose levels of selenomethionine were investigated. Irinotecan was given intravenously at a fixed standard weekly dose, starting on the first day of maintenance SLM.
Results Thirty-one patients were treated on study. Dose limiting diarrhea complicated by sepsis was noted in one of six patients at each of the dose-levels 1 and 7. Dose-levels ≥ 5 (4,800 mcg/dose loading maintenance) resulted in day 8 Se concentrations >15 μM while dose-level 7 (7,200 mcg/dose loading and maintenance) resulted in day 8 Se concentrations > 20 μM. No significant variations in SN-38 or biliary index were noted between weeks 1 and 4 of treatment. Despite achieving target Se concentrations, gastrointestinal and bone marrow toxicities were common and irinotecan dose modification was prevalent. Objective responses were seen in two patients and nine patients had disease control for 6 months or longer.
Conclusions Selenomethionine can be escalated safely to 7,200 mcg BID × 1 week followed by 7,200 mcg QD in combination with a standard dose of irinotecan. No major protection against irinotecan toxicity was established; however, interesting clinical benefits were noted-supporting the investigation of this combination in future efficacy trials.

Keywords Selenomethionine · Irinotecan · Pharmacokinetics · Phase I · Colon cancer

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Introduction

Selenium is an essential trace element with a worldwide average nutritional intake of 50–350 μg/day. Dietary selenium deficiency has been associated with an increased risk of carcinogenesis and of increased mortality [1, 2]. Multiple other epidemiological studies have suggested that higher Se blood levels are protective against the development of various solid tumors [3–12]. Clark et al. [13] investigated the use of 200 μg/day of Se (as selenized yeast) as a chemoprevention agent for non-melanoma skin cancer.

Selenium supplementation resulted in a significant reduction in total cancer mortality and total cancer incidence including lung, colorectal, and prostate cancers [13, 14]. Furthermore, a retrospective analysis of baseline Se levels in patients with non-Hodgkin's lymphoma showed a positive correlation between plasma Se levels and chemotherapy dose-delivery and outcome [15]. In a multivariate analysis, Se was the most important factor affecting survival with a hazard ratio of 0.76 for every 0.2 μM increase in concentration [15].

Given the favorable epidemiological data and the decreased chemotherapy-related toxicity in patients with normal or elevated serum selenium concentrations, our group has investigated the utility of high dose selenium supplementation with chemotherapy in pre-clinical models. We have shown that the organic selenium compounds SLM and methylselenocysteine (MSC) decrease irinotecan-induced toxicity in nude mice while at the same time increasing irinotecan antitumor activity [16]. The optimal protective effects of SLM against chemotherapy toxicity were noted when SLM was started 1 week prior to chemotherapy [16]. Furthermore, this protection against normal tissue toxicity was found to be dose dependent for both MSC and SLM with the threshold protective Se concentration with SLM being 15 μM [17, 18].

Based on these encouraging preclinical data, we initially conducted a phase I clinical trial of a fixed dose of SML at 2,200 μg/day (μg of elemental Se) in combination with escalating doses of weekly irinotecan [19]. Contrary to our expectations, we were not able to escalate irinotecan beyond its established recommended dose of 125 mg/m^2/week [19]. However, interesting clinical responses were noted on this study, particularly in a previously irinotecan-resistant patient who achieved the highest plasma concentration of Se [19]. Pharmacokinetic analysis on our initial phase I study confirmed that plasma concentrations of Se after 1 week of 2,200 μg/day of SLM loading (on the first dose of irinotecan administration) were suboptimal (< 10 μM), suggesting that higher doses of SLM are needed to test adequately for normal tissue toxicity protection [19].

We have thus designed and conducted a sequel phase I clinical trial to determine the optimal dose of SLM that results in Se concentrations exceeding the 15 μM threshold for protection against toxicity. Based on our prior pharmacokinetic modeling and the estimated 1 month lag for steady state Se concentration with daily administration of SLM [19], we elected to investigate a 1 week twice daily (BID) SLM loading schedule followed by a lower once daily (QD) SLM maintenance dosing with the goal of achieving our target 15 μM Se concentration by the end of the loading phase. We maintained irinotecan dosing at a fixed recommended dose of 125 mg/m^2/week to be started on the eighth day of SLM, i.e. after completion of the loading SLM phase. Our two main objectives were to determine the minimum and the maximum safe dose of SLM that results is Se plasma concentrations exceeding 15 μM. Our rationale behind escalating SLM beyond doses achieving the target protective threshold were based on the selenium dose-dependent antitumor synergy detected between irinotecan and organic selenium compounds [17, 18].

Materials and methods

This phase I, open-label, dose-escalation study of SLM in combination with a fixed dose of irinotecan was conducted at Roswell Park Cancer Institute (Buffalo, NY). The primary objective of the study was to determine the lowest and highest safe doses of SLM among seven dose-levels that result in Se plasma concentrations exceeding 15 μM on days 8 and 29 of SLM when combined with a fixed dose of weekly intravenous irinotecan. Secondary objectives included the evaluation of pharmacokinetics of SLM and irinotecan, the description of treatment-related toxicities, and the description of any observed clinical responses.

Patient criteria

Patients with a histologically or cytologically confirmed solid tumor that was metastatic or unresectable and for which standard curative or palliative measures did not exist or for whom single agent irinotecan constituted a reasonable treatment option were eligible for the trial. The last chemotherapeutic or radiation treatment was at least 4 weeks (6 weeks for nitrosureas or mitomycin C) prior to trial enrollment. Other criteria included age ≥ 18 years of age, ECOG performance status ≤ 1, estimated life expectancy > 12 weeks, no central nervous system involvement, adequate bone marrow function (neutrophils ≥ 1,500/μL, hemoglobin ≥ 8.0 g/dull, platelets≥100,000/μL), adequate hepatic function [serum bilirubin ≤ upper limit of normal range (ULN), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 × ULN], and adequate renal function (creatinine ≤ 1.5 ULN or creatinine clearance ≥ 60 mL/min). The study excluded patients unable to receive oral medications, patients with brain metastases, patients with a history of Gilbert's syndrome, and patients with active inflammatory bowel disease or chronic diarrhea. HIV positive patients were not eligible because of possible pharmacokinetic interaction with anti-retroviral drugs. Patients with≥G2 neuropathy (NCI CTC 3.0) were excluded because of concerns about possible exacerbation of neurotoxicity with SLM. Patients with reproductive potential had to agree to use adequate contraception prior to study entry and for the duration of study participation. The study and consent form

were approved by the institutional scientific and review committee (SRC) and the institutional review board (IRB) prior to its activation. All patients provided signed informed consent before study entry. The study was conducted in accordance with the good clinical practice guidelines as issued by the international conference on harmonization and the declaration of Helsinki.

Study design and treatment plan

Dose escalation

Three patients were entered at each dose level. In the absence of dose limiting toxicity (DLT), the next dose level was explored. If DLT was seen in one patient, three further patients were added at that dose level and, if no additional DLT was seen, escalation to the next dose level occurred. If at least two patients had DLT at a given dose level, accrual to that dose level was stopped; this was the maximally administered dose. Further patients were then added, as required, to the previous dose level (and if necessary to lower dose levels) to establish the highest dose at which < 2/6 patients had DLT. This was the maximum tolerated dose (MTD). No dose escalation was allowed beyond dose level 7 as the number of pills to be administered per day beyond that level was thought to be limiting. In the event that escalation to dose level 7 was feasible and no DLT were noted in the first 3 patients at that dose level, dose level 7 was to be expanded to 6 patients to determine if this was the MTD among the 7 levels investigated. No intra-patient escalation was allowed.

Treatment schedule

Selenomethionine was given orally (PO) twice daily (loading phase) starting 1 week prior to the first dose of irinotecan and subsequently once daily (maintenance phase). SLM was administered in the form of 800 or 400 mcg capsules (Sabinsa Inc.). Seven dose levels of SLM were to be investigated (Table 1). Irinotecan was administered intravenously (i.v.) at a fixed dose of 125 mg/m^2 in 500 cc of normal saline (NS) over 90 minutes once weekly × 4 every 6 weeks (one cycle) [20]. Patients were medicated with dexamethasone 10 mg (i.v.) and palonosetron 0.25 mg (i.v.) prior to irinotecan.

Dose limiting toxicities

A dose limiting toxicity was any of the following attributable to study treatment on cycle 1: any non-hematological grade (G) 3 or 4 toxicity, with the exception of G3 diarrhea lasting less than 48 h; any G4 thrombocytopenia or any G3 thrombocytopenia lasting more than 6 days; any G4 neutropenia lasting more than 6 days or any G4 neutropenia associated with fever; any dose-delay secondary to toxicity that lasts 2 or more weeks or results in giving less than 3 of the 4 scheduled weekly irinotecan treatments on the first cycle. G3 hypomagnesemia, G3 hypophosphatemia, G3 hypokalemia, and sodium levels of 128–130 mmol/l were not considered DLT unless they were persistent for more than 48 h despite medical intervention or in case they resulted in hospitalization.

Table 1 Dose levels of selenomethionine

Phase I escalation schema			
Dose level	SLM loading (D-7–D-1)[a] (mcg PO BID)	SLM maintenance (D1 and on) (mcg PO QD)	Irinotecan (mg/m^2) Q week (start on D1)
1	3,200	2,800	125
2	3,200	3,200	125
3	4,000	3,200	125
4	4,000	4,000	125
5	4,800	4,800	125
6	5,600	5,600	125
7	7,200	7,200	125

[a] Day 1 follows day-1 (no day 0)

Dose modifications

Dose modifications for irinotecan were required for G2 and higher toxicities (Table 2). Treatment was interrupted for any G3 or higher toxicity; missed treatments were not made up. A cycle was not to be started unless the absolute neutrophil count (ANC) recovered to ≥ 1,500/ml and the platelets to ≥ 75,000/ml and non-hematological treatment related toxicities improved to ≤ G1. Patients were instructed to take loperamide 4 mg PO at the onset of diarrhea and 2 mg every 2 h until diarrhea resolved. No growth factors were allowed on the study with the exception of recombinant erythropoietin.

No dose modification was allowed for SLM; however, the total daily dose of SLM could have been divided into 2–3 doses/day in case of dyspepsia.

Clinical evaluation and follow-up

A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count (CBC), and comprehensive chemistry profile (electrolytes, BUN, creatinine, magnesium, lactate dehydrogenase, ALT, AST, bilirubin) were obtained within a week prior to treatment initiation. Baseline CT scans were obtained within 4 weeks prior to initiation of treatment. CBC and comprehensive chemistry were repeated on a

Table 2 Irinotecan dose modifications for hematological and non-hematological toxicities

	During same cycle	During next cycle
No Toxicity	Maintain dose level	Maintain dose level
Grade 1	Maintain dose level	Maintain dose level
Grade 2[a]	Reduce by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above	Maintain dose level for dose level 1. Reduce to prior dose level for dose level 2 and above
Grade 3	Omit until Grade 2 or less and then decrease by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above	Decrease by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above
Grade 4	Omit until Grade 2 or less and then decrease by 50 mg/m^2 for dose level 1. Decrease dose by 40 % for dose level 2 or above	Decrease by 50 mg/m^2 for dose level 1. Decrease dose by 40% for dose level 2 or above

No dose-modification indicated for correctable electrolyte disturbances, hyperglycemia, vomiting that has not been treated with the maximum antiemetic therapy, alopecia, or toxicities that are not related to study drugs (irinotecan and SLM) but do not interfere with drug metabolism or clearance (example grade 3 pain secondary to bony metastases)

[a] Grade 2 hematological toxicities did not require dose modification on the next cycle

weekly basis on the first cycle (including the two-week break) and prior to planned irinotecan treatments on subsequent cycles. Medical history, physical examination, and toxicity assessment as per NCI CTC 3.0 were performed weekly on the first cycle and on weeks 1 and 3 of subsequent cycles. CT scans were repeated every 2 cycles (12 weeks) to assess response. Responses were categorized according to the RECIST criteria [21].

SLM treatment compliance was evaluated via a combination of a study specific patient diary and a monthly pill count.

Pharmacokinetics: sample collection, preparation, and analysis

Sample collection

Trough samples of blood for Se levels were collected in trace element free heparinized tubes on days 1, 2, 8, and 28 prior to the administration of SLM. Multiple samples of blood for phamacokinetic determinations of CPT-11, SN-38 and SN-38G were collected in separate heparinized tubes on week 1 of irinotecan administration and again on week 4 to evaluate potential effects of Se on CPT-11 pharmacokinetics and metabolism.

Selenium measurements

Selenium in plasma was measured by graphite furnace atomic absorption spectrophotometry using PE ZL4100 or PE analyst as has been described previously [19]. Quality assurance was maintained by running quality control samples assayed every time the patient samples are run as described earlier [19].

Measurement of irinotecan (CPT-11), SN-38 and SN-38G

CPT-11, SN-38 and SN-38G were measured using reverse phase HPLC with fluorescence detection as described on our prior phase I clinical trial with identical sample preparation and HPLC conditions [19].

Pharmacokinetic data analysis

PK analysis of the concentration-time data for CPT-11, SN-38 and SN-38G was carried out using non-compartmental methods using WinNonlin version 5.0 (Pharsight Corporation, Lexington, KY). Specific concepts behind the calculations of some of the derived parameters have been described before [19]. Summary statistics and comparisons wee made using SAS statistical software (PROC Mixed, SAS version 8.02, Cary, NC).

Results

Demographics

Between October 2004 and June 2006, 31 patients (27 evaluable) were entered on study. Four patients were not evaluable for treatment induced toxicity and are detailed below. One patient withdrew from study on her third week of treatment because of symptoms of progressive disease. One patient with a large ventral hernia developed a bowel incarceration early in her treatment that was deemed unrelated to study drugs and was taken off study. One patient was taken off study because of non-compliance with SLM treatment in the loading part of the study. The last non-evaluable patient was taken off study on day 1 of irinotecan

Table 3 Patient characteristics

Patient characteristics (n = 27 evaluable)	
Gender (male/female)	20/7
Age (median/range)	57/21–74 years
ECOG (0/1)	16/11
Primary tumor	
Colorectal	22
Small lung cancer	2
Non-small cell lung cancer	1
Sarcoma	1
Urachal	1
Prior chemotherapy	27
Prior irinotecan chemotherapy	12
Prior radiation therapy	10

as she was found to have pre-existing hallucinations that were related to narcotic treatment. These four patients were excluded from the toxicity analysis and efficacy analysis, as they were withdrawn from study prior to completing the first cycle of treatment. None of these four patients had evidence of treatment related toxicity at the time of their withdrawal from study. The characteristics of the 27 evaluable patients are listed in Table 3.

Treatment administration

Thirty-one patients received treatment on study, of whom, 27 are evaluable. All seven-dose levels of SLM were investigated. The median number of cycles administered was 2 (range 1–8), with a total of 90 cycles administered on study. Six patients received 6 or more cycles. All patients received all intended SLM treatment without any scheduling modification. However, dose modification of irinotecan was common. Nineteen patients required irinotecan dose interruption or reduction during the first cycle due to treatment toxicity-as mandated per study protocol.

Toxicity

Twenty-seven patients were evaluated for treatment-related toxicity. Only ≥ G2 toxicity data attributed to study treatment are reported. Treatment-related G2–G4 toxicities are summarized in Tables 4 and 5.

Hematological toxicity

Neutropenia was the predominant hematological toxicity. Cycle 1 G3 neutropenia was noted in one patient at dose level 1, one patient at dose level 3, and 3 patients at dose level 7. No G2 or above thrombocytopenia was noted on treatment. Hematological toxicities are detailed in Table 4.

Non-hematological toxicity

The most common ≥ G2 non-hematological adverse event was diarrhea. Six patients experienced G2 diarrhea and 5 experienced G3 diarrhea on cycle 1. G3 diarrhea occurred in one patient at DL1, 2 patients at DL5, and 2 patients at DL7. Grade 3 diarrhea lasted > 24 h in only 2 patients (DLT defining), one on DL1 and the other on DL7. Other common non-hematological toxicities included nausea and vomiting, fatigue, and abdominal cramps. Non-hematological toxicities for cycle 1 and for all cycles are detailed in Table 5.

Selenomethionine toxicity

Selenomethionine was well tolerated in all patients. The only toxicity attributed to SLM was mild garlic-like odor (breath and urine) and was limited to G1 in about 50% of the patients. This was seen more commonly during the induction SLM week and tended to ameliorate or disappear with prolonged treatment. No skin or nail toxicities secondary to SLM were documented.

Dose limiting toxicities, maximum tolerated dose, and recommended dose

Two patients experienced a dose limiting toxicity as defined by the study protocol. One patient with extensive peritoneal carcinomatosis experienced G3 abdominal pain, G3 nausea and vomiting, G3 diarrhea, G3 neutropenia, and G3 infection on his 3 week of treatment on cycle 1. Although his symptoms were partly attributed to disease progression

Table 4 Hematological toxicities (≥ grade 2)

Toxicity	DL1 (6pts) (G2/G3/G4)	DL2 (3pts) (G2/G3/G4)	DL3 (3pts) (G2/G3/G4)	DL4 (G2/G3/G4)	DL5 (G2/G3/G4)	DL6 (G2/G3/G4)	DL7 (G2/G3/G4)
Neutropenia cycle 1	1/1/0	2/0/0	0/1/0	0/0/0	0/0/0	0/0/0	1/3/0
Neutropenia all cycles	1/1/0	1/1/0	0/1/0	0/0/0	0/0/0	0/0/0	1/3/0
Anemia cycle 1	1/0/0	0/0/0	1/0/0	1/0/0	0/0/0	0/0/0	0/0/0
Anemia all cycles	1/0/0	0/0/0	1/0/0	1/0/0	0/0/0	0/0/0	0/0/0

Table 5 Non-hematological toxicities

Toxicity	DL1[a] (G2/G3/G4) $n = 6$	DL2 (G2/G3/G4) $n = 3$	DL3 (G2/G3/G4) $n = 3$	DL4 (G2/G3/G4) $n = 3$	DL5[b] (G2/G3/G4) $n = 3$	DL6 (G2/G3/G4) $n = 3$	DL7[bc] (G2/G3/G4) $n = 6$
Diarrhea cycle 1	2/1/0	1/0/0	1/0/0	1/0/0	0/2/0	1/0/0	0/2/0
Diarrhea all cycles	4/1/0	2/0/0	2/0/0	1/0/0	0/2/0	1/0/0	0/3/0
N/V Cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	2/0/0	3/0/0
N/V all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	2/0/0	3/1/0
Fatigue cycle 1	0/1/0	1/0/0	1/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Fatigue all cycles	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	3/0/0
Anorexia cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	3/0/0
Anorexia all cycles	0/1/0	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	4/0/0
Abdominal cramps cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Abdominal cramps all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Constipation cycle 1	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	0/0/0	2/0/0
Constipation all cycles	0/0/0	1/0/0	1/0/0	0/0/0	1/0/0	0/0/0	2/0/0
Infection cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Infection all cycles	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Hyponatremia cycle 1	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Hyponatremia all cycles	0/0/0	0/0/0	0/1/0	0/0/0	0/0/0	0/0/0	0/1/0
Dyspepsia cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	0/0/0
Dyspepsia all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	0/0/0
Hypotension cycle 1	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	0/0/0	0/0/0
Hypotension all cycles	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	0/0/0	0/0/0

[a] One patient on dose level 1 experienced a dose limiting toxicity consisting of diarrhea, nausea and vomiting, fatigue, sepsis (complicating grade 3 neutropenia), dyspepsia, abdominal cramps/pain, and anorexia. All G3 toxicities on dose level 1 involved the same patient

[b] Two G3 diarrhea on dose level 5 and one G3 diarrhea on dose level 7 lasted less than 24 h and thus did not constitute a DLT

[c] One patient of dose level 6 experienced a dose limiting toxicity consisting of diarrhea, sepsis (complicating G3 neutropenia), and hyponatremia

with partial small bowel obstruction, treatment related toxicity could not be ruled out as a contributing factor. DL1 was expanded to 6 patients without any further DLT. Further escalation of SLM did not result in any further DLT until DL7. At DL7, none of the first 3 patients experienced a DLT. Since DL7 was the highest dose level to be investigated, this cohort was expanded to 6 patients to determine if it fits the maximum tolerated dose (MTD) definition. The sixth patient on DL7 experienced a DLT. This patient, similar to the patient with DLT on DL1, suffered from extensive peritoneal carcinomatosis. On her second week of treatment, she experienced symptoms of partial small bowel obstruction with G2 N/V and DLT defining G3 infection, neutropenia, diarrhea, and hyponatremia.

The non-tolerable dose of SLM was not defined on this study. The MTD of SLM among the seven dose levels investigated was defined as DL7, consisting of SLM at 7,200 mcg PO BID × 1 week followed by 7,200 mcg PO QD. Given the tolerability of DL7 and the achievement of the target Se concentrations at that dose level (see PK section), DL7 was also declared the recommended dose for future studies.

Antitumor activity

Twenty-five patients were evaluable for radiographic response. The two patients with DLT did not have confirmed radiographic progression but suffered from symptoms of peritoneal carcinomatosis progression with the development of partial small bowel obstruction. Two patients had a partial response (PR). Both patients had a diagnosis of colorectal cancer and both had previously progressed on 5-FU, leucovorin, oxaliplatin, and bevacizumab but had no prior irinotecan exposure. The responses lasted for 7 and 10 months from initiation of study treatment. Twelve patients had stable disease (SD), 6 of which were confirmed with subsequent CT scans as per RECIST criteria. The 6 confirmed SD lasted between 7 and 12 months. Five of the 6 patients had a diagnosis of metastatic colorectal cancer and one patient had non-small cell lung cancer. Four patients with confirmed SD had prior irinotecan exposure including one case with prior irinotecan refractory disease.

Pharmacokinetics

Day 8 Se levels are available for 31 patients and Day 28 levels for 21 patients.

Pharmacokinetic data for CPT-11, SN-38 and SN-38G are available for 26 patients on Week 1 of irinotecan administration (7 days after the loading dose administration of SLM) and 11 patients on week 4 of irinotecan administration (28 days after the maintenance dose of SLM).

Selenium levels in plasma

Levels of Se in plasma of patients after a loading dose of SLM for 7 days and after 3 weeks of maintenance dosing are presented by dose level of SLM administered in Table 6. As evident from the data, while there is a significant inter-patient variability in selenium levels achieved by day 8, all patients had Se levels greater than 15 μM by dose level 5 (4,800 μg BID). All patients at dose level 7 had greater than 20 μM Se concentrations in plasma by day 8. The mean ± SD of Se levels at dose levels 5 ($n = 3$), 6 ($n = 4$) and 7 ($n = 6$) on day 8 were 20.8 ± 3.0, 16.94 ± 2.5 and 30.7 ± 10.6 μM, respectively. There was no significant further accumulation of Se on the maintenance dose administered for further 3 weeks until dose level 7, as evident from the Se levels in plasma measured on day 28. At dose level 7, for the 4 patients where data were available on day 8 and day 28, the mean ± SD of Se in plasma on day 8 and 28 were 26.7 ± 7.6 and 34.09 ± 9.0 μM respectively.

Serum Se levels were measured every 6 weeks in patients who remained on study for more than one cycle. Selenium levels reached a plateau in these patients at levels similar or slightly higher than the day 28 levels.

Irinotecan pharmacokinetics

With a goal to determine whether chronic administration of Se affects the PK and metabolism of irinotecan, the pharmacokinetics of CPT-11 and its major metabolites SN-38 and SN-38G have been evaluated after the first dose of irinotecan (week 1), and again after the fourth dose (week 4) (Table 7). It is apparent from the data that all the derived PK parameters for CPT-11 and the metabolites are unchanged between week 1 and week 4 when the patients are on the daily dose of SLM for 3 weeks. A trend towards decline in SN-38 AUC is suggested from the data and calculated biliary index, but is not statistically significant ($p = 0.31$). While the biliary index is a measure of conversion of SN-38–SN-38G relative to its synthesis from CPT-11, the data do not suggest any increase in SN-38G AUC.

Day 8 plasma selenium concentration and toxicity

The protective effects of plasma Se on irinotecan induced gastrointestinal and bone marrow toxicity in the evaluable population was explored by stratifying patients into 3 groups according to their Day 8 (day of first irinotecan dose) Se concentration. Since diarrhea and neutropenia are the most prevalent irinotecan related toxicities, only these two toxicities were captured for this exploratory analysis. Eight patients had a suboptimal Se concentration of < 15 μM on Day 8 of SLM; 2/8 had G3 and 5/8 had ≥ G2 diarrhea or neutropenia. Eleven patients had Day

Table 6 Se levels on day 8 and day 29 in patience receiving selenomethionine

Dose level (loading mcg BID/ maintenance mcg QD)	Pt no	Day 8 selenium level		Day 28 selenium level	
		ng/ml	μM	ng/ml	μM
DL1 (3,200/2,800)	1	785	9.94	866	10.96
DL1 (3,200/2,800)	2	1,647	20.85	1,598	20.23
DL1 (3,200/2,800)	3	1,199	15.18	No sample[a]	–
DL1 (3,200/2,800)	4	1,210	15.32	No sample[a]	–
DL1 (3,200/2,800)	5	900	11.39	1,237	15.66
DL1 (3,200/2,800)	6	1,225	15.51	1,216	15.39
DL2 (3,200/3,200)	7	633	8.01	721	9.13
DL2 (3,200/3,200)	8	1,110	14.05	No sample[a]	–
DL2 (3,200/3,200)	9	1,041	13.18	1,227	15.53
DL2 (3,200/3,200)	10	1,247	15.78	1,195	15.13
DL3 (4,000/3,200)	11	1,028	13.01	No sample[a]	–
DL3 (4,000/3,200)	12	1,298	16.43	No sample[a]	–
DL3 (4,000/3,200)	13	1,250	15.82	1,011	12.80
DL3 (4,000/3,200)	15	1,525	19.30	No sample[a]	–
DL4 (4,000/4,000)	17	1,563	19.78	No sample[a]	–
DL4 (4,000/4,000)	18	919	11.63	1,006	12.73
DL4 (4,000/4,000)	19	1,481	18.75	1,395	17.66
DL5 (4,800/4,800)	20	1,430	18.10	1,413	17.89
DL5 (4,800/4,800)	21	1,608	20.35	1,297	16.42
DL5 (4,800/4,800)	22	1,899	24.04	2,276	28.81
DL6 (5,600/5,600)	23	1,355	17.15	1,481	18.75
DL6 (5,600/5,600)	24	1,605	20.32	1,894	23.97
DL6 (5,600/5,600)	25	1,937	24.52	No sample[a]	–
DL6 (5,600/5,600)	26	1,245	15.76	1,528	19.34
DL7 (7,200/7,200)	27	NC[b]	–	1,662	21.04
DL7 (7,200/7,200)	28	2,089	26.44	2,608	33.01
DL7 (7,200/7,200)	29	2,280	28.86	No sample[a]	–
DL7 (7,200/7,200)	30	2,350	29.75	3,173	40.16
DL7 (7,200/7,200)	31	1,294	16.38	1,719	21.76
DL7 (7,200/7,200)	32	2,700	34.18	3,272	41.42
DL7 (7,200/7,200)	33	3,838	48.58	No sample[a]	–

[a] Day 29 sample was not collected

[b] Non-compliant

8 plasma Se concentrations ranging ≥ 15 and < 20 μM; 3/11 had G3 and 7/11 had ≥ G2 diarrhea or neutropenia. Eight patients had Day 8 plasma Se concentrations ≥ 20 μM; 4/8 had G3 and 5/8 had ≥ G2 diarrhea or neutropenia. Although no formal statistical analysis was performed, higher Se concentrations prior to initiation of irinotecan did not seem predictive of protection against diarrhea or neutropenia.

Discussion

We had previously demonstrated that the administration of daily SLM or MSC, starting one week prior to initiation of weekly irinotecan therapy, reduces irinotecan-induced toxicity and improves antitumor activity in preclinical models [16, 17]. In a previous phase I study we tested the ability of SLM to attenuate irinotecan toxicity by assessing the feasibility of irinotecan escalation beyond the previously recommended MTD of 125 mg/m^2 when combined with a fixed dose of SLM of 2,200 mcg/day in patients with advanced solid tumors [19]. Escalation of irinotecan was not possible on that study secondary to DLT consisting of prolonged G3 diarrhea [19]. However, Se concentrations on the day of initiation of irinotecan were suboptimal in all patients (< 10 μM), significantly less than the optimal concentration of 15 μM and higher [19]. Other findings included interesting clinical benefits in a variety of solid tumors and a reduction in biliary index when comparing week 4–week 1 of irinotecan pharmacokinetics.

We thus conducted this sequel phase I trial to determine the dose of SLM that results in Se concentrations that

Table 7 Summary of PK parameters for CPT-11, SN-38, and SN-38G

	C_{max} (ng/ml/mg/m^2)	Half-life (h)	V_D (L/m^2)	CL (L/h/m^2)	AUC_{inf} (ng h/ml)	Biliary Index[a]
CPT-11						
Week 1	15.8 (28)	8.6 (22)	91 (37)	11.9 (31)	11,582 (33)	5,450 (122)
Week 4	14.6 (25)	8.3 (13)	86 (21)	13.1 (29)	10,398 (32)	3,123 (47)
SN-38						
Week 1	0.9 (37)	19.5 (64)	2,422 (53)	132.8 (61)	1,256 (60)	–
Week 4	0.9 (64)	16.8 (40)	2,850 (58)	157.6 (57)	1,059 (61)	–
SN-38G						
Week 1	1.8 (40)	14.7 (44)	798 (58)	37.2 (58)	3,483 (49)	–
Week 4	2.1 (34)	14.2 (23)	748 (54)	42.6 (46)	3,446 (38)	–

All values are mean (CV%)

Week 1, $N = 26$; Week 4, $N = 11$

[a] Biliary index = $(AUC_{CPT\text{-}11}\ AUC_{SN\text{-}38})/AUC_{SN\text{-}38G}$

$P = 0.31$ for biliary index between week 1 and 4 (paired t-test)

exceed the threshold of 15 μM and to further evaluate the effects of prolonged SLM administration on irinotecan pharmacokinetics. Simulation analysis of the data from our initial study formed the basis for the design of the current escalation scheme for SLM. It is evident from the data presented that a BID dose of SLM for one week can produce a level of 15 μM concentration in plasma. Much higher levels in the order of ~25 μM are produced at the BID dose of 7,200 mcg, although an occasional individual still shows poor absorption of Se. The accumulation of Se appears to be limited as evident from the day 28 levels except at the highest dose of 7,200 μg SLM.

We evaluated the changes in irinotecan pharmacokinetics by comparing week 4 to week 1 of cycle 1. As evident from the data, no significant changes in PK parameters for either CPT-11 or its metabolites are found from week 1 to week 4. A slight decline in SN-38 AUC is suggested from the data and calculated biliary index, but is not statistically significant ($p = 0.31$). While the biliary index is a measure of conversion of SN-38 to SN-38G relative to its synthesis from CPT-11, the data do not suggest any changes in SN-38G AUC. Since the calculation of biliary index is also tied to the AUC of CPT-11, it is conceivable that changes in biliary index could encompass other metabolic reactions involving CPT-11 itself. We still cannot rule out an effect of SLM on irinotecan PKs based on the current data as both week 1 and 4 PK parameters were obtained in the setting of SLM treatment. It is possible that the difference in biliary index noted in the predecessor study (only 6 patients evaluated) was related to Se interaction with irinotecan that became evident due to the differences in Se concentration between week 1 and 4 [19]. On this current study, there has been no significant difference between Day 8 (week 1 irinotecan) and Day 29 (week 4 irinotecan) Se concentrations and thus, possibly, the lack of evident interaction. The sole way to conclusively study SLM/irinotecan interaction would be to administer irinotecan alone followed by SLM plus irinotecan for a definitive PK interaction study. This was not feasible in our phase I SLM escalation design.

Despite the fact that our study was not designed to investigate the protective effects of SLM on irinotecan-induced normal tissue toxicity, the frequent attenuation of irinotecan dosing on cycle 1 secondary to toxicity suggests lack of major protection. In fact, 19 out of 27 patients required dose reduction in irinotecan in the first cycle of treatment. To explore the possibility that higher Se concentrations may be more protective, we stratified our patient population according to their Se level on the day of their first irinotecan dose. Surprisingly, the rate of G2 and above toxicity did not seem to decrease with higher selenium concentrations. We continued to see significant irinotecan-induced toxicities even at the highest SLM cohort where Se concentrations of 30 μM were seen. This suggests that if SLM has any protective effects against irinotecan toxicity, those protective effects would be minimal. This is in contrast with the pre-clinical data generated by our group where SLM clearly allows the doubling of the maximum tolerated dose of irinotecan in nude mice. The discrepancy between our clinical and pre-clinical findings is poorly understood. It is likely that SLM may have different mechanisms of activity in mice in comparison to humans. Furthermore, despite the correlation between Se concentration and protective effects of SLM in mice, it is unclear that this endpoint is a valid surrogate endpoint of SLM activity in humans. Methylselenol has been previously established as the active metabolite of SLM; however, plasma Se concentrations do not reflect the concentrations of this active metabolite in patients [22]. Due to the instability and volatility of methylselenol, no validated clinical assay has been formulated yet to test this metabolite.

We have seen a large number of disease stabilizations and two partial responses on this study. Some of the stabilizations were prolonged and occurred in patients with previously documented irinotecan-refractory disease. Although these should be considered anecdotal, these findings would not been inconsistent with the synergy described between irinotecan and organic Se [17].

Future studies should determine which tumors are most likely to benefit for the addition of high dose of SLM to the treatment regimen. Selenomethionine has been shown to significantly alter the expression of 50 genes in the colorectal cancer cell line HCT116 [23]. Others have also shown that p53 status is predictive of the antitumor activity of Se compounds in vitro [24, 25]. Tumor molecular profiling before and after SLM treatment in future therapeutic trials may shed some insight on its mechanisms of activity and biological targets.

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