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Title: A phase I, pharmacokinetic and pharmacodynamic study of GSK2256098, a focal adhesion kinase inhibitor, in patients with advanced solid tumors

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ABSTRACT

Background: Focal adhesion kinase (FAK) is important in cancer growth, survival, invasion, and migration. The purpose of this study was to determine the maximum tolerated dose (MTD), safety, pharmacokinetics (PK), and pharmacodynamics (PD) of the FAK inhibitor, GSK2256098, in cancer patients.

Patients and methods: The dose of GSK2256098 was escalated in cohorts of patients with advanced cancer from 80 to 1500 mg, oral twice daily (BID), until the MTD was determined. Serial blood samples were obtained from all patients and the PK determined. Paired tumor biopsies were obtained in select patients and the level of phospho-FAK (pFAK) determined.

Results: Sixty-two patients (39 males, 23 females; median age 61 y.o., range 21-84) received GSK2256098. Dose-limiting toxicities of grade 2 proteinuria (1000 mg BID), grade 2 fatigue, nausea, vomiting (1250 mg BID), and grade 3 asthenia and grade 2 fatigue (1500 mg BID) were reported with the MTD identified as 1000 mg BID. The most frequent adverse events (AEs) were nausea (76%), diarrhea (65%), vomiting (58%), and decreased appetite (47%) with the majority of AEs being grade 1-2. The PK was generally dose proportional with a geometric mean elimination half-life range of 4-9 hours. At the 750, 1000, and 1500 mg BID dose levels evaluated, the pFAK, Y397 autophosphorylation site, was reduced by ~80% from baseline. Minor responses were observed in a patient with melanoma (-26%) and three patients with mesothelioma (-13%, -15%, -17%). In the 29 patients with recurrent mesothelioma, the median progression-free survival was 12 weeks with 95% CI 9.1, 23.4 weeks (23.4 weeks merlin negative, n=14; 11.4 weeks merlin positive, n=9; 10.9 wks merlin status unknown, n=6).

Conclusions: GSK2256098 has an acceptable safety profile, has evidence of target engagement at doses at or below the MTD, and has clinical activity in patients with mesothelioma, particularly those with merlin loss.

Introduction

Focal adhesion kinase (FAK, protein tyrosine kinase 2) is a non-receptor tyrosine kinase required for cancer cell growth, proliferation, survival, migration, angiogenesis, invasion and mesenchymal transformation [1]. Recent data indicate that FAK may be important in the maintenance of cancer stem cells and in macrophage activation [1, 2]. Over-expression of FAK (gene or protein) has been reported in several cancers, including breast, colorectal, head and neck, endometrium, lung, ovarian, pancreas, prostate, stomach, thyroid, and other solid tumors [3, 4] and hematologic cancers [5, 6]. FAK expression increases as tumors become more advanced and is associated with poor survival in ovarian, glioma, and acute myelogenous leukemia [6, 7, 8].

GSK2256098 is a potent, ATP-competitive inhibitor of FAK kinase activity and is highly selective for FAK with a ~1000 fold selectivity over the nearest family member *PYK2* [9]. Inhibition of FAK kinase activity has also been demonstrated in cells and *in vivo* as determined by decreased levels of pFAK in a concentration dependent manner [9]. *In vitro* cellular studies demonstrate that GSK2256098 inhibits cancer cell growth and induces apoptosis in cell selective and growth-dependent conditions [9]. GSK2256098 also inhibits cell migration, invasion [10], and angiogenesis [GSK internal data and 10]. As a single agent and in combination with other anticancer agents, GSK2256098 has demonstrated activity in *in vivo* models of ovarian cancer and glioblastoma [9, 11, 12].

The purpose of this first in cancer patient study was to determine the maximum tolerated dose (MTD), safety, pharmacokinetics (PK), pharmacodynamics (PD) and preliminary clinical activity of GSK2256098 in patients with advanced solid tumors.

PATIENTS AND METHODS

Patient selection

Signed, written informed consent was obtained from all patients and the study was approved by

independent ethics committee. Patients, ≥ 18 years of age, with histologically confirmed, advanced

solid tumors that were not responsive to standard therapy were eligible. For Part 1 of the study (see

study design below), patients with advanced solid tumors reported in the medical literature to

overexpress FAK were eligible. For Parts 2 and 3, patients with mesothelioma or cancers of the ovary,

pancreas, head and neck, stomach, endometrium, non-small cell lung, and prostate were eligible. Other

eligibility criteria included ECOG performance status of 0-1, adequate organ function (hematologic,

hepatic, renal), and ability to swallow oral medications. Female patients of child-bearing potential were

required to comply with protocol-defined contraceptive methods. Patients with symptomatic brain

metastases requiring steroids or anti-convulsant therapy were not eligible to participate.

Study design

This was a three-part Phase I study; Part 1 (Dose Escalation), Part 2 (Safety Expansion), and Part 3 (PD)

(NCT01138033). Patients received GSK2256098, orally twice daily, with a light meal (see online

supplemental material for details), until unacceptable toxicity, disease progression, or withdrawal of

consent. For Part 1, Dose Escalation, a Modified Acceleration Titration design [13] was used permitting

100% dose increases in single patient dose cohorts until a total of two patients in any cohort developed

Grade 2 toxicities within the first 21-day dosing period or one subject developed a DLT. At that point, a

standard 3 + 3 design was used. The MTD was defined as the dose level where ≤ 1 of up to 6 patients

had a dose-limiting toxicity within the first 21 days. Dose limiting toxicity (DLT) was identified as NCI

CTCAE v4.0 grade 3 or 4 non-hematologic toxicity (excluding nausea, vomiting, diarrhea without

adequate supportive care), grade 4 neutropenia > 5 days, febrile neutropenia, grade 4

anemia/thrombocytopenia, toxicity that resulted in ≥ 7 days of drug interruption (continuous or not) in the first 21 days, or any toxicity ≥ Grade 2 that in judgment of the study investigator was dose-limiting.

Study endpoints and assessments

Adverse events (AEs) were assessed continuously through the study with CTCAE v4.0. Hematology, urinalysis, clinical chemistry, and electrocardiograms were assessed at baseline, on Day 1, 8, 15, 22, then every three weeks thereafter. Fasting lipid panels were performed at baseline and day 22, day 43 then every 6 weeks. Disease assessments were performed at baseline and every six weeks and response assessed using RECIST 1.1 [14]. In patients with malignant pleural mesothelioma, their scans were also reviewed independently using Modified RECIST for Mesothelioma [15].

Translational research

Pharmacokinetics

Whole blood samples (2 mL) were collected during Parts 1-3 of the study and specific details regarding PK studies are found in the Supplemental materials online.

Tumor biopsy collection and determination of pFAK levels

Tumor biopsies were mandatory for patients in Part 3 and optional for those in Parts 1 and 2. Paired tumor biopsies were collected prior to dosing on Day 1 and on a day between Days 8-15, one to six hours after dosing.   Details regarding the pFAK analysis methods are found in the Supplemental materials online.

Evaluation of circulating tumor, endothelial, and endothelial progenitor cells

Details and references [16, 17, 18] regarding the evaluation of CTCs, CECs, and CEPs are found in the Supplemental materials online.

Determination of merlin status

Paraffin-embedded, archival tumor samples were required for all patients. Merlin (the protein product of the gene neurofibromin 2 or *NF2*) status was determined by immunohistochemistry of formalin fixed paraffin embedded (FFPE) archival samples collected from patients with mesothelioma (n=29) and a patient with melanoma (n=1). Details regarding the analysis of archival tumor samples for merlin expression are found in the Supplemental materials online.

Statistical analysis

The primary focus was the determination of the MTD, the safety profile, to identify a range of biologically active doses, and to determine the PK and PD of GSK2256098 in patients with solid tumors. The analyses were primarily descriptive or exploratory for toxicity, DLTs, and MTD. An exploratory analysis of progression-free survival (PFS) was conducted for the group of patients with mesothelioma. PFS was defined as the time from date of first dose of study drug to the date of first documented disease progression according to radiological or clinical assessment, or to date of death due to any cause. For patients who did not progress or die, PFS was censored at the time of last radiological disease assessment. Patients who discontinued the study with no post treatment tumor assessment were censored at date of first dose of study drug. Summaries of PFS and Kaplan-Meier curves were produced for all mesothelioma patients together and separately by merlin status.

RESULTS

Sixty-two patients were entered into the study and received at least one dose of GSK2256098. All patients had progressive disease of their tumor at study entry. Patient characteristics are provided in Table 1. Mesothelioma was the most common tumor type (n=29), and the rationale for enrollment of this tumor type is found below in Results and Discussion sections below. There were 26, 26, and 10 patients enrolled in Parts 1 (Dose Escalation), 2 (Safety Expansion), and 3 (Pharmacodynamics) of the study respectively.

Determination of the MTD

A summary of the dose levels evaluated and DLTs during Part 1 are provided in Table 2. One DLT was observed at the 1000 mg dose level, defined by reversible Grade 2 proteinuria (elevation in urine protein:creatinine (UPC) ratio requiring a protocol mandated dose reduction). The cohort was expanded to six patients at 1000 mg BID and was well-tolerated. Three patients were enrolled at the 1500 mg BID dose level, one had a DLT of Grade 3 asthenia. An additional two patients were enrolled and one had Grade 2 fatigue that was also considered dose limiting. Since the MTD was exceeded, an intermediate dose cohort of 1250 mg BID was enrolled with three patients. One patient had a DLT of grade 2 nausea, vomiting, and fatigue and a further 2 were enrolled. As overall tolerability to drug was poor at this level, no further enrollment occurred and 1000 mg BID was declared as the MTD.

Safety

A summary of AEs by dose, regardless of attribution, is provided in Table 3. The majority of AEs were grade 1-2 in severity with the four most frequent AEs being nausea, diarrhea, vomiting, and decreased appetite. The most frequent grade 3 AEs were hypertriglyceridemia, occurring in 3 patients (5%) and all at 1000 mg BID and hypokalemia occurring in 3 patients (5%), one at 750 mg BID and two at 1000 mg

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BID.   Two grade 4 AEs were reported, one patient with elevated blood creatinine phosphokinase and one patients with a cerebrovascular accident. Neither of these events was attributed to GSK2256098. Clinical laboratory AEs ≥ 20% included proteinuria (26%), hyperbilirubinemia (23%), and hypercholesterolemia (21%).

Dose reductions and interruptions

Dose reductions due to AEs occurred in seven (11%) patients, with nausea being the commonest reason (three patients, 5%). At the MTD of 1000mg BID (41 patients), there were six patients (15%) with dose reductions due to AEs with nausea being the most common reason (5%). Dose interruptions due to AEs occurred in 17 patients (27%). The AEs leading to dose interruptions included fatigue (6%), nausea (5%), vomiting (5%), decreased appetite (3%), diarrhea (3%), pleural effusion (3%), and pleuritic pain (3%). At the MTD of 1000mg BID (41 patients), 15 patients (37%) had dose interruptions due to AEs with fatigue, diarrhea, pleural effusion, and pleuritic pain (each 5%) being the most common reasons.

Pharmacokinetic analyses

When administered with a light meal on Day 1, GSK2256098 was rapidly absorbed (median tmax 1.5 to 4 hrs). The geometric mean half-life ranged between 4.0 and 9.0 hours. The Cmax and AUC, over the dose range of 80 to 1500 mg, were generally dose proportional after single and repeat dosing. A summary of the pharmacokinetics of GSK2256098 on Day 1 and 15 are provided in Table 4. The Cmax and AUC of GSK2256098 were lower after repeat dosing compared to Day 1 values.

Pharmacodynamic analyses

The percent decrease in Y397 pFAK/total FAK in paired pre- and on-treatment biopsy samples was determined from six patients at dose levels 750 mg BID , 1000 mg BID, and 1500 mg BID and was 80% or greater in 5 of 6 patients (Figure 1).

Circulating cells

CTCs, CECs, and CEPs were collected and analyzed at one clinical research center (GR). CECs were not affected by GSK2256098 treatment. CTCs were very low before and following treatment and no change was observed. However, a median decrease of 19% in CEPs from baseline values was noted.

Merlin analysis

Tumor tissue from 23 patients (79%) with mesothelioma was available for merlin evaluation by IHC analysis. Samples were either not available or not evaluable for 6 patients. Tissue from 14 patients (48%) stained negative for merlin indicating the putative loss of protein in these samples and the tissue from nine patients stained positive. One melanoma subject tested was identified as merlin negative

Clinical activity

A best response of stable disease was achieved in 28 patients (45%). In patients with measurable disease, changes in tumor size from baseline by RECIST, duration on treatment, and patient tumor type are shown in Figure 2. A summary of minor responses or prolonged stable disease are provided in Table 5. One patient with nasopharyngeal cancer had a 31% decrease from baseline in his target lesions but at the same scan date had a new lesion and was removed from the study due to progressive disease. In patients with malignant pleural mesothelioma, the overall median PFS (95% CI) was 12 weeks (9.1, 23.4). In patients with merlin negative mesothelioma (n=14), merlin positive (n=9), or unknown (n=6), the median PFS (95% CI) was 23.4 (6.0, 28.1), 11.4 (4.3, 22.6), and 10.9 (9.1, not determined) weeks respectively.

DISCUSSION

The importance of FAK in multiple biological processes of cancer, including invasion and metastases means that targeting FAK is a rational treatment strategy. An earlier single-dose, dose ranging, first time

in human study evaluated the pharmacokinetics, safety, and food effect in healthy volunteers (NCT00996671). The current study described here is the first in cancer patient and repeat-dose study of GSK2256098, an oral selective inhibitor of FAK, in a patient population with advanced and metastatic cancers. In this study the safety, PK, and clinical activity were evaluated over a dose range of 80 to 1500 mg BID and tumor PD was performed at doses of 750, 1000, and 1500 mg BID.

GSK2256098 had an acceptable safety profile at and below the MTD. Overall, the majority of AEs were Grade 1-2 in severity. Gastrointestinal AEs were the most common AEs and were the major reason for dose reductions and interruptions. Reversible proteinuria, seen at doses of 750 mg twice daily and higher, was present in 26% of patients and was observed during preclinical studies at high doses in 28-day preclinical safety studies in rats and dogs (GSK internal data). Increases in total and direct bilirubin were observed. Increased total bilirubin was also seen in 28-day preclinical animal safety studies although only total bilirubin was measured (GSK internal data). *In vitro*, GSK2256098 is an inhibitor of UGT1A1 at concentrations achieved in this study. Elevated cholesterol and triglycerides was also seen in the current study and in preclinical animal safety studies. The mechanism for this increase is unclear.

At the MTD dose of 1000 mg BID, a reduction in Cmax and AUC were observed on Day 15 compared to Day 1, while a comparison of the the terminal elimination phases appeared similar (i.e. were parallel) between the two days. This finding suggests a change in bioavailability, perhaps due to changes in absorption with repeat dosing, rather than an alteration in systemic clearance. Autoinduction of key drug metabolizing enzymes in the gut resulting in a reduction in Cmax and AUC is one potential explanation for the reduction. Additional pharmacokinetic sampling and *in vitro* data are required to fully understand the mechanism of these changes.

Target engagement (decreased pFAK from baseline) was observed in multiple tumor types and was similar across the dose range of 750, 1000, and 1500 mg BID, the only doses at which biopsies were

obtained. No correlation was observed between different measures of GSK2256098 systemic exposure

and pFAK inhibition, possibly due to concentrations being in the range of maximal response on the dose-

response curve for target engagement. Given that minor tumor responses were seen across the range of

doses evaluated, including at the very first dose evaluated at 80 mg BID, it would be of interest to see if

target inhibition is occurring at lower doses. An ongoing clinical study of GSK2256098 is evaluating pFAK

inhibition at lower GSK2256098 doses (FAK114746) in combination with trametinib. At doses of 250 mg

and 500 mg twice daily of GSK2256098, pFAK is reduced by more than 80 and 60% respectively [19].

During the conduct of the study, a patient with malignant pleural mesothelioma in the 300 mg BID

cohort, with four prior regimens, was noted to have a 15% decrease in tumor size. Upon treatment with

GSK2256098, this patient continued on therapy for 191 days. Analysis of the patient's archival tumor

sample indicated that the tumor was merlin negative. Merlin is a tumor suppressor frequently lost (40-

50%) in mesothelioma [20]. In mesothelioma cell lines, merlin negative cells have increased

invasiveness and FAK expression [21]. Auger et al demonstrated that merlin negative mesothelioma cell

lines were >100X more sensitive that a merlin positive cell line to GSK2256098 [9]. Shapiro et al have

also noted increased sensitivity of merlin negative mesothelioma cells to a small molecule inhibitor of

FAK [22].   Although merlin negative mesothelioma cells have greater sensitivity to GSK2256098,

antitumor activity is also seen in a merlin positive mesothelioma cell line [9]. Based on the clinical and

laboratory findings noted above, additional enrollment of patients with mesothelioma was encouraged.

PFS in recurrent mesothelioma is poor with a recent Phase 3 study of vorinostat versus placebo in

recurrent mesothelioma reporting a median 6 week PFS in the treatment and placebo groups [23]. In

both merlin negative and merlin positive mesothelioma patients, the PFS for both groups was greater

was noted in the vorinostat study thus supporting the finding in the *in vitro* studies of GSK2256098

noted above [9] that activity is present in both merlin groups. The current study is unable to determine

whether merlin negativity is a prognostic or predictive biomarker and well-designed, prospective, clinical studies are needed to answer this question.

Merlin negativity may result in increased sensitivity of other tumor histologies to the FAK inhibitor GSK2256098. A patient with metastatic melanoma in the very first cohort (80 mg BID) was noted to have a minor response (26% decrease). This patient had progressed on two prior investigational small molecules and radiation therapy before receiving GSK2256098. The archival tumor from this patient was merlin negative. Additional laboratory and clinical studies, including evaluation of additional patient tumor specimens, are required to validate this hypothesis. Approximately 43% of meningioma has inactivated NF2 [24]. A recent study of GSK2256098 has been initiated in patients with recurrent meningioma that has mutant NF2 (NCT02523014)

Defactinib (VS-6063), a small molecule inhibitor of FAK and Pyk2, is in clinical development [25]. A Phase 2, double-blind, placebo-controlled study of defactinib as maintenance therapy for mesothelioma following first-line treatment (COMMAND), with patients stratified based on merlin status, was stopped for futility due to an insufficient level of efficacy (www.verastem.com). Given that defactinib targets FAK and Pyk2 [25], while GSK2256098 is selective for FAK alone, it is unclear if this difference in target selectivity may result in different antitumor activity between the two compounds.

A recent positive Phase 3 trial of bevacizumab in mesothelioma supports the potential use of a FAK inhibitor since FAK signals through VEGF pathway and VEGF/VEGFR act as an autocrine loop in mesothelioma [26] so the use of a FAK inhibitor may be rational.

This study provides preliminary evidence that GSK2256098 is active in patients with recurrent, mesothelioma with potentially enhanced clinical activity in merlin negative mesothelioma. Future

strategies could include preselecting patients for GSK2256098 by tumor merlin expression or using

GSK2256098 in a treatment combination. Preliminary results from a Phase Ib combination study of

GSK2256098 and trametinib (MEK inhibitor) is ongoing and is being evaluated in multiple tumor types

including mesothelioma [19].

REFERENCES

References are found in the Supplemental materials online.

Table 1. Patient Characteristics

Characteristic	No. of Patients 62 (%)
Age (years)	
Median (Range)	61 (21-84)
Gender	
Males/Females	39/23 (63/37)
ECOG Performance Status	
0/1	27/35 (44/56)
Race	
White (European ancestry	54 (87)
Black (African ancestry)	2 (3)
Southeast Asian	2 (3)
Arabic/North African	2 (3)
South Asian	1 (2)
Mixed Race	1 (2)
Median No. of Prior Therapies (range)	2 (1-8)
Tumor Types	
Mesothelioma	29 (46)
Ovary	8 (13)
Pancreas	6 (10)
Colon/rectum	3 (5)
Kidney	3 (3)
Melanoma	2 (3)
Non-small cell lung	2 (3)

Thyroid   2 (3)

Other[1]   7 (11)

[1] - Includes one each of angiosarcoma, bile duct cancer, bone cancer, bronchial cancer,
   hepatocellular carcinoma, cancer of the mouth, and cancer of the nasopharynx

Table 2. Determination of the maximum tolerated dose

Cohort	Dose (mg twice daily)	N	DLT
1	80	1	None
2	160	1	None
3	300	3	None
4	600	2	None
5	1000	6*	Grade 2 proteinuria with dose interruption
6	1500	5	Grade 3 asthenia Grade 2 fatigue
7	1250	5	Grade 2 fatigue, nausea and vomiting

*- Three additional patients enrolled (9 total) following dose escalation, no additional DLTs occurred.

Table 3. Adverse Events ≥ 20% - All Doses Regardless of Causality

Adverse Event	All Grades n (%)	Grade 3 n (%)
Any event	62 (100)	Grade 3: 24 (39) Grade 4: 2 (3)
Nausea	47 (76%)	0
Diarrhea	40 (65%)	0
Vomiting	36 (58%)	1 (2%)
Decreased Appetite	29 (47%)	0
Proteinuria	16 (26%)	0
Fatigue	15 (24%)	1 (2%)
Asthenia	14 (23%)	1 (2%)
Increased Total Serum Bilirubin	14 (23%)	0
Constipation	13 (21)	0

Increased Total Cholesterol	13 (21%)	1 (2%)

Additional Grade 3 adverse events n (%): Hypertriglyceridemia 3 (5), dyspnea 2 (3), hypercalcemia 2 (3), hypercholesterolemia 2 (3), hyperlipasemia 2 (3), hypokalemia 2 (3), lymphopenia 2 (3), pleuritic pain 2(3), upper abdominal pain 1 (2), agitation 1 (2), increased amylase 1 (2), bile duct obstruction 1 (2), chest pain 1 (2), increased blood creatinine phosphokinase 1 (2), increased serum GGT 1 (2), headache 1 (2), hypercalcemia 1 (2), hyperglycemia 1 (2), hypertension 1 (2), hypoacusis 1 (2), hypokalemia 1 (2), hypophospatemia 1 (2), interstitial lung disease 1 (2), loss of consciousness 1 (2), musculoskeletal pain 1 (2), neutropenia 1 (2), osteoarthritis 1 (2), pericardial effusion 1 (2), pathological fracture 1 (2), peripheral sensory neuropathy 1 (2), pleural effusion 1 (2), pleural neuroplasm 1 (2), sciatica 1 (2), tumor pain 1 (2)

Grade 4 adverse events n (%): Increased blood creatinine phosphokinase 1 (2), cerebrovascular accident 1 (2)

Table 4: Summary of GSK2256098 pharmacokinetic parameters after single and repeat dose administration of GSK2556098 on day 1 and day 15 (Parts 1-3)

Dose regimen Day 1	n	C_{max},[a] ng/mL	t_{max},[b] h	$AUC_{0-\infty}$,[a] h•ng/mL	$t_{1/2}$,[a] h
80 mg	1	203	3.0	1013	4.5
160 mg	1	392	4.0	1910	5.5
300 mg	3	2439 (30)	1.5 (1.0, 2.0)	9962 (39)	9.0 (62)
600 mg	2	6006 (45)	2.3 (1.5, 3.0)	20094 (45)	4.7 (23)
750 mg	3	4035 (22)	3.2 (1.5, 3.5)	19258 (42)	4.7 (34)
1000 mg	39	7058 (46)	2.1 (1.0, 6.1)	33528 (45)	4.4 (25)
1250 mg	5	8557 (57)	3.0 (2.5, 4.0)	40136 (65)	5.1 (40)
1500 mg	5	10452 (47)	3.0 (2.1, 4.0)	50266 (48)	4.0 (16)
Dose regimen Day 15	n	C_{max},[a] ng/mL	t_{max},[b] h	$AUC_{0-\tau}$,[a] h•ng/mL	C_{τ},[a] ng/mL
80 mg BID	1	239	3.0	1110	21.2
160 mg BID	1	482	4.0	2783	0
300 mg BID	3	1766 (29)	2.0 (1.5, 2.0)	8603 (37)	233 (56)
600 mg BID	2	2635 (164)	1.4 (1.3, 1.5)	9549 (134)	337 (31)
750 mg BID	3	4130 (6.3)	3.0 (1.1, 4.0)	15062 (30)	242 (63)
1000 mg BID	34	5946 (33)	2.1 (1.0, 8.0)	24758 (26)	465 (67)
1250 mg BID	3	6433 (55)	3.0 (2.1, 3.0)	24803 (81)	285 (56)
1500 mg BID	3	8806 (6.3)	2.0 (1.5, 3.0)	32223 (9.0)	316 (88)

[a] Data reported as geometric mean (CV%)
[b] T_{max} reported as median (range).

Table 5. Response Characteristics of Selected Patients

Tumor type	Merlin status	Dose (mg twice daily)	Best response	% Decrease in Tumor from Baseline	Duration on Study (days)
Melanoma	Negative	80	SD	26	377
Mesothelioma	Negative	300	SD	13	191
Mesothelioma	Positive	1000	SD[1]	17	294
Mesothelioma	Negative	1000	SD	15	169
Nasopharynx	ND[2]	1000	SD	25	209
Kidney	ND[2]	1000	SD	6	452

[1] - By independent review using modified RECIST for mesothelioma, this patient had an unconfirmed PR (34% decrease from baseline)

[2] - Not determined

Figure Legends

Figure 1. Inhibition of pFAK activity in tumor at 750, 1000, and 1500 mg twice daily BID in patients receiving GSK2256098.

Figure 2. Three dimensional plot of maximum reduction in tumor size (RECIST 1.1), duration on treatment, and tumor type. Merlin status is provided in patients with mesothelioma and in one patient with melanoma (positive, negative, or unknown).

SUPPLEMENTAL ONLINE MATERIAL

Administration of GSK2256098

Patients were instructed to take GSK2256098 twice daily, orally 30 minutes after a light meal was completed. While in clinic for PK sampling periods, GSK2256098 was administered with a light meal. A "light" breakfast might consist of a bowl of cereal and two pieces of toast with spreads (honey, jam, butter, etc). A standardized dinner may consist of a small portion of meat (chicken, beef, lamb) plus vegetables/salad and either rice or a small bread roll would be the standard. Otherwise, a serving of pasta or a slice of lasagne with a bread roll and salad would be appropriate. Dessert can also accompany the main meal (a small tart, piece of cake, small cheese platter). For a given subject, the morning and evening meals were to be as similar as possible to standardize conditions associated with PK sample collection. When subjects are not in clinic, they were instructed to take GSK2256098 twice daily, orally after a light meal of their choosing as long as it complied with the exclusionary food list (subjects must abstain from consuming red wine, Seville oranges, grapefruit or grapefruit juice and/or kumquats, pummelos, exotic citrus fruit (i.e., star fruit, bitter melon), grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication and throughout the remainder of the time on study.

Translational research

Pharmacokinetics

Whole blood samples (2 mL) were collected in tubes containing EDTA and then spotted on dried blood spot cards. In Part 1, samples were collected prior to dosing on Day 1 and Day 15, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours after dosing. In Part 1, a single dose of GSK2256098 was administered on Day 1 and from Day 2 onwards was administered twice daily (BID) on a continuous dosing schedule.

Samples were also collected from patients in Part 2 (Day 1 serial PK for 12 hours post-dose and single pre-dose samples on Days 8, 15, 22, and 43) and Part 3 (Day 15 serial PK for 12 hours post-dose, pre- and post-dose on day of tumor biopsy). Human blood samples were analyzed for GSK2256098 using a validated analytical method based on extraction from dried blood spots on Whatman FTA paper, followed by ultra high-performance liquid chromatography with tandem mass spectrometric detection analysis (UHPLC/MS/MS). The lower limit of quantification for GSK2256098 was 10 ng/mL, using a 3 mm punch taken from a 15 μL dried human blood spot, with a higher limit of quantification of 10,000 ng/mL.

GSK2256098 pharmacokinetic parameters were calculated after single and repeat dosing using standard noncompartmental methods. Dose proportionality was assessed using a power model and declared based on the pre-specified criteria (90%CI) of 0.5 to 2.0 based on the slope. To estimate the accumulation after repeat dosing and to assess time invariance, ratios of area under the concentration-time curve over the dosing interval ($AUC_{0-\tau}$) on Day 15 to AUC(0-τ) AUC(0-12) on Day 1 and area under the concentration-time curve from time zero extrapolated to infinity ($AUC_{0-\infty}$) on Day 1, respectively were calculated.

Tumor biopsy collection and determination of pFAK levels

Tumor biopsies were mandatory for patients in Part 3 and optional for those in Parts 1 and 2. Paired tumor biopsies were collected prior to dosing on Day 1 and on a day between Days 8-15, one to six hours after dosing.   Samples were analyzed for pFAK and total FAK by a proprietary Collaborative-Enzyme Enhanced Reactive-immunoassay (CEER) (Prometheus Laboratories, California, USA). A proximity based immunoassay, the CEER assay leverages a multiplexed immune-microarray platform combined with triple-antibody-enzyme channeling signal amplification. Specific signal amplification occurs when target proteins (pFAK and FAK) captured on an antibody microarray co-localize with two

additional detector-antibodies linked with channeling enzymes (horseradish peroxidase, HRP and glucose oxidase, GO). pFAK levels were normalized to total FAK.

Evaluation of circulating tumor, endothelial, and endothelial progenitor cells

Blood was collected prior to dosing on Day 1, 15, 30, and 60 for the isolation of circulating tumor cells (CTCs). Measurement of CTCs was performed using the CellSearch method (Veridex, Raritan, NJ, USA). In addition, a telomerase activity based method which can detect CTCs with a high sensitivity and specificity was also used [16]. Additional blood was collected prior to dosing on Day 1 and on Days 30 and 60 and enumeration of circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs) was performed. Measurement of CECs was performed using previously published methods [17, 18]. Measurement of CEPs was performed using four color flow cytometry after progenitor cell enrichment [17].

Determination of merlin status

Paraffin-embedded, archival tumor samples were required for all patients. Merlin (the protein product of the NF2 gene) status was determined by immunohistochemistry of formalin fixed paraffin embedded (FFPE) archival samples collected from patients with mesothelioma (n=29) and a patient with melanoma (n=1). The immunohistochemistry assay was developed and conducted by Mosaic Laboratories (Lake Forest, California, USA). The primary antibody included an NF2 antibody (C-18): sc332, Santa Cruz Biotechnology (Dallas, Texas, USA). A cell line tissue microarray comprising a total of six high, moderate and low merlin expressors and a rabbit IgG served as the positive and negative control, respectively. Merlin status from archived tumor tissue was recorded as the percentage of cells that stain at +2, +3 using the above assay and then dichotomized as either "Merlin Negative" if the percentage of neoplastic cells stained in the intensity staining category 3 and 2 equaled 0 or was less than 10 in category 2, or "Merlin Positive" otherwise.

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