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A Phase 1 Study of 7-t-butyldimethylsilyl-10-hydroxycamptothecin (AR-67) in Adult Patients with Refractory or Metastatic Solid Malignancies

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Abstract

Purpose—AR-67 is a novel third generation camptothecin selected for development based on the blood stability of its pharmacologically active lactone form and high potency in preclinical models. Here we report the initial phase I experience with intravenous AR-67 in adults with refractory solid tumors.

Experimental Design and Methods—AR-67 was infused over 1 hour daily $\times$ 5, every 21-days, using an accelerated titration trial design. Plasma was collected on the 1st and 4th day of cycle 1 to determine pharmacokinetic parameters.

Results—Twenty six patients were treated at 9 dosage levels (1.2–12.4mg/m²/day). Dose limiting toxicities (DLTs) were observed in 5 patients and consisted of grade 4 febrile neutropenia, grade 3 fatigue, and grade 4 thrombocytopenia. Common toxicities included: leukopenia (23%), thrombocytopenia (15.4%), fatigue (15.4%), neutropenia (11.5%), and anemia (11.5%). No diarrhea was observed. The maximum tolerated dosage (MTD) was 7.5 mg/m²/day. The lactone form was the predominant species in plasma (>87% of AUC) at all dosages. No drug accumulation was observed on day 4. Clearance was constant with increasing dosage and hematologic toxicities correlated with exposure ($p < 0.001$). A prolonged partial response was observed in one subject with non-small cell lung cancer (NSCLC). Stable disease was noted in patients with small cell lung cancer (SCLC), NSCLC, and duodenal cancer.

Conclusions—AR-67 is a novel, blood stable camptothecin with a predictable toxicity profile and linear pharmacokinetics. The recommended phase II dosage is 7.5mg/m²/day $\times$ 5 q 21 days.

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Translational Relevance

Topoisomerase I interacting agents have demonstrated antineoplastic activity that is closely associated with the stability of their α -hydroxy- δ -lactone pharmacophore. AR-67 is a novel 3rd generation camptothecin analog with increased lipophilicity and blood stability, relative to the clinically approved analogs topotecan and irinotecan. This phase I study was conducted to determine the maximum tolerated dose, dose limiting toxicities and pharmacokinetics of AR-67 given daily for 5 days during a 21 day cycle to patients with advanced solid tumors. Four patients experienced stable disease and one patient had prolonged partial response (>16 months) indicating preliminary evidence of antitumor activity. Hematologic toxicities were manageable and notably none of the patients experienced diarrhea. AR-67 exhibited increased blood stability and as compared to other lipophilic investigational analogs it did not appear to accumulate in plasma with repeat dosing. AR-67 warrants further evaluation to assess its efficacy alone or in combination with other agents.

Keywords

Camptothecin analog; cytotoxic therapy; phase I trial; pharmacokinetics; pharmacodynamics

INTRODUCTION

Camptothecins are potent DNA/topoisomerase-I interacting agents and combine the merits of both cytotoxic and molecularly targeted agents(1–3). Their potency is controlled by their α -hydroxy- δ -lactone pharmacophore, which hydrolyses to the open ring or carboxylate form in a pH dependent, but reversible manner. Although both the lactone and carboxylate forms have been shown to interact with the DNA/Topoisomerase-I complex, the lactone moiety is considered the active one (1,3,4). This may be attributed to the higher lipophilicity of the lactone form that could facilitate cell penetration as compared to the charged carboxylate (5). In addition to the intrinsic hydrolysis rate of each analog, the carboxylate binds human serum albumin with high affinity and facilitates an equilibrium shift toward hydrolysis, thus further compromising the lactone form by creating sink conditions (6–9).

Given that camptothecin pharmacokinetics (PK) present a significant challenge to achieving lactone exposure, there has been a tremendous effort to create novel analogs with improved stability and potency (10). One such effort was initiated by the research groups of Drs. Thomas Burke and Dennis Curran that successfully achieved lactone stabilization by introducing substituents that promote lipid bilayer partitioning (hence protecting the drug from hydrolysis) and minimized albumin binding (7,9,11). Several silatecans and homosilatecans were synthesized and tested for their blood stability and potency (12–15). Among those, AR-67 (7-*t*-butyldimethylsilyl-10-hydroxycamptothecin), also known as DB-67, emerged as the most blood stable and potent analog and was chosen for further preclinical and clinical development (12,15,16).

We initiated this phase I trial to determine the maximum tolerated dosage (MTD), and describe the dose limiting toxicities (DLT) of intravenous AR-67 administered once daily for 5 days of an every 21 day schedule to adults with refractory and metastatic solid tumors. In addition, we evaluated antitumor activity, PK and explored correlations between AR-67 exposure and toxicity.

EXPERIMENTAL DESIGN AND METHODS

Patient Eligibility

Patients (>18 years) with refractory solid malignancies were eligible if their disease was metastatic or unresectable and standard curative or palliative measures no longer existed or were no longer effective. Other eligibility requirements included Eastern Cooperative Group (ECOG) performance status ≤ 2 , adequate hematologic (leukocytes $>3,000/\mu\text{L}$, absolute neutrophil count (ANC) $>1,500/\mu\text{L}$, platelet count $>100,000/\mu\text{L}$), hepatic, and renal function. Objective measurable disease was not required. No prior chemotherapy, molecularly targeted agents or radiation therapy was allowed within 2 weeks (6 weeks for mitomycin C or nitrosoureas), and no major surgery was allowed within 3 weeks; all therapy-related toxicity should have resolved to less than grade 1. Prior camptothecin therapy was allowed, but patients with allergic reactions attributed to compounds of similar chemical or biologic composition to AR-67 or subjects with prior grade 3 or 4 anaphylactic reaction to any product formulated with cremophor (i.e., paclitaxel) were excluded. Patients with known brain metastases that had been treated and were clinically stable were eligible for this clinical trial. Other exclusions included subjects with: uncontrolled intercurrent illness that would limit study compliance, HIV disease,

QTc prolongation over 450 msec, evidence of ongoing and significant consumptive coagulopathy, or pregnant or nursing females.

Study Drug

AR-67 was supplied by the NCI (RAID program) in vials that contained 10mg/2mL in cremophor ethanol diluent. After reconstitution with 5% dextrose for injection in non-PVC bags (0.05 – 0.5 mg/mL), the drug was administered at a constant rate over one hour into a free flowing intravenous (iv) line (non-PVC tubing) of 5% dextrose via a standard infusion pump. The drug was given within 4 hours of reconstitution to ensure lactone stability.

Study Treatments and Dose Escalation

Since it is established that 25 – 30% of those receiving cremophor-formulated compounds will experience grade 3 or 4 allergic infusion reactions(17), all patients were premedicated to prevent potential cremophor toxicities as follows: dexamethasone 20 mg orally (PO), 12 hr prior to the first dose of AR-67 and 30 min prior to each dose on days 1–5, ondansetron 8 mg PO, loratidine 10 mg PO, famotidine 20 mg PO, and diphenhydramine 25 mg PO. Institutional equivalents were allowed. Investigators could reduce the dose of dexamethasone if no allergic reactions were noted in the previous cycle.

The phase-I starting dose was 1.67 mg/m²/day, iv, daily × 5. The first patient (prostate cancer) at dose level 1 developed grade 4 thrombocytopenia and consumptive coagulopathy, and his dose was reduced to level –1 (1.2 mg/m²) for safety. Ultimately, toxicities were attributed to a disease progression with extensive bone metastasis and myelophthistic marrow changes and consumptive coagulopathy. This phase I trial was designed to have an accelerated dose escalation followed by a modified Fibonacci design when certain parameters were met. Accordingly, after two subjects developed grade 2 toxicity early in the study, the study reverted to a modified Fibonacci design, as required by protocol. After 8 more subjects were accrued and demonstrated no grade 2 or greater toxicities, a secondary accelerated phase design was proposed and approved by the IRB in order to expose the minimal number of subjects to potentially sub-therapeutic dosage levels. Two dosage level escalations occurred during this secondary accelerated phase (at 4.5 mg/m² and 6.3 mg/m²), with subsequent expansion to a modified Fibonacci design at dosage level 5.5 (7.5 mg/m²). All dosage levels are described in Table 1.

Assessments, Follow-Up, and Monitoring

Toxicities were defined using the National Cancer Institute Common Toxicity Criteria 3.0 (CTCAE 3.0). The toxicity of a given AR-67 dosage level was considered a dose limiting toxicity (DLT) if any of the following were observed during cycle 1: (1) any Grade 5 toxicity, (2) ANC < 500/μL for longer than seven days or associated with fever or infection of any duration, (3) platelet < 25,000/μL of any duration, (4) grade 3 or 4 non-hematopoietic toxicity according to the CTCAE version 3.0 with the exception of grade 3 nausea and/or vomiting, grade 3 diarrhea of less than 3 days after treatment with loperamide or grade 3 fever (with or without neutropenia). The recommended phase II dose and the MTD were defined as the dosage level below the dosage that induced a DLT in two or more patients during cycle 1. Routine use of colony stimulating factors were not permitted during cycle one. Treatment was resumed once the ANC had recovered to ≥ 1,500/μL and a platelet > 100,000/μL (measured within 1 day of treatment) and resolution of all non-hematologic toxicity to less than grade 2. If these parameters were not met, therapy was delayed for up to 3 weeks for recovery. AR-67 dosage reductions by one dosage level were made for any DLT, any grade 4 neutropenia or thrombocytopenia or grade 3 or 4 non-hematologic toxicity in the previous cycle. A maximum of two dose reductions were allowed per subject and no dosage escalations were allowed. In the absence of treatment delays due to adverse events, treatment continued until one of the

following occurred: disease progression, unacceptable adverse event(s), request of patient to withdraw from study, general or specific changes in the patient's condition that rendered further treatment unacceptable or treatment delay of greater than 3 weeks. Tumor measurements were performed by computer tomography (CT) scan or magnetic resonance imaging (MRI) every two cycles of therapy. Disease was assessed according to the response evaluation criteria in solid tumors (RECIST v1.0) (18). Treatment was continued for 6 cycles in the absence of disease progression, provided all toxicities remained acceptable and patients were willing to continue on study.

Pharmacokinetic and Pharmacodynamic Methods

Blood was collected from all patients in heparinized tubes at pre-dose, 5 min, 45 min, 65 min and 1.5, 2, 4, 6, 8, and 24 hrs after the start of infusion on days 1 and 4. Day 4 samples were collected to determine if repeat dosing of AR-67 would result in drug accumulation as previously noted with other 3rd generation lipophilic camptothecin analogs (19). AR-67 lactone and carboxylate concentrations were determined in plasma by a validated high-performance liquid chromatography method with fluorescence detection based on a previously published assay (20). Plasma PK parameters were estimated by noncompartmental methods with WinNonlin (version 5.2; Pharsight, Mountain View, CA). Clearance values were compared by the Wilcoxon signed rank paired test to assess differences between days 1 and 4. Relationships between drug exposure and toxicity were explored using sigmoid E-max and logistic regression models (Graph-Pad Prism V5, La Jolla, CA). Spearman correlation was used to evaluate relationships between hematologic toxicities and exposure in terms of AUC, dosage level, and absolute dose.

RESULTS

Patient characteristics

Between November 2, 2006 and December 15, 2008, 26 subjects were enrolled and all were assessable for toxicity. Patient demographics are listed in Table 2. Overall, 61 courses of AR-67 over 9 dosage levels were delivered. One patient at dose level 1 received only 2 days of drug administration prior to progressive disease manifested by bowel obstruction that required hospitalization, and was removed from study. This subject was not evaluable for response, but was evaluable for toxicity.

Toxicity

Dose limiting toxicities were observed in 5 patients: two of two subjects at 12.4mg/m²/day (highest dosage level) experienced DLTs; one grade 4 febrile neutropenia and one grade 3 fatigue; two of four subjects at 8.9 mg/m²/day exhibited grade 4 thrombocytopenia and one of seven subjects at 7.5 mg/m²/day manifested grade 4 thrombocytopenia. All DLTs resolved without permanent sequelae. Table 3 summarizes all CTCAE toxicities at least possibly related to AR-67. Common Grade 3 and 4 toxicities included: leukocytopenia (23%), thrombocytopenia (15.4%), fatigue (15.4%), neutropenia (11.5%), and anemia (11.5%). Notably, none of the patients experienced infusion related allergic reactions or diarrhea.

Efficacy

The secondary endpoint of response per RECIST v1.0 was evaluable in 22 subjects. One subject with squamous cell carcinoma of the lung received ten cycles of therapy and had a prolonged partial response at dosage level 5.5. This response continues as of this writing, seven months after cessation of therapy (See supplemental Figure S1). Four subjects had stable disease: one patient at dosage level 7 with duodenal cancer (82 days), two patients at dosage levels 5.5 and

6 with small cell lung cancer (refractory disease, 91 days; sensitive relapse, 112 days) and one patient with non small cell lung cancer at dose level 5.5 (74 days).

Pharmacokinetics

This compound was designed to promote lactone stability in-vivo with the presumption that high lactone stability would lead to improved efficacy. Our results demonstrate that in human plasma the AR-67 lactone form accounts for 87.5% ($\pm 8.5\%$, SD) of the total AR-67 AUC (lactone + carboxylate). Summary PK parameters of total AR-67 at each dosage level and lactone AUCs are presented in Table 4. Mean plasma concentrations for the MTD cohort are presented in Figure 1A. The drug is eliminated with a bi-exponential profile and there is no evidence of accumulation on day 4. Greater than 80% of the total concentration is in the lactone form (Figure 1B) at each time point. The values of the lactone and total AUC (Figure 1C) were highly correlated (Spearman correlation: $r=0.99$, $p<0.0001$ and $r=0.99$, $p<0.0001$ for day 1 and 4, respectively). Given this strong correlation and the high lactone to total AR-67 ratio, further analysis was done based on the total AR-67 concentrations. Linear regression and correlation analysis demonstrated that the increase in $C_{\max}$ (data not shown) and AUC were dose dependent, while clearance was constant. As shown in Figure 1D no correlation was observed between dosage and clearance suggesting that clearance was constant across dosage levels. However, the mean clearance of total AR-67 increased 20% ($p=0.0031$, Wilcoxon signed rank two-tailed t-test) from day 1 ($14.5 \pm 4.1 \text{ L/hr/m}^2$, $N=26$) to day 4 ($17.4 \pm 5.2 \text{ L/hr/m}^2$, SD, $N=25$). It should be noted that the non-compartmental analysis may slightly overestimate the clearance since the true $C_{\max}$ may not have been captured by the 45-minute sample, which was collected during the infusion.

Pharmacodynamics

Given the high correlation and low variability in the lactone to total AR-67 ratio in all subjects, we used the total AR-67 PK parameters to explore relationship between exposure and toxicity. Total AR-67 exposure and the observed hematologic DLTs were examined using a sigmoid E-max model (Equation 1). Where $E(d)$ is the effect as a function of AUC or dosage, H is the curve shape factor (i.e., Hill coefficient), and ED_{50} is the AUC or dosage at which 50% of the effect is observed.

$$E(d) = \frac{E_{\max} \cdot d^H}{ED_{50} + d^H} \quad \text{Equation 1}$$

Figure 2 depicts the relationship between dosage level (panel A) or AUC (panel B) and the % decrease in ANC and platelets from baseline. The dosage level ED_{50} (95% CI) of 6.1 (4.3 – 8.7), and 4.9 (3.2 – 7.6) mg/m^2 were estimated for the % decrease in ANC and platelets, respectively. The estimated AUC ED_{50} (95% CI) values were 323.6 (231.9 – 451.6) and 364.2 (249.4 – 531.9) $\text{hr} \cdot \text{ng/mL}$ for ANC and platelets, respectively.

To determine the probability for the occurrence of each toxicity during cycle 1, we performed logistic regression (Equation 2), where P is the probability, $\times$ (values of 1 or 0) denotes the presence or absence, respectively, of toxicity determined by a nadir value below the respective lower limit set for ANC ($<1500/\text{mm}^3$) and platelets ($<100,000/\text{mm}^3$), and α and β are model parameters (α representing the log-odds of toxicity occurrence when $X=0$ and β is the increment in the log-odds of toxicity when $X=1$). Furthermore, the parameter β describes the steepness of the s-shaped curve or the rate of change in probability with increasing drug exposure.

$$P = \frac{1}{1 + \exp^{-(\alpha + \beta \cdot X)}}$$

Equation 2

As depicted in Figure 2 (panel C), during cycle 1 the occurrence of neutropenia was more likely than thrombocytopenia at exposures resulting from the MTD dosage level. Strong correlations could also be demonstrated between exposure and nadir values of ANC and platelets (see supplemental Figure S2). This analysis demonstrates that the correlations were similar when considering exposure in terms of AUC and dosage level (mg/m²). Similar correlations were also observed with leucopenia and the probability for observing leucopenia was similar to that for neutropenia (data not shown). At the MTD level ANC and platelet nadir levels were observed on days 10–14 during cycle 1 but patients recovered prior to day 22 (day 1 of cycle 2).

DISCUSSION

AR-67 administered daily for five days of an every 21-day cycle was well tolerated in this study. DLTs were thrombocytopenia, febrile neutropenia and fatigue and the MTD was defined as 7.5 mg/m²/day. Notably, no diarrhea or allergic infusional reactions occurred in the 26 people exposed to this compound. At the recommended phase II dose of 7.5 mg/m²/day (n=7), the regimen produced only modest and manageable side effects. While fatigue and hematologic toxicities are hallmarks of camptothecins, the lack of diarrhea seen in the present trial is notable compared to other drugs of this class, particularly irinotecan. Interestingly, although AR-67 is lipophilic and shares some structural characteristics with the active metabolite of irinotecan (i.e., SN-38), it does not undergo UGT1A1 mediated glucuronidation, but is extensively metabolized by UGT1A8, which is primarily expressed in the gastrointestinal tract (21,22). This may partly explain the lack of diarrhea observed in this study. Complete studies related to the metabolism and transport pathways of this compound are underway and will be presented elsewhere.

Pharmacokinetic studies demonstrated that 87.5% (±8.5%) of the drug is in the lactone form. This represents a significant improvement in effective drug delivery from other clinically approved camptothecins with reported lactone AUC ratios of 30–76% for irinotecan and irinotecan derived SN-38 (23–25) and for topotecan (26). With respect to 3rd generation analogs, the AR-67 lactone ratio is lower than the reported values for gimatecan and karenitecin, both in early clinical trials, which exist in plasma as 90–98% (19,27) and ~90–95% (28,29) in the lactone form, respectively. However, the AR-67 elimination half-life (~1.4 hr) may explain the lack of accumulation observed with gimatecan and karenitecin, which have documented half-lives of 77 hr and 15 hr, respectively (27–29). Interestingly, despite their long half-lives, these analogs have not proven more effective than the clinically approved camptothecins, which exhibit shorter half-lives. Thus, the prolonged exposures required for efficacy in rapidly growing preclinical models are not necessarily important for improved clinical efficacy.

AR-67 clearance is linear and correlates with hematologic toxicity. Interestingly, AR-67 clearance increased on day 4 of cycle 1 suggesting that this may be due to dexamethasone co-treatment inducing CYP3A4 activity. This is consistent with our *in-vitro* data demonstrating that AR-67 is a substrate for CYP3A4 and with previous clinical evidence demonstrating that dexamethasone treatment for 5 days induces CYP3A4 activity (21,30). However, CYP3A4 induction was highly variable in that study (30) as compared to our data that demonstrates a consistent ~20% decrease in AUC between days 1 and 4 in all patients. Thus, it is possible that the drug is also inducing its own metabolism to some extent.

The use of the cremophor-ethanol excipient is known to be associated with hypersensitivity reactions and has the potential to cause non-linear pharmacokinetics (17,31). However, the amount of cremophor at the MTD is less than 4% of that administered to a patient receiving a typical paclitaxel dose of 200 mg/m² and no patients exhibited hypersensitivity reactions. Nonetheless, patients treated in upcoming Phase II studies will continue to receive prophylactic premedication to prevent potential hypersensitivity reactions.

The partial response seen in NSCLC is notable because of the rapid tumor regression demonstrated by CT scan (see supplemental Figure S1). This subject continued to benefit, despite two dose reductions for a total of 10 cycles, and ultimately stopped treatment due to grade 3 fatigue. The partial response was ongoing seven months after cessation of therapy. In addition, stable disease was noted in two patients with SCLC (refractory and sensitive relapse for over three months) and one patient each with NSCLC and duodenal cancer. Camptothecins as a class have been proven effective in each of these cancer types, and further exploration of AR-67 in these groups is warranted (10).

In conclusion, AR-67 given daily for 5 days in an every-21-day cycle is well tolerated, with acceptable myelosuppression and fatigue as DLTs. At the MTD, toxicities were manageable and no diarrhea or hypersensitivity reactions were seen. Critically important was the demonstration of high lactone stability (~87.5% of total AR-67) in human plasma. Interestingly, a confirmed partial response was noted in a patient with non-small cell lung cancer, who remained on therapy for ten cycles. We also demonstrated linear PK and correlation of AUC and dosage with toxicity. Further clinical testing of AR-67 is warranted and ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Pharmacokinetic analysis. (A) Mean plasma concentration-time profiles of total AR-67 in the MTD cohort (n=7). (B) The mean percent lactone in plasma at each time point for all patients. (C) Correlation plot of total and lactone AUC. (D) Relationship of administered AR-67 dosage with clearance. Solid lines in panels C and D represent linear regression lines.

Figure 2. Pharmacodynamic analysis of neutropenia and thrombocytopenia during cycle 1 in all patients. Percent decrease from baseline as a result of (A) increasing dosage level and (B) AUC (day 1 of cycle 1). Lines represent fit of a sigmoid Emax model to the data. (C) Logistic regression analysis demonstrating the probabilities of manifesting neutropenia and thrombocytopenia with increasing AR-67 exposure. The gray area encompasses the range of AR-67 AUC achieved at the MTD during day 1 of cycle 1.

Table 1

Dose – escalation scheme and dose limiting toxicities.

Dose Level	Dose daily $\times$ 5	n	DLT per Cohort	Type of DLT
Level -1	1.2 mg/m ² /day	2*	0	
Level 1	1.67 mg/m ² /day	3	0	
Level 2	2.34 mg/m ² /day	3	0	
Level 3	3.2 mg/m ² /day	3	0	
Level 4	4.5 mg/m ² /day	1	0	
Level 5	6.3 mg/m ² /day	1	0	
Level 5.5	7.5 mg/m ² /day	7	1/7	Grade 4 thrombocytopenia
Level 6	8.9 mg/m ² /day	4	2/4	Grade 4 thrombocytopenia (2)
Level 7	12.4 mg/m ² /day	2	2/2	Grade 4 febrile neutropenia Grade 3 fatigue

* One subject did not receive 5 days of therapy and was replaced

Table 2

Patient characteristics.

Characteristic	No of Patients (N = 26)
Median age, years	62
Range	30–79
Performance Status	
0	13
1	12
2	1
Male/Female	15/11
Median prior chemotherapy regimens	3
Range	(1–6)
Race	
Caucasian	25
African-American	1
Tumor types	
Colon	8
Non-Small Cell Lung	4
Small Cell Lung	3
Soft Tissue Sarcoma	3
Head and Neck	2
Prostate	2
Bladder	1
Duodenal	1
Esophageal	1
Pancreas	1

Table 3

CTCAE toxicities possibly, probably, or definitely related to AR-67

Category	Adverse Event	Grade				Total
		1	2	3	4	
Blood/bone marrow	Hemoglobin	8	12	6	0	26
	Leukocytes (total WBC)	18	15	8	0	41
	Monocytopenia	0	0	0	1	1
	Neutrophils/granulocytes (ANC/AGC)	3	10	5	1	19
	Platelets	13	8	9	7	37
Cardiac general	Hypotension	0	1	0	0	1
Constitutional symptoms	Fatigue (asthenia, lethargy, malaise)	4	14	8	0	26
	Fever (in the absence of neutropenia)	0	1	0	0	1
	Insomnia	2	5	0	0	7
	Weight loss	2	0	0	0	2
	Pruritus/itching/Dry skin/Flushing	7	0	0	0	7
Dermatology/skin	Nail changes	1	0	0	0	1
	Rash/desquamation	2	1	0	0	3
Gastrointestinal	Anorexia/Taste Alteration	4	7	1	0	12
	Constipation/Dehydration	6	8	0	0	14
	Mucositis/stomatitis	2	0	0	0	2
	Nausea	14	0	0	0	14
	Hemorrhage, GI - Duodenum	0	0	1	0	1
Infection	Febrile neutropenia	0	0	1	2	3
	Infection - ANC grade 0-2	3	4	0	2	9
	Infection with unknown ANC	2	1	0	0	3
Metabolic/laboratory	ALT, SGPT, Alkaline Phosphatase	8	0	0	0	8
	Electrolyte abnormalities	8	0	0	0	8
Musculoskeletal	Extremity-lower (gait/walking)	1	0	0	0	1
	Muscle weakness - Lower extremity	0	3	1	0	4
Neurology	Dizziness	0	1	0	0	1
	Neuropathy: sensory	1	0	0	0	1

Category	Adverse Event	Grade				Total
		1	2	3	4	
Pulmonary	Dyspnea	1	0	0	0	1
	Hiccoughs (hiccups, singultus)	2	1	0	0	3
Total		112	92	40	13	257

Abbreviations: AGC, absolute granulocyte count; ALT, alanine aminotransferase; GI, gastrointestinal.

Table 4
Comparison of total AR-67 pharmacokinetic parameters between days 1 and 4 of cycle 1.

Pharmacokinetic parameter													
Dosage Level		Total AUC _(0-∞)		Lactone AUC _(0-∞)		C _{max, total} [#]		V _{ss, total}		T(1/2) _{total} [*]		Clearance _{total}	
Day	(mg/m ²)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1.2 (n=2)	88	11	81	8	77.6	2.9	7.4	2.2			13.8	1.8
	1.67 (n=3)	128	19	117	21	96.9	20.1	13.1	4			13.2	2
	2.34 (n=3)	201	118	186	110	158.9	97.3	13.5	6.9			14.1	6.3
	3.23 (n=3)	290	72	269	64	221.9	55.9	9.6	0.7			11.6	3
	4.5 (n=1)	214		179		117.8		32.7				21	
	6.3 (n=1)	490		334		168.5		51.6				12.9	
	7.5 (n=7)	581	82	498	66	349.7	114	18.7	7.3	1.4	0.3	13.1	1.8
	8.9 (n=4)	502	158	471	270	290.6	153.5	30.1	9.6	1.6	0.2	18.9	5.1
	12.4 (n=2)	786	64	686	77	464.7	105.4	18.1	3.8	1.1	0.1	15.8	1.3
All dosage levels													
4	1.2 (n=1)	51		49		51.6		15.6		1.4	0.1	14.5	4.1
	1.67 (n=3)	115	57	109	58	74.6	11.3	16.3	4.4			23.6	
	2.34 (n=3)	154	10	137	9	137.8	6.1	7.9	3.4			16.7	6.5
	3.23 (n=3)	255	70	236	61	195	58.8	10.6	4.3			15.3	1
												13.3	3.5
	4.5 (n=1)	186		149		82.5		45.3				24.2	
	6.3 (n=1)	429		327		166.4		35.3				14.7	
	7.5 (n=7)	478	127	411	104	308.6	122.9	25.6	16.3	1.8	0.7	16.8	5
	8.9 (n=4)	507	145	397	119	276.7	69.5	27.5	6.7	1.4	0	18.6	4.9
12.4 (n=2)	663	216	551	146	358.5	77.5	28.9	5.4	1.7	0.1	19.8	6.5	
All dosage levels													
										1.6	0.4	17.4	5.2

Abbreviations: AUC area under the plasma concentration-time curve; SD, standard deviation; V_{ss}, volume of distribution at steady-state.

* Half-life estimates are reported for dosage levels at which estimation of terminal slopes could be obtained by at least 3 data points in the elimination phase.

C_{max} values are the observed values at 45 minutes during the 1-hr infusion.