Clinical Cancer Research

Phase I Study of GSK461364, a Specific and Competitive Polo-like Kinase 1 Inhibitor, in Patients with Advanced Solid Malignancies

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Abstract

Purpose: GSK461364 is an ATP-competitive inhibitor of polo-like kinase 1 (Plk1). A phase I study of two schedules of intravenous GSK461364 was conducted.

Experimental Design: GSK461364 was administered in escalating doses to patients with solid malignancies by two schedules, either on days 1, 8, and 15 of 28-day cycles (schedule A) or on days 1, 2, 8, 9, 15, and 16 of 28-day cycles (schedule B). Assessments included pharmacokinetic and pharmacodynamic profiles, as well as marker expression studies in pretreatment tumor biopsies.

Results: Forty patients received GSK461364: 23 patients in schedule A and 17 in schedule B. Dose-limiting toxicities (DLT) in schedule A at 300 mg (2 of 7 patients) and 225 mg (1 of 8 patients) cohorts included grade 4 neutropenia and/or grade 3–4 thrombocytopenia. In schedule B, DLTs of grade 4 pulmonary emboli and grade 4 neutropenia occurred at 7 or more days at 100 mg dose level. Venous thrombotic emboli (VTE) and myelosuppression were the most common grade 3–4, drug-related events. Pharmacokinetic data indicated that AUC (area under the curve) and C_{max} (maximum concentration) were proportional across doses, with a half-life of 9 to 13 hours. Pharmacodynamic studies in circulating tumor cells revealed an increase in phosphorylated histone H3 (pHH3) following drug administration. A best response of prolonged stable disease of more than 16 weeks occurred in 6 (15%) patients, including 4 esophageal cancer patients. Those with prolonged stable disease had greater expression of Ki-67, pHH3, and Plk1 in archived tumor biopsies.

Conclusions: The final recommended phase II dose for GSK461364 was 225 mg administered intravenously in schedule A. Because of the high incidence (20%) of VTE, for further clinical evaluation, GSK461364 should involve coadministration of prophylactic anticoagulation. *Clin Cancer Res; 17(10); 3420–30. ©2011 AACR.*

Introduction

The polo-like kinase (Plk) family comprises 4 members (Plk1–4), of which Plk1 has a prominent mitotic role: It is required for centrosome maturation, chromosome condensation, and cytokinesis (1), as well as other functions including cell polarity, DNA damage response, and p53 pathways (2–4). Plk1 is overexpressed at mRNA and protein levels in many human tumors such as esophageal and ovarian cancer, in which expression correlates with poorer survival (5–7). The constitutive expression of *PLK1* in NIH3T3 fibroblasts drives malignant transformation and results in tumor formation in nude mice (8). It is therefore an attractive target for cancer treatment, and a number of Plk inhibitors (Plki), with varying specificity for Plk isoforms, are currently in clinical trials (9, 10).

GSK461364 is a potent, selective, and reversible ATP-competitive Plk1 inhibitor ($K_i = 2.2$ nmol/L) with at least 390-fold greater selectivity for Plk1 than for Plk2 and Plk3 and 1,000-fold greater selectivity than for a panel of 48

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Translational Relevance

GSK461364 is a small-molecule, ATP-competitive inhibitor of the cell-cycle regulating protein polo-like kinase 1 (Plk1). A strategy of Plk1 inhibition is based on the finding that *PLK1* has been shown to be amplified in esophageal cancer and the protein is overexpressed in a number of hematologic and solid malignancies. This phase I study was conducted to determine the maximum tolerated dose, dose-limiting toxicities, and pharmacokinetics of GSK461364 given in 2 competing IV schedules. Compared with other Plk inhibitors that have undergone early clinical trials, GSK461364 shows greater specificity to Plk1 (than to Plk2 and Plk3). For the first time in trials of this class of agents, proof-of-principle pharmacodynamic markers were included, such as predictive markers of response and assays by using circulating tumor cells to provide "real-time" assessment of drug activity. Although no objective responses were observed, GSK461364 was associated with prolonged stable disease (>16 weeks) in 6 patients, including 4 patients with esophageal cancer. Although hematologic toxicity was mild, there was a higher than expected incidence of thromboembolic events (20%). This trial shows the effectiveness of PLK1 inhibition in the treatment of solid tumors but recommends the use of GSK461364 alongside prophylactic anticoagulants in future trials.

other kinases. In preclinical testing, the drug showed antiproliferative activity against multiple (>120) tumor cell lines and potently inhibited the proliferation of greater than 83% and 91% of these cell lines, with IC_{50} values lower than 50 and 100 nmol/L, respectively (11, 12). GSK461364 did not impact normal or slowly proliferating human cells. Increasing concentrations of GSK461364 caused misaligned chromosomes (10 nmol/L), mitotic arrest with severely perturbed mitotic spindles (250 nmol/L), and G_2 delay followed by delayed entry in mitosis and prometaphase arrest (>300 nmol/L). Mitotic arrest due to prolonged Plk1 inhibition led to cell death by mitotic catastrophe or aberrant mitotic exit with severe micronucleation (11, 12). Intraperitoneal administration of GSK461364 caused regression or tumor growth delay in different xenograft models, including Colo205 xenografts. Suppression of Plk1 *in vivo* by using GSK461364 resulted in mitotic arrest with aberrant mitotic figures consisting of monopolar or collapsed mitotic spindles. Also, elevation of phosphorylated histone H3 (pHH3) and suppression of Plk1 were observed in tumor xenografts (11, 13). The toxicities reported in preclinical toxicology studies were reversible hematopoietic and gastrointestinal changes, associated to dose and infusion duration. Reversible QTc prolongation related to *hERG* channel inhibition and acute hemodynamic changes were also observed and related to C_{max} (maximum concentration). Nevertheless, the overall favorable preclinical safety profile and antitumor activity observed with GSK461364 led to this phase I study in patients with advanced solid tumors. The primary objectives included evaluating safety and tolerability to define the dose-limiting toxicities (DLT) and the maximum tolerated dose (MTD). Secondary objectives included pharmacokinetic and pharmacodynamic evaluation alongside tumor response.

In this study, to fully characterize the activity of GSK461364, pharmacodynamic endpoints were obtained in addition to pharmacokinetic assessments. These consisted of tumor tissue levels of Plk1, pHH3, and Ki-67 and the evaluation of pHH3 expression in circulating tumor cells (CTC) before and during treatment.

Patients and Methods

Patient selection

Patients eligible for this study were 18 years or older with a confirmed diagnosis of advanced solid tumor for which no effective treatment was available. Other key eligibility criteria included provision of informed consent; Eastern Cooperative Oncology Group (ECOG; ref. 14) performance status of 2 or less; adequate bone marrow, renal, and hepatic functions; no residual toxicities [Common Terminology Criteria for Adverse Events (CTCAE) version 3.0; ref. 15] grade more than 1; no prior anticancer treatment less than 3 weeks of study entry; and absence of significant comorbidities.

Study design and dosing

The trial was an open-labeled, nonrandomized, dose-escalation, and dose-finding phase I study of GSK461364 conducted at The Royal Marsden NHS Foundation Trust (Sutton, UK), Imperial College Healthcare NHS Trust (London, UK), and Queen's University Belfast Cancer Centre (Belfast, UK) in accordance with the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice and was approved by the relevant regulatory and independent ethics committees.

Optimal preclinical *in vitro* and *in vivo* antitumor activity of GSK461364 required extended exposure and frequent dosing. The relationship with toxicity was more complex. In general, toxicity was observed with increasing dose and more frequent dosing; however, the therapeutic window also seemed to increase with more frequent dosing. Thus, 2 schedules were evaluated, both with identical cycle length (28 days), with drug administered 3 of 4 weeks. In schedule A, GSK461364 was administered once a week on days 1, 8, and 15. In schedule B, GSK461364 was administered twice a week on days 1, 2, 8, 9, 15, and 16. Dose escalation for schedules A and B were started at 50 and 25 mg, respectively. The starting dose was to be escalated by 100% increments in 2 patient cohorts until 2 or more patients experienced study drug-related grade 2 adverse events (AE) during the first 28 days of treatment, whereupon dose escalation was to be reduced to 50% in 3 patient cohorts. If 1 or more patients experienced non–dose-limiting grade

3–4 drug-related AEs during cycle 1, the dose was to be escalated by 33% in 3 patient cohorts. Cohort expansion to a minimum of 6 patients was required if 1 DLT was reported and dose escalation was to cease if 2 or more patients experienced DLTs. GSK461364 treatment was continued in all patients until discontinuation criteria were met, including clinical or radiologic disease progression, withdrawal of consent, or unacceptable toxicity.

DLT and MTD definition

DLTs were to be assessed during cycle 1 and were defined as follows: grade 4 neutropenia more than 7 days; grade 4 thrombocytopenia, or grade 3 or more nonhematologic toxicity with the exception of alopecia. Grade 3–4 nausea, vomiting, or diarrhea were considered a DLT if they persisted despite optimal medical management. The inability to receive at least 80% of the planned doses during cycle 1 due to unresolved toxicity also qualified for DLT. The MTD was to be defined as the highest dose at which no more than 1 of 6 of subjects experienced a DLT.

Formulation, administration, and concomitant medications

During this study, GSK461364 was administered in 2 different formulations, acetate and Captisol. Initially, patients were treated with the acetate formulation, but from February to April 2009, all new and ongoing patients were given GSK461364 in the Captisol formulation. GSK461364, solubilized in either acetate or Captisol, was diluted in 5% dextrose and administered in a 4-hour intravenous infusion. This infusion time was selected on the basis of simulations by using animal pharmacokinetic data and on toxicity findings to obtain optimal C_{max} and AUC (area under the curve) values for humans. Subsequent to the occurrence of venous thromboembolic (VTE) events, administration of subcutaneous prophylactic low-molecular-weight heparin (LMWH) was introduced from December 2008.

Tumor response and safety

AEs were recorded from day 1 until 30 days after the last administered dose and graded according to CTCAE version 3.0. Cardiac monitoring was conducted in all patients for 24 hours during infusion days in cycle 1. Tumor measurements were done every 7 to 8 weeks from study day 1. Tumor response was evaluated by using Response Evaluation Criteria in Solid Tumors (RECIST; ref. 16). In addition, immunochemical studies were done in archived tumor samples and included pHH3, Ki-67, and Plk1 expression (Supplementary Methods).

Pharmacokinetic studies

Blood samples for pharmacokinetic analysis were initially obtained from patients predose, and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours from the start of infusion on day 1 and at the same time points on day 2 (only schedule B) of week 1. Additional blood samples were obtained at the same time points during weeks 2 or 3 of cycle 1 and in patients who switched from acetate to Captisol formulation. Samples were processed within 30 minutes of collection, and plasma samples were frozen (–20°C). Plasma samples were assayed for GSK461364 by using a validated method based on liquid–liquid extraction, followed by high-performance liquid chromatography tandem mass spectroscopy analysis developed by GlaxoSmithKline Worldwide Bioanalytical. GSK461364 plasma concentrations were analyzed by noncompartmental analysis by using WinNonlin 5.2 by GlaxoSmithKline Clinical Pharmacokinetics/Modeling and Simulation. Summary statistics were assessed by using SAS 8.2 by GlaxoSmithKline Oncology Discovery Biometrics.

Pharmacodynamic studies

Additional blood samples were taken from trial patients with either breast or prostate cancer to evaluate CTCs by using the CellSearch System (Veridex) as previously described (17, 18). In addition, CTCs were assayed by using Alexa Fluor 488–labeled antibody against (Ser10) pHH3 (Cell Signaling Technology), a mitotic-specific marker elevated during mitotic arrest (19).

Results

Patient characteristics

A total of 40 patients were enrolled between October 2007 and March 2009 and all were included in the safety analysis. Patient baseline characteristics and demographics are summarized in Table 1. The median age was 62 years (range: 28–80). The most common tumor diagnoses were colorectal adenocarcinoma (30%) and esophageal cancer (15%). All but 1 patient had received at least 1 prior systemic chemotherapy. Patients were enrolled alternately into both schedules. Patients enrolled in schedule A ($n = 23$) were treated at 5 escalating dose levels: 50 mg ($n = 2$), 100 mg ($n = 3$), 150 mg ($n = 3$), 225 mg ($n = 8$), and 300 mg ($n = 7$); patients enrolled in schedule B ($n = 17$) were treated at 4 escalating dose levels: 25 mg ($n = 2$), 50 mg ($n = 2$), 75 mg ($n = 6$), and 100 mg ($n = 7$). A total 105 cycles (54 in schedule A and 51 in schedule B) were administered to these 40 patients. A total of 22 patients received prophylactic LMWH (dalteparin, tinzaparin, or enoxaparin) either intermittently (every other day) or continuously (daily).

DLTs and MTD

Schedule A. The MTD for schedule A was 225 mg. Three patients experienced DLTs in this schedule. At the 225 mg cohort, 1 patient developed persistent grade 4 neutropenia at 7 or more days and discontinued treatment. The 225 mg cohort was subsequently expanded to 8 patients with no further DLTs observed. In the 300 mg cohort, 1 patient had prolonged grade 4 neutropenia and thrombocytopenia and a second had persistent grade 3 thrombocytopenia and consequently missed more than 20% of planned doses during cycle 1. Both recovered and continued on treatment after a dose reduction to 225 mg.

Schedule B. The MTD for schedule B was 75 mg. Two patients experienced DLTs in the 100 mg cohort, 1 patient

Table 1. Demographics and baseline characteristics

Description	Schedule A ($n = 23$)	Schedule B ($n = 17$)	Total ($n = 40$)
Age, median (range), y	62.0 (31–80)	62.0 (28–75)	62.0 (28–80)
Gender			
Male	13 (57)	12 (71)	25 (62)
Female	10 (43)	5 (29)	15 (38)
Tumor type at initial diagnosis			
Colorectal adenocarcinoma	6 (26)	6 (35)	12 (30)
Esophageal carcinoma	2 (9)	4 (24)	6 (15)
Malignant melanoma	3 (13)	2 (12)	5 (13)
Breast cancer	1 (4)	2 (12)	3 (8)
Ovarian adenocarcinoma	1 (4)	1 (6)	2 (5)
Renal cancer	1 (4)	1 (6)	2 (5)
Other[a]			
Treatment history			
Surgery	20 (87)	15 (88)	35 (88)
Radiotherapy	8 (35)	7 (41)	15 (38)
Systemic chemotherapy	22 (96)	17 (100)	39 (98)
1 prior regimen	6 (26)	4 (24)	10 (25)
2 prior regimens	6 (26)	7 (41)	13 (33)
$\geq$3 prior regimens	10 (44)	6 (35)	16 (40)
Hormonal therapy	2 (9)	1 (6)	3 (8)

NOTE: Please given are number (percentage), unless otherwise specified.

[a]Other cancer types with 1 patient each were endometrial adenocarcinoma, retroperitoneal liposarcoma, gastric adenocarcinoma, anorectal squamous carcinoma, pleural mesothelioma, pancreas adenocarcinoma, castration-resistant prostate cancer, hepatocarcinoma, cholangiocarcinoma, and unknown primary adenocarcinoma.

developed persistent grade 4 neutropenia but recovered and continued on study on a reduced dose of 50 mg. A second patient was diagnosed with pulmonary emboli (PE) and discontinued study drug. Following these 2 DLTs within the 100 mg cohort, GSK461364 dose was deescalated to 75 mg and 6 patients were treated at this dose level with no DLTs observed in cycle 1.

Safety and tolerability

Drug-related grade 3 or 4 events across schedules included neutropenia (10%), thrombocytopenia (10%), and leukopenia chest pain (3% each). The most common nonhematologic drug-related AEs were infusion site reactions and phlebitis occurring in 6 (15%) and 5 (13%) patients, respectively. A total of 6 (35%) patients on schedule B experienced VTE events comprising 5 PE and 1 portal vein thrombosis. Two of these patients had a history of VTE, and 3 were already receiving intermittent prophylaxis with LMWH. Four of these 6 patients discontinued GSK461364 following these VTE events. In addition, 2 (9%) patients on schedule A experienced lower-limb vein thrombosis, 1 in patient with a history of PE. VTE, phlebitis, and/or infusion site reactions were observed across a range of doses. In addition to the 22 patients who received prophylactic LMWH, 8 patients were initiated on LMWH during the study in relation to AEs. Drug-related AEs are summarized in Table 2.

Pharmacokinetic analysis

During the first week of cycle 1, blood samples were obtained from 21 of the 23 subjects in schedule A and 15 of the 17 subjects in schedule B. The pharmacokinetic parameters were similar between the 2 schedules, GSK461364 C_{max} and $AUC_{0-\infty}/AUC_{0-24}$ were dose proportional. Expected accumulation was observed in schedule B with a plasma concentration higher on the second dose per week than the first. Following the end of the 4-hour infusion, GSK461364 plasma concentrations declined biexponentially (Fig. 1A and B). GSK461364 was characterized by an initially rapid distribution phase with plasma concentrations decreasing to approximately 15% to 20% of the C_{max} within 8 hours of cessation of infusion. This initial distribution was followed by an elimination phase with a mean/median terminal half-life of 9 to 13 hours. Clearance (CL) and steady-state volume of distribution (V_{ss}) were similar for both schedules. The mean CL values ranged from 73.1 to 110.0 L/h and V_{ss} values ranged from 656 to 1,081 L. Derived pharmacokinetic parameters were plotted and summarized by schedule, cycle, visit, and dose in Figure 1 and Table 3, respectively.

Pharmacodynamic analysis

Blood samples for CTC isolation were obtained from 4 patients (3 MBC, 1 prostate cancer), but CTCs counts were determined only in 3 patients. Mean CTC counts were

Table 2. Summary of drug-related AEs[a]

Preferred term	Schedule A (once weekly)												Schedule B (twice weekly)										Overall study	
	50 mg (*N* = 2) Grade		100 mg (*N* = 3) Grade		150 mg (*N* = 3) Grade		225 mg (*N* = 8)		300 mg (*N* = 7) Grade		Total (*N* = 23) Grade		25 mg (*N* = 2) Grade		50 mg (*N* = 2) Grade		75 mg (*N* = 6) Grade		100 mg (*N* = 7) Grade		Total (*N* = 17) Grade		Total (*N* = 40) Grade	
	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4
Neutropenia																								
Cycle 1 (all cycles)	0	0	0	0	0	0	0 (1)	1 (1)	0	2 (2)[b]	0 (1)	3 (3)	0	0	0	0	0	0	1 (2)	1 (1)	1 (2)	1 (1)	1 (3)	4 (4)
Anemia																								
Cycle 1 (all cycles)	0	0	0	0	0	0	1 (1)	0 (1)	2 (2)	0	3 (3)	0 (1)	0	0	0	0	0	0	1 (2)	0	1 (2)	0	4 (5)	0 (1)
Infusion site reaction																								
Cycle 1 (all cycles)	0	0	0	0	0 (1)	0	1 (1)	0	2 (2)	0	3 (4)	0	0	0	1 (1)	0	1 (1)	0	0	0	2 (2)	0	5 (6)	0
Phlebitis																								
Cycle 1 (all cycles)	0	0	0	0	1 (1)	0	2 (2)	0	0	0	3 (3)	0	0	0	0	0	1 (1)	0	1 (1)	0	2 (2)	0	5 (5)	0
Thrombocytopenia																								
Cycle 1 (all cycles)	0	0	0	0	0	0	0	0	0	3 (3)	0	3 (3)	0	0	0	0	0	0	0 (1)	1 (1)	0 (1)	1 (1)	0 (1)	4 (4)
Thromboembolism																								
Cycle 1 (all cycles)	0	0	0	0 (1)	0	0	0	0 (1)	0	0	0	0 (2)	0	0 (1)	0	0	0	1 (3)	0	1 (2)	0	2 (6)	0	2 (8)
Chest pain																								
Cycle 1 (all cycles)	0	0	0	0	0	0	0	1 (1)	0	0	0	1 (1)	0	0	0	0	0	0	0	0	0	0	0	1 (1)

[a]All grade 3–4 related AEs, all VTE events regardless of relationship to study drug, and all grade 1–2 AEs regardless of relationship to study drug occurring at least in 5% of patients.
[b]A patient treated at 300 mg in schedule A presented simultaneously grade 4 neutropenia and thrombocytopenia at cycle 1. Leukopenia grade 3 of nonclinical significance was also seen in 1 patient at 300 mg in schedule A.

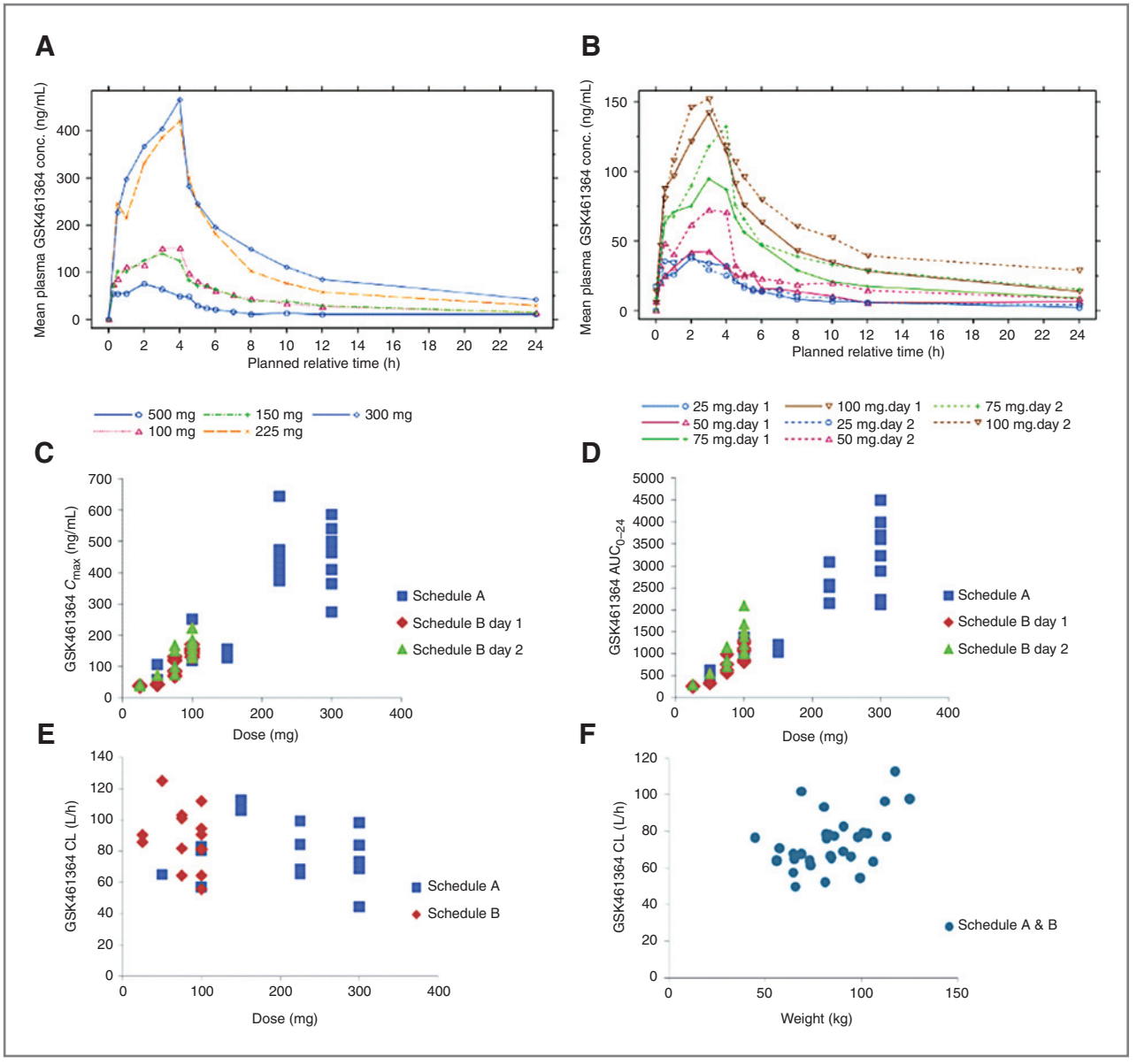

Figure 1. Pharmacokinetics. A, each color line represents mean plasma GSK461364 levels (linear scale) along cycle 1 day 1 for the different dose-escalation levels: 50 mg ($n = 2$), 100 mg ($n = 2$), 150 mg ($n = 3$), 225 mg ($n = 7$), and 300 mg ($n = 6$). B, schedule B, each color represents a different dose-escalation level: 25 mg ($n = 2$), 50 mg ($n = 2$), 75 mg ($n = 7$), and 100 mg ($n = 6$). Continuous lines and dashed lines represent mean plasma concentration of GSK461364 for day 1 and day 2, respectively. In general, following the end of the 4 hours infusion, GSK461364 plasma concentrations declined bi-exponentially. Dose (mg) versus (C) C_{max} (ng/mL) and (D) AUC_{0-24} (ng h/mL) values. Both C_{max} and AUC_{0-24} increased with dose, and in schedule B increased from day 1 to day 2, supporting proportional drug exposure and accumulation. E, CL (L/h) values versus dose (mg). Apparent clearance values were similar irrespective of dose. F, CL (L/h) versus body weight (kg). A poor correlation ($r^2 = 0.20$) between apparent clearance and body weight was seen. These results support the use of a fixed dose.

shown to decline 24 hours following GSK461364 administration. pHH3-positive CTCs were not detected at baseline but were detected 24 hours after treatment in all 3 patients (range: 36%–60%) consistent with induction of mitosis arrest in tumor cells (Fig. 2).

Tumor evaluation

Thirty-two patients were evaluable for antitumor activity by RECIST. Eight patients withdrew trial early because of adverse events ($n = 3$) or rapid clinical progression ($n = 5$). Stable disease (SD) for ≥4 or more months was the best response in 6 patients, all them treated at MTD or doses above MTD, and included 3 esophageal cancer patients (SD for 23, 23+, and 56 weeks), an endometrial carcinoma patient (43 weeks), and an ovarian cancer patient (19 weeks). An additional esophageal cancer patient, progressing at study entry, withdrew from the study after 15 weeks because of toxicity but remained in follow-up at the investigator site

Table 3. Summary of pharmacokinetic parameters summary of selected pharmacokinetic parameters

Dose	PK parameter[a]						
	n,[b] Sb/PK	C_{max}, ng/mL	AUC,[c] ng h/mL	Elimination $t^1/_2$, h	C24, ng/mL	CL, L/h	V_{ss}, L
Schedule A (once weekly)							
50 mg	2/4	61.2 (57.6–106)	665 (512–769)	13.1 (12.0–13.7)	8.22 (5.56–14.5)	75.2 (65.0–97.7)	1,049 (759–1,288)
100 mg	3/7	139 (119–252)	1,369 (1,204–2,654)	12.9 (10.7–17.5)	16.1 (12.6–55.5)	73.1 (37.7–83.1)	962 (543–1,277)
150 mg	2/4	149 (128–189)	1,362 (1,213–1,415)	9.61 (9.26–13.7)	13.8 (11.5–15.4)	110 (106–124)	1,081 (960–1,521)
225 mg	7/13	411 ± 91.1 (22%)	2,953 ± 805 (29%)	8.99 ± 4.69 (44%)	27.2 (8.94–56.1)	82.0 ± 23.6 (29%)	656 ± 283 (38%)
300 mg	7/14	456 ± 86.3 (21%)	3,774 ± 1,150 (30%)	9.18 ± 2.11 (22%)	34.5 (16.3–112)	85.9 ± 24.4 (30%)	745 ± 210 (27%)
Schedule B (twice weekly)[d], [e]							
25 mg (day 1)	2/4	37.6 (34.4–41.4)	263 (244–266)	8.80 (7.22–10.8)	2.23 (1.95–3.70)	87.0 (79.2–90.4)	682 (577–888)
25 mg (day 2)	44.3 (37.9–46.3)	304 (287–312)	8.22 (7.54–19.4)	2.64 (2.34–5.40)	–	–	
50 mg (day 1)	2/3	70.9 (42.4–71.2)	495 (326–616)	12.7 (8.63–14.1)	7.25 (6.20–12.0)	78.6 (59.9–125)	1,049 (930–1,339)
50 mg (day 2)	71.9 (69.1–72.2)	611 (546–666)	14.0 (8.64–9.95)	9.42 (8.64–9.95)	–	–	
75 mg (day 1)	5/13	100 ± 21.7 (21%)	767 ± 168 (22%)	11.4 ± 2.15 (19%)	9.29 (6.92–13.7)	83.9 ± 16.4 (20%)	945 ± 251 (29%)
75 mg (day 2)	5/9	120 ± 30.4 (26%)	980 ± 205 (22%)	12.7 ± 5.71 (39%)	14.2 (11.6–20.6)	–	–
100 mg (day 1)	7/10	151 ± 31.9 (22%)	1,044 ± 193 (19%)	9.50 ± 2.93 (33%)	11.0 (5.51–23.8)	85.3 ± 17.7 (22%)	787 ± 255 (33%)
100 mg (day 2)	6/9	180 ± 38.6 (21%)	1,462 ± 374 (26%)	12.4 ± 4.72 (31%)	23.4 (13.9–58.4)	–	–

Abbreviations: PK, pharmacokinetic.

[a]Values are median (ranges) for 25, 50, 100 (schedule A), and 150 mg doses. All other values are mean ± SD and coefficient of variation. All C24 values are median (ranges).

[b]Sb/PK = no. of subjects/no. of sets of PK parameters. A subject may have more than 1 set of PK parameters. AUC, elimination $t^1/_2$, CL, and V_{ss} may have few sets of PK parameters than PK number.

[c]AUC = $AUC_{0-\infty}$ for all AUC values except for day 2 where it is AUC_{0-24}.

[d]Day 1 and day 2 were used to designate first day and second day of dosing in a week.

[e]CL and V_{ss} values were not included for day 2, as these values are not valid.

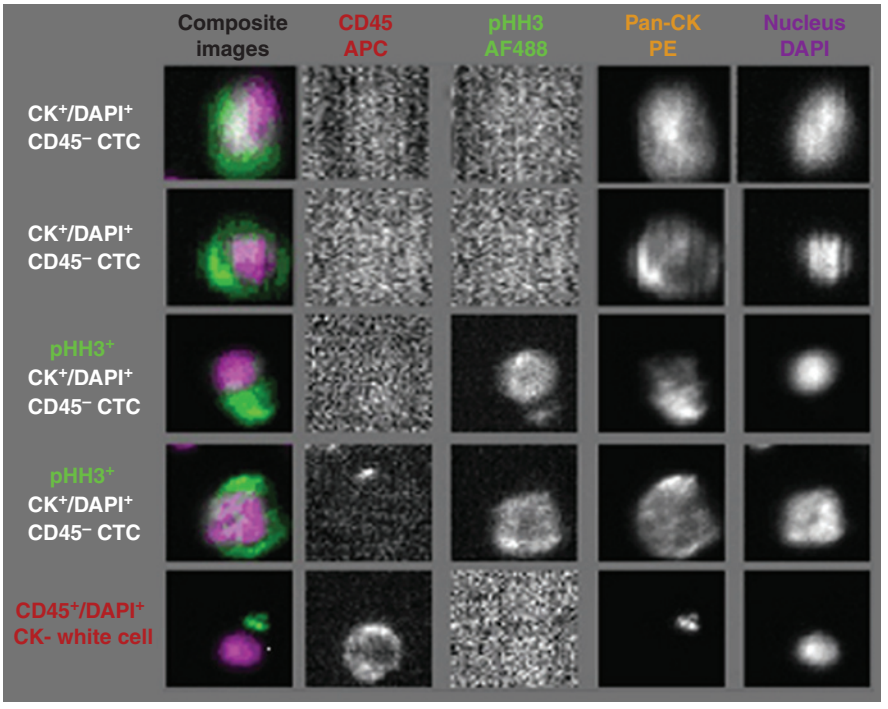

Figure 2. Molecular characterization of CTCs collected pre- and post-GSK461364 treatment. The images show 5 cell events from a patient with MBC 24 hours after the first administration of GSK461364. The first and second rows show images for an event qualifying for a CTC: nucleated, positive for pan-cytokeratin (CK) antibody and negative for the common leukocyte antigen CD45. The third and fourth rows show 2 CTCs with the above-mentioned characteristics, as well as phosphorylation of Ser473 histone H3 (pHH3), suggesting mitotic arrest. The bottom row shows a nucleated white cell that is positive for CD45 and negative for CK and pHH3.

with clinical benefit and RECIST SD for a further 10 weeks. Archived tumor samples from 22 patients were analyzed for Ki-67, pHH3, and Plk1 expression. Patients with prolonged SD for more than 16 weeks ($n = 4$) had a significantly higher percentage of positive cells for proliferative and mitotic markers [Ki-67 ($P = 0.042$), pHH3 ($P = 0.006$), and Plk1 ($P = 0.018$)] than those who progressed earlier. An esophageal cancer patient with SD for 23+ weeks was found to have very high expression of Ki-67 and PLK-1, and elevated pHH3 levels with a 20% shrinkage of his target tumor lesions after 4 cycles of GSK461364 (Fig. 3).

Discussion

This is the first trial of a Plki to be prospectively designed to provide proof-of-principle pharmacodynamic endpoints alongside tumor response evaluation. Here, assays of pHH3 in CTCs along with CTC counts confirmed preclinical evidence that GSK461364 effectively targets Plk1 in tumor. This study was also the first to provide full pharmacokinetic assessment of 2 competing schedules of GSK461364, a weekly (schedule A) versus a twice-weekly regimen (schedule B).

Observed toxicity was generally mild, with no neuropathy described. It is noteworthy that the toxicity profile of this drug was different from that observed in phase I trials of 2 other Plk inhibitors (BI2536 and ON01910.Na). Neutropenia was observed in 18% of patients on GSK461364 (and was related to a DLT in 3 patients). This differs from the phase I trial of BI2536 (20), in which neutropenia was observed in 45% of patients. In contrast, neutropenia was not a side effect described with ON01910.Na (21). Myelotoxicity is in keeping with the mechanism of action of these agents (10). Other toxicities such as fatigue or nausea reported with GSK461354 were also common with BI2536 (20) and ON01910.Na (21).

Grade 1–2 infusion site reaction and phlebitis occurred in a significant proportion of patients with both schedules, even after the less irritating Captisol formulation was substituted; thus, many patients opted to receive the drug via central venous catheter rather than peripherally. Nonfatal PE and portal vein thrombosis were seen in 6 patients enrolled in schedule B; 4 were considered drug related by the investigator. Therefore, from the safety perspective, schedule A was the more favorable regimen. Neither thrombophlebitis nor VTE was observed with BI2536 (20) or ON01910.Na, suggesting that this may be an off-target toxicity (21).

The finding of VTE in patients on this study is in marked contrast to the toxicity profile observed in preclinical testing of GSK461364. The underlying etiology of these thrombotic events is unknown, and VTE events have not been observed in trials of other Plki (20, 21). It is widely accepted that cancer patients have a predisposition to VTE (22), but at 20%, the incidence of VTE in this study was considerably higher than 2% to 4.2% rate of events previously observed in a retrospective study of cancer patients (23). Decreased mobility due to weekly overnight admissions that included cardiac monitoring could have exacerbated VTE risk in patients on schedule B. Alternatively, the prevalence of VTE and thrombophlebitis observed in this study may be due to off-target or on-target effects of GSK461364 on endothelial cells.

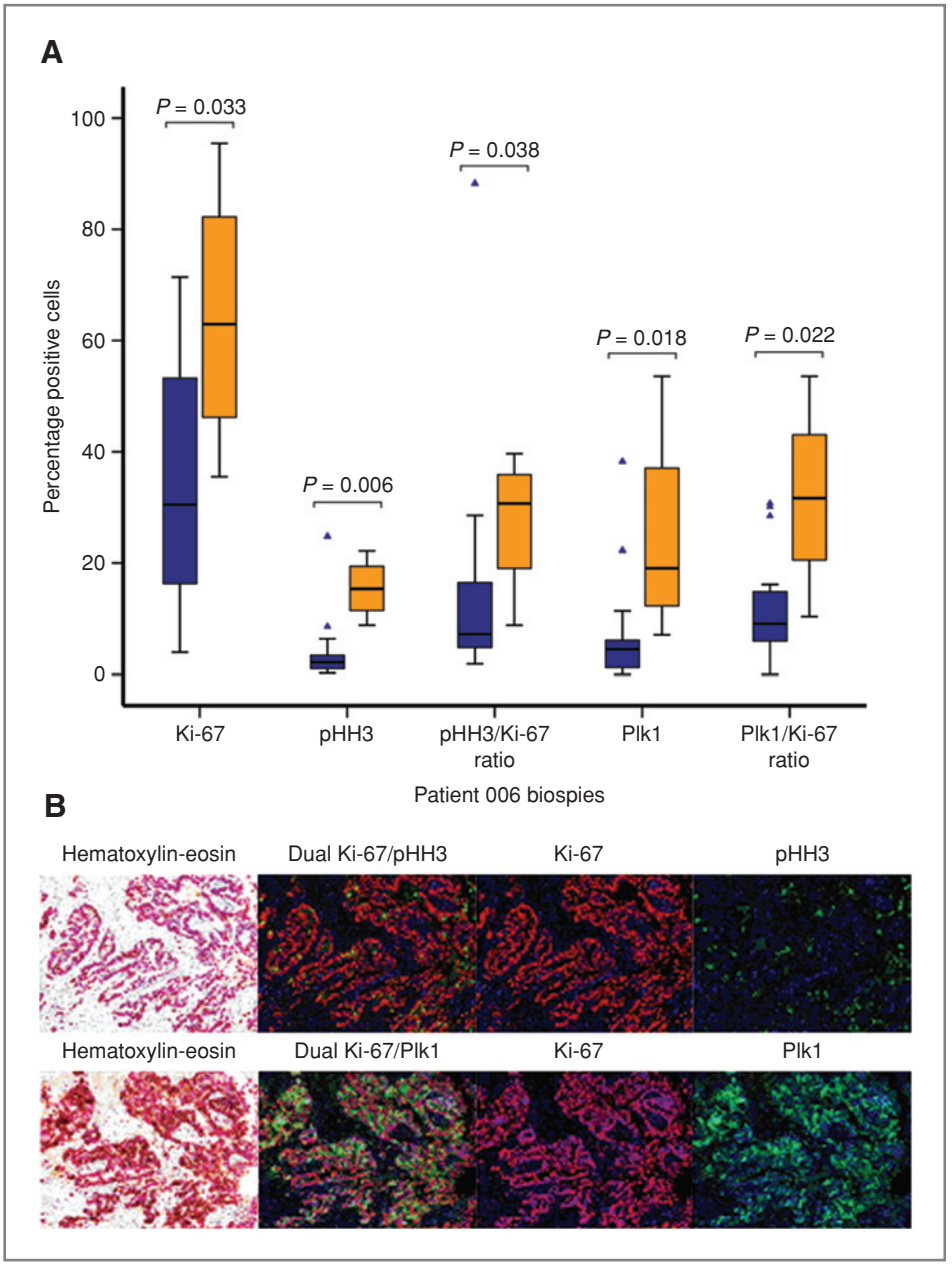

Figure 3. A, box-plot diagrams showing percentage staining intensity scores of archived formalin-fixed paraffin-embedded tumor samples ($n = 22$) as determined by immunohistochemistry (IHC). Results are separated according to time to progression: blue boxes represent patients progressing less than 16 weeks ($n = 18$), whereas orange boxes represent patients progressing more than 16 weeks (prolonged SD) after starting treatment with GSK461364 ($n = 4$). Boxes define the 25th and 75th percentiles; the horizontal line into the boxes indicates the median; and bars define the lowest and highest values within 1.5 times of the interquartile range. The Δ represents outlying values. Statistical analysis: Mann–Whitney *U* test, all *P* values were 2-sided. This shows that the proliferative marker Ki-67 was significantly higher ($P = 0.042$) in the patients with prolonged SD (median: 63%; range: 35.5–95.4) versus the patients with rapid progression (median: 30.5%; range: 4.0–71.4). The mitotic marker, pHH3, was also significantly higher ($P = 0.006$) in patients with prolonged SD (median: 15.4%; range: 8.8–22.2) than in those with shorted disease control (median: 2.2%; range: 0.3–24.8). Finally, Plk1 median positive cells percentiles were also significantly elevated ($P = 0.018$) in patients with prolonged SD (median: 19.1%; range: 7.1–53.6) compared with the other group (median: 4.5%; range: 0%–38.2%). There was also a significant difference in the Ki-67/pHH3$^+$ ratio ($P = 0.038$) and for the Ki-67/Plk1 ratio ($P = 0.039$). B, images of IHC. Left to right, bright field images after IHC staining of a squamous esophageal carcinoma that had a decrease in target lesions by 20% and progressed clinically after 8 months of GSK461364 (top and bottom left); dual staining images for Ki-67 and pHH3 (second top left) and for Ki-67 and Plk1 (second bottom left) and single staining images for Ki-67 (second top and bottom right), pHH3 (top right), and Plk1 (bottom right). All stainings were done using hematoxylin as a counterstain. With exception of the bright field images on the left which show the original colors after IHC staining, all the other images are the recolored images as described in Patients and Methods for ease of enumeration. Ki-67–positive cells appear as red, pHH3- or Plk1-positive cells appear as green, and nuclei in blue.

Infusion site reactions, phlebitis, and VTE occurred across multiple doses. It is unknown whether these events are related; however, they may be due to an unintended, off-target effect. When considering on target effects, a recent meta-analysis of antiangiogenic drug trials showed an 11.9% incidence of VTE in patients with cancer receiving the anti-VEGF antibody bevacizumab (24). Enhanced angiogenesis was observed in *PLK3*$^{-/-}$mice and Plk3 is thought to be a negative regulator of HIF1α and thus VEGF (25). An hypothesis is that members of the Plk family exert competing control over vascular signaling and that targeted depletion of Plk1 disrupts this balance in favor of the antiangiogenic influence of Plk3 (25). However, there is no preclinical data to support this hypothesis and VTE events have not been observed as a class effect. It is noteworthy that, in our study, no VTE events occurred in patients who were receiving continuous prophylactic LMWH, suggesting that this provides adequate VTE prophylaxis.

GSK461364 showed dose-related RECIST antitumor activity in both schedules, with a best response of SD lasting greater than 4 months in 6 (15%) patients overall, 3 (13%) in schedule A and 3 (17%) in schedule B. Two patients, one with endometrial cancer and one with esophageal adenocarcinoma, had substantial prolonged RECIST SD for 43 and 56 weeks from the start of treatment. An additional 3 esophageal cancer patients had SD lasting 23, 23+ and 25+ weeks, respectively. Of the 6 responders, 2 had previously received taxane-based treatment. Interestingly, esophageal cancer is the only cancer type in which *PLK1* gene has been found to be amplified, rather than overexpressed, at mRNA or protein level (7).

CTCs were shown here to reliably confirm GSK461364 antimitotic activity in the patients from whom they were successfully obtained. This was reflected in a decrease in overall number of tumor cells and an increase in pHH3 within 24 hours of drug treatment. In addition, markers of mitotic activity in archived tumor tissue, such as Ki-67, pHH3, and Plk1, were shown to be significantly associated with clinical response (SD >16 weeks) among subjects with available archived tumor sample (4 of 22 patients; 18%). These results, although preliminary, imply that kinase inhibitors such as GSK461364 can be targeted toward patients with tumors of high mitotic activity. In addition, where technically feasible, CTCs can provide an early marker of drug activity (18).

This first-in man trial shows that GSK461364 effectively targets Plk1, has antitumor activity, and provides further support for this class of kinase inhibitors as effective anticancer agents. In view of the low incidence of myelotoxicity and neuropathy observed using GSK461364, it has potential for future use in combination with other cytotoxics, such as paclitaxel. Patients most likely to obtain clinical benefit from GSK461364 can be preselected by using markers of mitotic activity such as Ki-67 and pHH3 in tumor specimens. If feasible, CTCs can be used as pharmacodynamic markers of Plk1 inhibition, by enumerating total and pHH3-positive cells following drug exposure. In view of the high incidence of VTE observed, particularly in patients receiving bi-weekly dosing, we recommend that GSK461364 is administered as a weekly schedule at a dose of 225 mg given intravenously on days 1, 8, and 15 of a 28-day treatment cycle. We also recommend for future studies that patients are treated with concomitant daily LMWH as VTE prophylaxis.

Disclosure of Potential Conflicts of Interest

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