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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 was well tolerated, had evidence of target engagement at doses at or below the MTD, and had clinical activity in patients with mesothelioma, particularly those with merlin loss.

Key words: focal adhesion kinase, Phase I, pharmacokinetics, pharmacodynamics, mesothelioma, merlin, NF2

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 were required to demonstrate lack of child-bearing potential or comply with protocol-defined contraceptive methods. Patients with symptomatic brain metastases requiring steroids or anti-convulsant therapy were not permitted.

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, 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 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). 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. 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 in the Discussion.

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 which 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 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). 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%).

Pharmacokinetic analyses

Following administration 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 were 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%). A summary of minor responses or prolonged stable disease of interest 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. This study describes the first in cancer patient study of GSK2256098, an oral selective inhibitor of FAK, in a patient population with advanced and metastatic cancer. 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. An earlier single dose, dose ranging, first time in human study (healthy volunteers) evaluated the pharmacokinetics, safety, and food effect in healthy volunteers (NCT00996671).

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 resulting in Gilbert's like effect. 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 was observed on Day 15 compared to Day 1, while the terminal phase appeared similar between the two days. This suggests a change in bioavailability, perhaps due to changes in absorption with repeat dosing rather than an alteration in systemic clearance.

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]. 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 [23]. 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 [24].

Merlin negativity may result in 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 are required to validate this hypothesis.

This study provides evidence that GSK2256098 is active in patients with recurrent, merlin negative mesothelioma. In view of the good tolerability observed, future strategies could include preselecting patients for GSK2256098 by tumor merlin expression or using GSK2256098 in a treatment combination.

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Disclosure

At the time of the clinical study, D. Gibson, V. Peddareddigari, S. Murray, N. Nebot, J. Mazumdar, L. Swartz, K.R. Auger, and R.A. Fleming were employees of GlaxoSmithKline.

All remaining authors have declared no conflict of interest.

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: 26 (42) 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%)

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 volume (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).