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ORIGINAL ARTICLE

First-time-in-human study of GSK923295, a novel antimitotic inhibitor of centromere-associated protein E (CENP-E), in patients with refractory cancer

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Abstract
Purpose GSK923295 is an inhibitor of CENP-E, a key cellular protein important in the alignment of chromosomes during mitosis. This was a Phase I, open-label, first-time-in-human, dose-escalation study, to determine the maximum-tolerated dose (MTD), safety, and pharmacokinetics of GSK923295.
Patients and methods Adult patients with previously treated solid tumors were enrolled in successive cohorts at GSK923295 doses ranging from 10 to 250 mg/m^2. GSK923295 was administered by a 1-h intravenous infusion, once weekly for three consecutive weeks, with treatment cycles repeated every 4 weeks.
Results A total of 39 patients were enrolled. The MTD for GSK923295 was determined to be 190 mg/m^2. Observed dose-limiting toxicities (all grade 3) were as follows: fatigue (n = 2, 5%), increased AST (n = 1, 2.5%), hypokalemia (n = 1, 2.5%), and hypoxia (n = 1, 2.5%). Across all doses, fatigue was the most commonly reported drug-related adverse event (n = 13; 33%). Gastrointestinal toxicities of diarrhea (n = 12, 31%), nausea (n = 8, 21%), and vomiting (n = 7, 18%) were generally mild. Frequency of neutropenia was low (13%). There were two reports of neuropathy and no reports of mucositis or alopecia. GSK923295 exhibited dose-proportional pharmacokinetics from 10 to 250 mg/m^2 and did not accumulate upon weekly administration. The mean terminal elimination half-life of GSK923295 was 9–11 h. One patient with urothelial carcinoma experienced a durable partial response at the 250 mg/m^2 dose level.
Conclusions The novel CENP-E inhibitor, GSK923295, had dose-proportional pharmacokinetics and a low number of grade 3 or 4 adverse events. The observed incidence of myelosuppression and neuropathy was low. Further investigations may provide a more complete understanding of the potential for GSK923295 as an antiproliferative agent.

Keywords Cancer · Antimitotic · CENP-E · Pharmacokinetics

Translational relevance

The novel antimitotic, GSK923295, is an allosteric inhibitor of the motor domain of centromere-associated protein E (CENP-E). CENP-E is expressed only in cells undergoing mitosis and is an integral protein in the kinetochore complex that helps to regulate proper chromosomal separation

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and cell division. Inhibition of CENP-E activity can lead to cell cycle arrest and apoptosis and consequently offers an attractive target for antitumor therapy. As a CENP-E inhibitor, GSK923295 has shown antiproliferative activity across a broad range of tumor types in preclinical studies. As a targeted antimitotic agent, GSK923295 is expected to have fewer off-target effects, including less neuropathy which is commonly associated with other antimitotics that interfere with microtubule polymerization or depolymerization, such as the taxanes and vinca alkaloids. This manuscript reports results from the first-time-in-human study that characterized safety and pharmacokinetics, determined the MTD, and demonstrated early signs of activity of GSK923295 administered on Days 1, 8, and 15 of a 28-day cycle. This manuscript is the first report of a clinical study of a CENP-E inhibitor.

Introduction

The mitotic machinery in the dividing cell is a well-validated target in cancer therapy. Currently, the vinca alkaloids and the taxanes are the most widely used antimitotic drugs and act by interfering with tubulin polymerization and depolymerization, respectively. Despite the success of these agents and an understanding of their mechanism of action, the complex molecular processes involved in mitosis have been poorly understood. In the past decade, these mechanisms have been under investigation, leading to the discovery of several new mitotic targets including centromere-associated protein E (CENP-E), kinesin spindle protein (KSP), polo-like kinase (PLK), aurora kinases, and other cellular proteins [1–3].

CENP-E is a kinesin motor protein that is expressed in dividing cells and is one of several proteins that form the kinetochore complex [2, 3]. The kinetochore assembles prior to cell division at the centromeric region of each chromatid pair, captures spindle microtubules, and aligns chromosomes in preparation for cell division. As a part of this complex, CENP-E functions as the key component in microtubule capture and instigation of downstream activities which results in satisfaction of the mitotic checkpoint and entry of the cell into anaphase [2–5]. Interruption of this complex process can result in mismatched copies of chromosomes, or aneuploidy, leading to tumor suppression [5].

Inhibition of CENP-E activity has been shown to result in varying levels of tumor suppression and inhibition of tumor cell growth [2, 4]. CENP-E expression is limited to those cells undergoing mitosis and its function is critical to proper cell division. Consequently, CENP-E represents an attractive antimitotic target for rapidly dividing tumors. Potential toxicities associated with other microtubule-inhibiting agents, including peripheral neuropathy, may also be less likely with drugs targeting CENP-E due to limited effects on non-dividing cells.

GSK923295 is a novel, potent, and specific allosteric inhibitor of human CENP-E with a K_i value of 3.2 nM that acts by inhibiting the microtubule-stimulated ATPase activity of the human CENP-E motor domain. In vitro studies suggest that GSK923295 binds to both ADP-inorganic phosphate (ADP-Pi)-bound and ATP-bound CENP-E. This binding induces a conformational change that appears to lock CENP-E onto microtubules inhibiting the movement of this motor protein by stabilizing the ADP-Pi state of the CENP-E–microtubule complex [2]. The resulting mitotic arrest and subsequent apoptosis are responsible for the antitumor activity of GSK923295.

GSK923295 has a broad spectrum of activity in vitro against a variety of human solid tumor and hematologic malignancy cell lines and in vivo against a variety of solid tumor xenograft models. Antiproliferative activity was shown in more than 200 solid tumor cell lines (median IC_{50} of 30 nM), including cancers of the colon, breast, pancreas, lung, ovary, prostate, bone, head and neck, fibrosarcoma, kidney, stomach, bladder, and endocervix, and in more than 80 hematologic cell lines, including acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin's and non-Hodgkin's lymphomas, and myeloma and lymphoma (median IC_{50} of 52 nM) [6, 7]. In addition, dose-dependent and sustainable antitumor activity was observed in lung, colon, breast, and ovarian xenografts grown subcutaneously in athymic nude mice [2, 8]. In 53 human breast cancer cell lines, cell viability and growth were assessed in the presence of GSK923295 [9]. The greatest sensitivity occurred in cell lines with the highest mitotic activity, while non-malignant and cell lines with low mitotic activity were more resistant to growth inhibition.

Antitumor activity of GSK923295 has been shown in normal primary human bone marrow progenitor cell assays with IC_{50} and IC_{90} of 133 and 219 nM, respectively. These estimates of IC_{50} are approximately 10 times higher than the Ki for human CENP-E. Based on the assumption that the media concentrations in these preparations represented the free concentration in the interstitial space and plasma, the potential therapeutic dose was estimated to be in the order of approximately 250 mg/m^2 based on allometric scaling of non-clinical pharmacokinetic data. The therapeutic dose estimate is consistent with results from in vivo pharmacology studies in xenograft nude mice, where stable disease and tumor regression were observed at 62.5 and 125 mg/kg doses, respectively [8]. Using the FDA dose calculator, these efficacious doses are equivalent to 190 and 380 mg/m^2 in humans, bracketing the therapeutic estimate of 250 mg/m^2.

Human CENP-E is most similar to dog CENP-E with respect to sequence homology and Ki values. Preclinical dog data were used to determine the starting dose in the FTIH study. In dog studies of up to 3 weeks in duration (once-weekly, 1-h intravenous infusion), the principle dose-limiting toxicities were hematopoietic and gastrointestinal changes, consistent with sequelae of antimitotic activity. The highest non-severely toxic dose (HNSTD) in dogs was 120 mg/m^2 (AUC of 15,000 ng.h/mL). Based on FDA guidance for estimating starting doses for FTIH studies and other preclinical toxicological studies, a starting dose of 10 mg/m^2 was proposed (less than 1/6th of the HNSTD) [10]. The purpose of this Phase I FTIH study in patients with refractory solid tumors was to determine the maximum-tolerated dose (MTD), safety, pharmacokinetics (PK), and preliminary clinical activity of GSK923295.

Patients and methods

Eligibility

Eligible patients were ≥18 years of age with histologically or cytologically confirmed diagnosis of solid tumors that were not responsive to accepted therapies or for which there was no standard therapy, and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1. Patients with significant cardiac, hematologic, hepatic, or renal dysfunction, gastrointestinal disorders, prior bone marrow transplant, infectious disease, symptomatic or untreated central nervous system involvement (i.e., brain metastases, leptomeningeal disease, and cord compression), peripheral neuropathy, or other neurological toxicity ≥grade 2 were excluded. There was no limit on the number of prior anticancer therapies; however, patients with any major surgery or prior anticancer therapy within the past 28 days (42 days for prior nitrosoureas or mitomycin C) were excluded. The protocol was reviewed and approved by appropriate institutional review boards, and all patients provided written informed consent according to Good Clinical Practice and applicable regulations.

Study design and treatments

This was an open-label, Phase I, multicenter, dose-escalation study of GSK923295. Treatment consisted of a 1-h intravenous administration of GSK923295, once weekly for three consecutive weeks, repeated every 4 weeks. Based on predicted plasma concentrations in humans and observed toxicity in preclinical studies of GSK923295, a starting dose of 10 mg/m^2 was selected. Adverse events (AEs) were graded using the NCI Common Toxicity Criteria for Adverse Events (CTCAE, version 3) [11]. Dose-limiting toxicity (DLT) was defined as any drug-related toxicity that was grade ≥3 that occurred during the first treatment cycle. Exceptions were alopecia and untreated vomiting. Non-hematologic toxicities of grade ≥2 that persisted into cycle 2 could also be considered dose limiting. The MTD was defined as the dose of GSK923295 at which no more than 1 of 6 patients experienced a DLT in the first treatment cycle.

Dose escalation took place according to an accelerated titration scheme that consisted of two patients per cohort with up to 100% dose escalation until at least two patients experienced grade ≥2 toxicity [12, 13]. After at least two patients experienced grade ≥3 toxicity during the first treatment cycle, dose escalation continued at a maximum of 33% with ≥3 patients per cohort following a standard 3 + 3 design.

Study assessments

Safety evaluations throughout the study included physical examination, vital signs, clinical laboratory tests (hematology, coagulation, clinical chemistry, and urinalysis), cardiac monitoring (12-lead ECG and telemetry), and adverse event reporting.

Serial blood PK samples were collected over 48 h on Days 1 and 15 for GSK923295 PK analysis. Plasma concentrations of GSK923295 were determined from a 50-μL aliquot of human plasma using a validated HPLC/MS/MS method performed by the Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline. The limits of quantification for GSK923295 ranged from 5 ng/mL to 5,000 ng/mL. Plasma PK parameters were calculated using standard non-compartmental methods (WinNonlin version 5.2, Pharsight, Mountain View, CA).

RECIST version 1.0 Guidelines were used to assess efficacy and disease status with the exception that evaluation of up to 3 selected lesions was considered sufficient for this FTIH study [14]. Tumor measurements were taken prior to the start of dosing and after every two cycles. Changes in tumor measurements that met the criteria for an objective response (complete response [CR] or partial response [PR]) by RECIST were confirmed by repeat assessments no less than 4 weeks after the criteria for response were first met. Stable disease (SD) measurements must have met the SD criteria at least once after study entry at a minimum of 8 weeks.

Statistical methods

No hypotheses were tested in this study. Safety, pharmacokinetics, and tumor response data were tabulated by treatment

group using descriptive statistics. Dose proportionality of GSK923295 PK parameters was assessed using a power model, and accumulation was assessed with ANOVA.

Table 1 Demographic characteristics

Characteristics	Total $N = 39$
Age (years) median (range)	63 (20–79)
Sex, *n* (%)	
Female	21 (54%)
Male	18 (46%)
Race, *n* (%)	
White	34 (87%)
Asian	4 (10%)
African American	1 (2%)
ECOG performance status at baseline[a], *n* (%)	
0	11 (28%)
1	27 (69%)
Tumor type in ≥ 2 patients[b], *n*(%)	
Pancreas	10 (26%)
Non-small cell lung	4 (10%)
Colon/rectum	4 (10%)
Head and neck	4 (10%)
Small cell lung	2 (5%)
Stomach	2 (5%)
Liver	2 (5%)
Esophagus	2 (5%)
Prior therapy, *n* (%)	
Surgery	39 (100)
Chemotherapy (cytotoxics, non-cytotoxics)	38 (97)
Radiotherapy	22 (56)
Biologic therapy (monoclonal antibodies, vaccines)	11 (28)
Immunotherapy	2 (5)
Hormonal therapy	1 (3)

[a] One patient was missing a baseline ECOG score

[b] Tumor types reported in a single patient ($n = 1$, 2.5%): gall bladder, kidney, bladder, ovary, prostate, gastroesophageal junction, breast, and ureter. One subject was reported with an unknown tumor type

Results

Patient characteristics

A total of 39 patients, consisting of an approximately equal number of men and women with a median age of 63 years, were enrolled at three clinical study sites (Table 1). The majority of patients had pancreatic cancer, and all but one patient had prior chemotherapy.

Safety

All 39 patients enrolled in this study received at least one dose of GSK923295. The median duration of exposure to GSK923295 was six doses or 2 cycles (range 1–12 cycles). Dose levels studied ranged from 10 to 250 mg/m^2. The first 3 cohorts of patients received GSK923295 doses of 10 mg/m^2 ($n = 2$), 20 mg/m^2 ($n = 2$), and 40 mg/m^2 ($n = 2$), respectively, without any clinically significant toxicity. Subsequent cohorts received GSK923295 doses of 80 mg/m^2 ($n = 7$), 105 mg/m^2 ($n = 3$), 140 mg/m^2 ($n = 8$), 190 mg/m^2 ($n = 7$), and 250 mg/m^2 ($n = 8$). A total of five patients experienced DLTs: one each at the 80 mg/m^2 (grade 3 AST increased) and 140 mg/m^2 (grade 3 hypoxia) dose levels, and 3 patients at the 250 mg/m^2 dose level with grade 3 fatigue ($n = 2$) and grade 3 hypokalemia ($n = 1$) (Table 2). Based on these results, the MTD was established at 190 mg/m^2 with this schedule.

All but one patient experienced one or more adverse event (Table 3). Twenty-eight (72%) patients experienced at least one AE that was considered at least possibly related to drug. The most commonly reported AE was fatigue which occurred in 22 of 39 (56%) patients. Of the 22 reported cases of fatigue, 13 cases were considered drug-related by the investigator. While the majority of patients experienced grade 1 fatigue, grades 2 ($n = 2$) and 3 ($n = 3$) fatigue were observed at doses ≥140 mg/m^2. There were no reports of grade 4 fatigue (Table 4). All cases of fatigue resolved within 3–96 days. Gastrointestinal toxicities of diarrhea ($n = 12$, 31%), nausea ($n = 8$, 21%), and vomiting

Table 2 Dose-limiting toxicities

Dose level (mg/m^2)	Dosed (*n*)	DLTs (*n*)	DLT	DLT Onset	Patient disposition/comment
80	7	1	AST increased, grade 3	Cycle 1/day 14	Continued dosing; patient withdrawn for progressive disease on cycle 1/Day 15
140	8	1	Hypoxia, grade 3	Cycle 1/Day 9	Continued dosing; patient withdrew consent on cycle 1/Day 22
250	8	3	Hypokalemia, grade 3	Cycle 1/Day 22	Dose reduced to 190 mg/m^2
			Fatigue, grade 3	Cycle 1/Day 26	Patient withdrawn
			Fatigue, grade 3	Cycle 1/Day 28	Patient withdrawn

Table 3 Summary of the adverse events reported in ≥4 patients, regardless of causality

Adverse Event, *n*	GSK923295 Dose (mg/m^2)								Total *N* = 39
	10 *N* = 2	20 *N* = 2	40 *N* = 2	80 *N* = 7	105 *N* = 3	140 *N* = 8	190 *N* = 7	250 *N* = 8	
Total number of patients with any event	2	2	2	7	3	8	7	7	38
Fatigue	2	0	1	3	3	4	4	5	22
Diarrhea	1	1	0	1	0	2	4	3	12
Decreased appetite	2	2	0	1	1	3	2	1	12
Hemoglobin decreased	2	0	0	4	1	2	1	2	12
Dyspnea	0	1	1	0	1	5	1	1	10
Hypoalbuminemia	2	0	0	2	2	2	0	2	10
Hyperglycemia	0	1	1	2	1	0	1	3	9
Nausea	2	2	0	1	1	1	1	0	8
AST increased	0	0	0	3	1	2	0	2	8
Hypokalemia	1	1	1	1	1	0	1	1	7
Vomiting	1	0	0	0	2	2	0	2	7
Dizziness	0	0	0	1	0	2	1	2	6
Constipation	0	0	0	0	0	5	1	0	6
Blood alkaline phosphatase increased	1	0	0	2	1	0	0	2	6
Edema peripheral	0	0	0	1	1	0	1	2	5
Abdominal pain	2	0	0	1	0	0	2	0	5
Back pain	1	1	0	0	1	2	0	0	5
Cough	0	0	0	0	0	3	2	0	5
Urinary tract infection	0	1	0	0	0	3	1	0	5
WBC count decreased	0	0	0	3	0	2	0	0	5
Sinus tachycardia	0	0	0	0	1	2	1	1	5
Tachycardia	0	0	0	0	1	3	1	0	5
Infusion site pain	0	0	0	0	1	1	1	1	4
Dehydration	0	0	0	0	1	2	1	0	4
Injection site reaction	0	0	0	0	0	3	0	1	4
Headache	0	1	0	0	0	1	0	2	4
Hypotension	0	0	1	0	0	0	1	2	4

Table 4 Summary of fatigue by maximum toxicity grade

Adverse event, *n* (%)	GSK923295 Dose (mg/m^2)								Total *N* = 39
	10 *N* = 2	20 *N* = 2	40 *N* = 2	80 *N* = 7	105 *N* = 3	140 *N* = 8	190 *N* = 7	250 *N* = 8	
Any Grade	2 (100)	0	1 (50)	3 (43)	3 (100)	4 (50)	4 (57)	5 (63)	22 (56)
Grade 1	2 (100)	0	1 (50)	3 (43)	3 (100)	3 (38)	2 (29)	2 (25)	16 (41)
Grade 2	0	0	0	0	0	1 (13)	1 (14)	1 (13)	3 (8)
Grade 3	0	0	0	0	0	0	1 (14)	2 (25)	3 (8)

(*n* = 7, 18%) were generally mild (grades 1 and 2) with no apparent dose relationship, and none resulted in a dose modification. Myelosuppression consisted predominantly of anemia (31%). Grade 3 neutropenia occurred in one patient each at the 80 mg/m^2 and 190 mg/m^2 dose levels. No overall trend was observed for an effect on neutrophils.

Peripheral neuropathy (grade 1) was reported in two patients, one at 20 mg/m^2 and one at 190 mg/m^2; however, both of these patients had a history of neuropathy at study entry. No mucositis or alopecia was reported. Grade 3 and grade 4 adverse events are summarized in Table 5. The only grade 3 or grade 4 AEs reported in more than one patient

Table 5 Summary of adverse events by maximum toxicity grade—grades 3 and 4 only

Adverse event, *n*	*N*	Grade 3	Grade 4	Grade 3 + 4
Any event, any dose total		12	3	15
10 mg/m^2—any event	2	0	0	0
20 mg/m^2—any event	2	1	0	1
Abdominal pain upper		1	0	1
Pleuritic pain		1	0	1
40 mg/m^2—any event	2	0	0	0
80 mg/m^2—any event	7	2	1	3
AST increased		1	0	1
Neutrophil count decreased		1	0	1
Pulmonary embolism		0	1	1
Lymphopenia		1	0	1
105 mg/m^2—any event	3	1	0	1
Vomiting		1	0	1
Hiccups		1	0	1
140 mg/m^2—any event	8	3	1	4
Hypoalbuminemia		1	0	1
Hyponatremia		0	1	1
ALT increased		0	1	1
AST increased		0	1	1
Hypoxia		2	0	2
Dyspnea		2	0	2
190 mg/m^2—any event	7	1	1	2
Fatigue		1	0	1
Hyperglycemia		1	0	1
Hemoglobin decreased		1	0	1
Neutrophil count decreased		1*	0	1
Neutropenia		0	1*	1
250 mg/m^2—any event	8	4	0	4
Fatigue		2	0	2
Hyperglycemia		1	0	1
Hypokalemia		1	0	1
Thrombosis		1	0	1

* Grade 3 neutrophil count decreased and grade 4 neutropenia occurred in the same patient

were fatigue ($n = 3$), AST increased, neutrophil count decreased, dyspnea, and hypoxia ($n = 2$ each). Overall, there were no clinically significant changes in laboratory parameters, ECGs or vital signs. While one patient had a DLT of elevated AST at 80 mg/m^2, there was no overall trend in elevated liver function tests observed during the study.

Pharmacokinetics

A summary of plasma GSK923295 pharmacokinetic parameters on Day 1 and Day 15 of cycle 1 is shown in Table 6, and a summary of median GSK923295 plasma concentration on Day 1 is illustrated in Fig. 1. Dose proportionality for AUC(0–∞) and Cmax on Day 1 and Day 15 was tested separately using a power model. The mean slope parameters ranged from 1.00 to 1.12, and the 90% confidence intervals for all tests included 1.00, suggesting dose proportional increases in exposure following single-dose administration over the 10–250 mg/m^2 dose range. The ratios of Day 1–Day 15 AUC(0–∞) and Cmax for dose levels enrolling more than 2 patients ranged from 0.85 to 1.22 and the 90% confidence intervals for all tests included 1.00, indicating no accumulation or time-dependence in GSK923295 pharmacokinetic parameters.

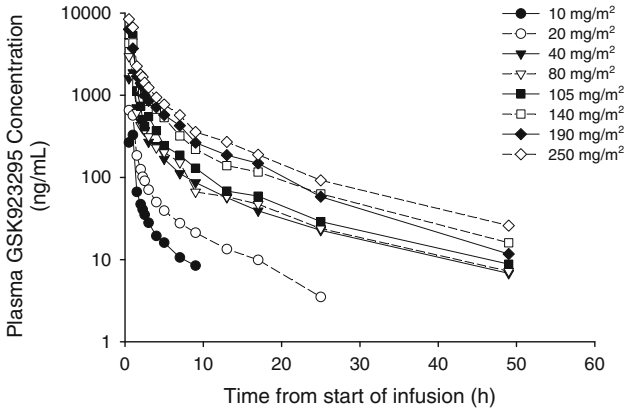

Fig. 1 Median Day 1 plasma GSK923295 concentrations during cycle 1 following single-dose administration on Days 1, 8, and 15

Clinical activity

Although clinical activity was not the primary objective of this study, patients were assessed for tumor response by the investigator using RECIST (Table 7). Based on these criteria, one patient, with urothelial carcinoma treated at 250 mg/m^2, had a PR after six cycles of treatment. The response was later confirmed, and at the time of the submission of this manuscript, this patient had completed 21 cycles of treatment with no evidence of disease progression. Prior treatment for this patient consisted of resection of a bladder tumor with adjuvant BCG (bacilli Calmette-Guerin), 2 cycles of loco-regional BCG, and nephroureterectomy with 4 cycles of adjuvant gemcitabine and carboplatin. Thirty-three percent of patients had a response of SD (median/range—51 days/8–183 days), and 54% had progressive disease (PD). Four patients had no post-treatment response evaluations performed.

Discussion

This study was designed to determine the safety, MTD, and PK of a novel antimitotic agent, GSK923295, in patients

Table 6 Geometric mean (CV%) of Day 1 and Day 15 Plasma GSK923295 pharmacokinetic parameters during cycle 1 following single-dose administration on Days 1, 8, and 15

Dose (mg/m^2)	Day	*n*	Cmax (ng/mL)	AUC(0–∞) (ng h/mL)	Half-life (h)	Clearance (L/h)
10	1	2	324 (22)	507 (27)	3.19 (23)	36.0 (24)
	15	2	305 (43)	541 (54)	6.04 (21)	33.6 (50)
20	1	2	647 (24)	1,260 (33)	9.38 (5)	33.2 (40)
	15	2	666 (43)	1,340 (54)	8.70 (4)	31.4 (62)
40	1	2	1,820 (50)	3,980 (99)	7.82 (92)	18.0 (110)
	15	2	2,020 (26)	4,110 (96)	10.7 (115)	17.4 (108)
80	1	7	2,830 (46)	4,980 (47)	10.7 (27)	29.0 (48)
	15	6	2,230 (51)	4,700 (53)	9.23 (41)	30.5 (58)
105	1	3	4,430 (36)	7,950 (15)	10.2 (30)	23.7 (28)
	15	3	3,630 (36)	7,220 (39)	8.16 (41)	26.0 (33)
140	1	8	5,200 (41)	12,500 (39)	10.1 (19)	20.1 (48)
	15	5	7,340 (68)	14,600 (50)	10.3 (31)	16.8 (60)
190	1	7	7,120 (60)	14,300 (33)	8.82 (26)	23.7 (30)
	15	6	7,120 (48)	14,700 (45)	8.16 (39)	23.2 (36)
250	1	8	8,320 (24)	21,000 (31)	10.7 (20)	21.7 (37)
	15	7	8,240 (33)	19,600 (34)	11.6 (13)	23.1 (38)

Table 7 Summary of investigator-assessed response

Response	GSK923295 Dose (mg/m^2)								
	10 *N* = 2	20 *N* = 2	40 *N* = 2	80 *N* = 7	105 *N* = 3	140 *N* = 8	190 *N* = 7	250 *N* = 8	Total *N* = 39
Complete Response	0	0	0	0	0	0	0	0	0
Partial response (PR)	0	0	0	0	0	0	0	1 (13%)	1 (3%)
Stable disease (SD)	0	1 (50%)	0	4 (57%)	0	3 (38%)	3 (43%)	2 (25%)	13 (33%)
Progressive disease (PD)	2 (100%)	1 (50%)	2 (100%)	1 (14%)	3 (100%)	5 (63%)	4 (57%)	3 (38%)	21 (54%)
No post-dose assessment	0	0	0	2 (29%)	0	0	0	2 (25%)	4 (10%)

with advanced refractory cancers. GSK923295 represents a first-in-class compound that selectively inhibits the motor domain of CENP-E. CENP-E is an integral protein in the kinetochore complex that is critical for proper chromosomal alignment and separation during cell division.

Based on the mechanism of action and preclinical toxicology studies, myelosuppression and gastrointestinal toxicity were expected following treatment with GSK923295 in humans. In this study, neither was observed to a significant extent. Approximately one-third of patients reported gastrointestinal toxicities of nausea, vomiting, and diarrhea. Myelosuppression was also infrequent, and there was no clear dose–response relationship in the incidence of hematological toxicities. Anemia was reported in 31% of patients, which is an expected rate in this patient population [15]. Episodes of low absolute neutrophil count (ANC) were infrequent and were typically observed in patients with low baseline ANCs. Overall, neutropenia was reported in approximately 13% of enrolled patients and considered drug-related in 8% of the patients. Because neutropenia was not a DLT in preclinical studies, the low occurrence of neutropenia in this clinical study is not surprising; however, it is a unique finding for an antimitotic. As expected for an agent targeting CENP-E, a protein that is not expressed in neuronal cells, the incidence of neuropathy was low, being observed in 2 of 39 patients (both grade 1). In addition, no mucositis or alopecia was reported.

The MTD for GSK923295 was determined to be 190 mg/m^2. At the highest dose studied (250 mg/m^2), DLTs were observed in 3 of 8 patients: 2 patients with grade 3 fatigue and one patient with grade 3 hypokalemia. The most common AE reported across all doses in the study was fatigue ($n = 22$, 56%). Fatigue was typically reported as grade 1, except at doses ≥140 mg/m^2 where grade 2 ($n = 3$, 8%) and 3 ($n = 3$, 8%) events were observed. Fatigue is commonly observed in patients with advanced cancer and with compounds that affect the cell cycle, e.g., flavopiridol [16], SB-715992 [17], BI2536 and ON01910 [18], and Ro 31-7453 [19]. Moreover, 54% of patients entered this trial with fatigue recorded as a baseline medical condition.

The number of patients enrolled at each dose level was small, so it is difficult to evaluate the impact of GSK923295 dose and underlying disease on the incidence of fatigue, especially in patients with advanced and progressing disease. However, the highest doses of 190 and 250 mg/m^2 were associated with the highest severity of fatigue. The two reported events of grade 3 fatigue that were observed at 250 mg/m^2 (25%) resulted in a significant impairment of daily living that required discontinuation of GSK923295. In both cases, the investigator observed improvement over a 2-week period after study drug was stopped.

Overall, the PK results of the study showed dose proportional increases in GSK923295 plasma exposure over the dose range 10–250 mg/m^2 following once-weekly intravenous administration for three consecutive weeks. The average terminal elimination half-life was 9–11 h. It is likely that the estimation of half-life and AUC(0–∞) at lower dose levels (<80 mg/m^2) was impacted by plasma GSK923295 data below the limit of quantification of the assay, resulting in underestimation of both parameters (and overestimation of clearance). Following weekly administration of GSK923295 during cycle 1, the systemic exposures were similar on Day 1 and Day 15, with no observed accumulation or time-dependence. On Day 1, the mean systemic plasma clearance and volume of distribution at steady-state (data not shown) between 80 and 250 mg/m^2 ranged from 20.1 to 29.0 L/h and 122-195 L, respectively. This clearance value is approximately 40–60% of liver plasma flow, with a distributional volume of 1.7–2.8 L per kilogram of body weight, and would suggest GSK923295 has a moderate plasma clearance and volume of distribution.

For the doses tested, there was no obvious correlation between individual exposure of GSK923295 and toxicity or response. Two patients experienced DLT of fatigue at 250 mg/m^2, with exposures (AUC(0–∞)) of 17,000 and 34,300 ng.h/mL, compared to a mean exposure for the group ($n = 8$) of 21,000 ng.h/mL. The patient with dose-limiting hypokalemia in this dose group had an AUC(0–∞) of 18,500 ng h/mL. Notably, the PK profile of the patient with the confirmed durable PR at the highest dose tested (250 mg/m^2) indicated that this patient had an AUC(0–∞) of 13,600 ng h/mL, which was 35% lower than the mean AUC(0–∞) for this dose level, and more similar to the mean AUC(0–∞) for the 190 mg/m^2 (MTD) dose group.

Estimates of the efficacious exposure of GSK923295 based on data from xenograft models and in vitro proliferation assays were previously presented while the study was ongoing [20]. It was estimated that exposures may need to be as high as 20,000 ng h/mL (i.e., similar to those at the 250 mg/m^2 dose level) to reach these preclinical exposures, but based on the emerging PK profile, these doses would still not maintain GSK923295 plasma concentrations above representative IC50 values for more than 24 h post-dose. It was also noted that the unbound plasma concentrations of GSK923295 in humans were approximately half that in the mouse, and in order to reach efficacious unbound exposures of GSK923295, doses may need to be in excess of 400 mg/m^2.

The low frequency and severity of expected gastrointestinal toxicity and myelosuppression events suggest that, at tolerable doses on this day 1, 8, 15 schedule, the agent may not have achieved sufficient inhibition of the target to significantly affect mitosis. Similarly, it is difficult to assess whether the low incidence or absence of neurotoxicity, mucositis, and alopecia is due to the absence of these toxicities with the drug, or lack of intended inhibition of the target. Pharmacodynamic studies, such as pre- and post-dose biopsy assessment of changes in markers of cell proliferation (antimitotic effect), were planned within this study. Unfortunately, the patients enrolled did not have disease amenable to biopsy or were not willing to undergo biopsy. Additionally, due to the relatively low MTD at the tested schedule (compared to exposures observed in preclinical studies) and low incidence of expected on-target toxicities, no additional patients were enrolled to conduct pharmacodynamic evaluations.

GSK923295 is a novel antimitotic agent targeting CENP-E. At doses ranging from 10 to 250 mg/m^2 administered once weekly for 3 weeks of a 4-week cycle, the compound exhibited dose-proportional pharmacokinetics, a low incidence of grade 3 and grade 4 adverse events and neurotoxicity, and no observed mucositis or alopecia. One patient achieved a durable, confirmed PR. However, given the lack of expected on-target toxicity, as well as a human exposure that is lower than the predicted preclinical minimum efficacious doses, further studies of GSK923295 should focus on evaluating different schedules of administration that might optimize GSK923295 exposure and explore potential on-target effects.

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