First-in-Human Study of AT13148, a Dual ROCK-AKT Inhibitor in Patients with Solid Tumors

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ABSTRACT

Purpose: AT13148 is an oral AGC kinase inhibitor, which potently inhibits ROCK and AKT kinases. In preclinical models, AT13148 has been shown to have antimetastatic and antiproliferative activity.

Patients and Methods: The trial followed a rolling six design during dose escalation. An intrapatient dose escalation arm to evaluate tolerability and a biopsy cohort to study pharmacodynamic effects were later added. AT13148 was administered orally three days a week (Mon–Wed–Fri) in 28-day cycles. Pharmacokinetic profiles were assessed using mass spectrometry and pharmacodynamic studies included quantifying p-GSK3β levels in platelet-rich plasma (PRP) and p-cofilin and p-MLC2 levels in tumor biopsies.

Results: Fifty-one patients were treated on study. The safety of 5–300 mg of AT13148 was studied. Further, the doses of 120–180–240 mg were studied in an intrapatient dose escalation cohort. The dose-limiting toxicities included hypotension (300 mg), pneumonitis, and elevated liver enzymes (240 mg), and skin rash (180 mg). The most common side effects were fatigue, nausea, headaches, and hypotension. On the basis of tolerability, 180 mg was considered the maximally tolerated dose. At 180 mg, mean C_{max} and AUC were 400 nmol/L and 13,000 nmol/L/hour, respectively. At 180 mg, ≥50% reduction of p-cofilin was observed in 3 of 8 posttreatment biopsies.

Conclusions: AT13148 was the first dual potent ROCK-AKT inhibitor to be investigated for the treatment of solid tumors. The narrow therapeutic index and the pharmacokinetic profile led to recommend not developing this compound further. There are significant lessons learned in designing and testing agents that simultaneously inhibit multiple kinases including AGC kinases in cancer.

Introduction

PI3Ks are key mediators of intracellular signaling between the membrane-bound receptor tyrosine kinases (RTK) and downstream effector molecules, such as AKT/PKB, m-TOR, GSK3β, S6K, and S6, which control a range of vital cellular functions deregulated in cancer cells, including cellular growth, proliferation, and survival (1). There are currently multiple drugs licensed for use in the treatment of cancer in this pathway, i.e., PI3Kα, PI3Kδ, and m-TOR inhibitors (2–5).

AGC kinases are a group of evolutionarily related serine threonine kinases, some of which are deregulated in cancer (6). PDK1/PKA/PKB and S6K are key components of the PI3K pathway and have been targeted using a wide range of PI3K pathway inhibitors (1). However, there remain other AGC kinases such as Rho-associated coiled-coil containing protein kinase (ROCK) that influence growth (7–9) and metastasis of cancer cells independent of the PI3K pathway (10). ROCK-myosin II signaling has also recently been shown to be important in modulating resistance to targeted therapy in melanoma and an immunosuppressive environment within tumors (11).

AT13148 was discovered using a high-throughput X-ray crystallography and fragment-based drug design as an AGC kinase inhibitor that inhibited AKT and other AGC kinases such as ROCK (12). It was hypothesized that additional AGC kinase activity would result in increased antiproliferative and antimetastatic efficacy and possibly reduce resistance to selective AKT inhibition (13, 14). Preclinical studies have evaluated the efficacy of AT13148 in blocking invasion and metastasis in melanoma and pancreatic cancer models (14, 15). In addition, AT13148 has been shown to inhibit PI3K signaling and induce cell death in a range of preclinical models, including gastric and prostate cancer models (13, 16). There are currently multiple selective AKT inhibitors in late stages of clinical development (17) and ROCK inhibitors are licensed for use in nononcologic indications, such as pulmonary hypertension (18), but have not been clinically tested in patients with cancer until now. Herein, we report the results from the first-in-human phase I study of AT13148, a potent dual ROCK-AKT inhibitor, in adults with advanced solid tumors.

Patients and Methods

Design

The study followed a rolling six dose escalation design (19). Following an amendment, two cohorts were added. The first included an intrapatient dose escalation in patients at dose levels 120–180–240 mg starting with one week dosing at the lowest dose followed by escalating to the next dose of 180 mg until the highest dose of 240 mg during cycle 1. The aim of this cohort was to evaluate whether the on-target

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

AGC (protein family A, G, and C) kinases are serine threonine kinases, which include ROCK, AKT, and p70S6K. They are key anticancer targets with relevance to motility, metastasis, survival of cancer cells and an immune-suppressive state within tumors. AT13148 is a potent ROCK and AKT inhibitor. The clinical tolerability profile was dominated by dose-limiting toxicities suggestive of ROCK inhibition (hypotension and headaches) and not AKT inhibition (hyperglycemia and rash). Careful consideration is warranted while developing multikinase inhibitors involving AGC kinases as they can have broad activity in preclinical models, but their clinical development using systemic routes of administration may be limited by side-effect profiles related to potent inhibition of ROCK.

hypotension would be better tolerated if the dose was increased in a stepwise fashion. The second amendment introduced a mandatory biopsy cohort where fresh biopsies were collected before and after treatment at the recommended phase II dose (RP2D) of 180 mg. The trial was conducted to ethical principles laid down by the Declaration of Helsinki and the protocol was reviewed by regulatory agencies (MHRA, UK) and a UK national research ethics committee. Written informed consent was obtained (patients were given an information sheet and signed a consent form) prior to patients enrolling in the clinical trial. Inclusion criteria included the fact that patients had received standard-of-care treatment for their metastatic solid tumors and had adequate renal and hepatic function (full inclusion and exclusion criteria in Supplementary Data). Key exclusion criteria included an abnormal fasting blood sugar and a history of hypertension on antihypertensive medication.

AT13148 was administered orally three days a week (Mon–Wed–Fri) in 28-day cycles. Patients were seen weekly for toxicity assessments and imaging to evaluate tumor response was conducted every 2 cycles.

Pharmacokinetics

A single dose of AT13148 was administered 7 days before cycle 1 for pharmacokinetic analysis. A full pharmacokinetic profile was also carried out on cycle 2, day 1. Blood was collected at baseline; 15 minutes; 30 minutes; and 1, 2, 6, 12, 24, 48, 72, and 96 hours postdose for analysis (the last two being optional from cohort 6, 160 mg, cycle 1). Plasma concentrations of AT1318 were measured by LC-MS (see Supplementary Data). Pharmacokinetic parameters were derived from Phoenix WinNonlin using noncompartmental analysis.

Pharmacodynamics

Blood samples for pharmacodynamics were taken at baseline, 2, 6, 24, and 48 hours after treatment on the run-in dose at day −47 to −4 and on cycle 2, day 1. Phosphorylation of GSK3β Ser9 (p-GSK3β) was quantified in platelet-rich plasma (PRP) extracted from serial blood samples using electrochemiluminescent immunoassays on the Meso-Scale Discovery (MSD) technology platform using previously validated techniques (20). Relative changes in p-GSK3β were reported as a ratio, calculated as the percentage of baseline levels normalized to total GSK3β. Phosphorylated cofilin protein levels in tumor biopsies predose and post-AT13148 treatment (between cycle 1, day 15 or cycle 2, day 1, 24 hours posttreatment) were measured using the Protein Simple WES Assay platform. Changes in phospho- and total cofilin normalized to GAPDH were measured in the form of chemiluminescence signals with the area under the curve (AUC) calculated for each analyte by the WES Compass software. Relative changes in phosphorylated cofilin were reported as a percentage of pre-dose protein levels.

Phosphorylation of MLC2 was quantified using IHC. Four-micron–thick sections were incubated at 60°C for 20 minutes and then subjected to antigen retrieval using Access Super Tris pH 9 buffer (A Menarini Diagnostics) at 110°C for 6 minutes in a Decloaking Chamber NxGen (Biocare Medical). Samples were blocked with Dual Endogenous Enzyme-Blocking Reagent (Dako) for 10 minutes and were then incubated with primary antibodies for 40 minutes at room temperature, washed, and then incubated with biotinylated secondary antibodies (rabbit, mouse or rat; 1:200; Vector Laboratories) for 30 minutes at room temperature. Signal was then amplified using a VECTASTAIN ABC HRP kit (PK-4000) for 20 minutes at room temperature and the reaction was developed using VIP substrate (SK-4600, Vector Laboratories) for 10 minutes at room temperature. Stainings were counterstained with hematoxylin. The slides were imaged using a NanoZoomer S210 slide scanner. Staining quantification was performed using QuPath 0.1.2. To quantify p-MLC2, the images were analyzed using positive cell detection and three different thresholds were applied according to the intensity scores (0, 1, 2, and 3). Next, the software was trained by creating a random trees classification algorithm combined with the intensity information to differentiate tumor from stroma, necrosis, and immune cells (21).

Results

Patient characteristics

A total of 56 patients with solid tumors who had previously received standard-of-care treatment were enrolled on the study from December 2012 to December 2017, of whom 51 patients received at least one dose of AT13148. The demographics of the patients included a male/female ratio of 25/31, age range of 34–76 years, and the most common tumor types were colorectal and breast cancer (Supplementary Table S1).

Safety

Patients were initially treated across a dose rage of 5–300 mg a day in 8 cohorts following a rolling six design during the dose escalation. Two further cohorts were then added: first, a cohort that included intrapatient dose escalation (cohort 9) over 3 dose levels (120 mg–180 mg–240 mg) to evaluate whether incremental increase of dose over a period of 3 weeks could improve tolerability; then, a mandatory biopsy cohort of patients was treated at the RP2D of 180 mg (cohort 10) to study pharmacodynamic effects in pre- and posttreatment biopsies (**Fig. 1**).

The dose escalation proceeded by dose doubling from 5 to 160 mg with no dose-limiting toxicities reported. At the dose of 160 mg (cohort 6), following multiple non–dose-limiting toxicity (DLT) grade 3 toxicities of hypotension and diarrhea, the dose escalation proceeded by a 50% increase to 240 mg (cohort 7). There was one DLT of elevated liver enzymes seen at 240 mg in addition to multiple episodes of grade 2 toxicities such as nausea, fatigue, anorexia, and hypotension. As only 1 in 6 evaluable patients had a DLT, the dose was escalated cautiously by a further 25% to 300 mg (cohort 8). At this dose level, two patients experienced grade 2 hypotension, one patient experienced grade 3 hypotension, and one patient experienced grade 4 hypotension with a systolic blood pressure of less than 40 mm Hg requiring inotropic support. No further patients were treated at the dose of 300 mg following the adverse event. In cohort 9 (intrapatient dose escalation cohort), two DLTs (grade 3 pneumonitis at 240 mg and grade 3 rash at 180 mg) were reported. However, multiple grade 1 and 2 toxicities

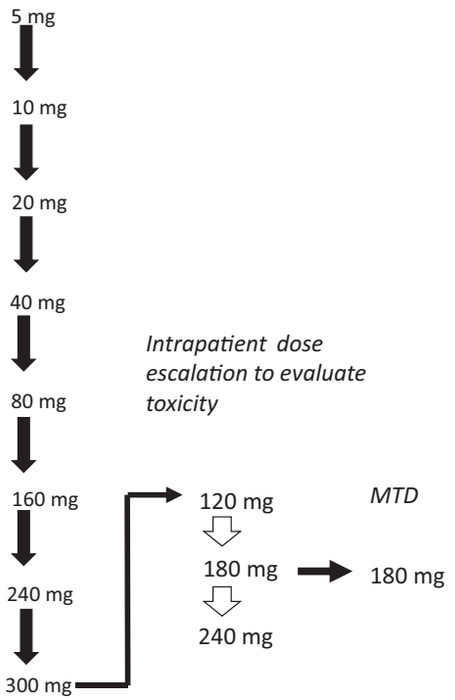

Figure 1.

The dose escalation scheme. Dose was doubled from 5 to 160 mg, followed by a more conservative increase in dose to 240 mg and 300 mg was evaluated. Following grade 4 hypotension being seen at 300 mg, an intrapatient dose escalation schedule was explored to evaluate whether this improved tolerability of higher doses. The dose of 180 mg was considered the MTD. Pre- and posttreatment biopsies were conducted at this dose to evaluate pharmacodynamic effects.

including grade 2 fatigue were seen at 240 mg and limited continuation of this approach. The dose was therefore deescalated and the MTD was established at a dose of 180 mg AT13148 orally given on days 1, 3, and 5 of each week. As a composite of the standard dose escalation and the intrapatient dose escalation cohort, a dose of 180 mg was considered the MTD of AT13148.

The most common treatment-related side effects were hypotension, nausea, headache, and fatigue (**Table 1**). There were four grade 3 or 4 events that were considered DLTs, which included hypotension at 300 mg, elevated transaminases and pneumonitis at 240 mg, and a maculopapular rash at 180 mg.

The most common known on-target toxicity of AT13148 included hypotension. This was often associated with headaches and occurred within 8 hours of dosing. Fatigue and nausea were commonly seen at or above the dose level 160 mg. Although one episode of elevated liver enzymes was described as a DLT in cohort 7 (240 mg), there were no dose-dependent trends of increased liver transaminases across different dose levels. Rash was seen in 6 of 51 (11.8%) patients but was dose-limiting in only one patient.

Pharmacokinetics

AT13148 was measured in plasma in all patients and data was obtained from 44 patients across nine cohorts (cohorts 1–8 and 10). Data were limited from cohort 8 (300 mg AT13148) as there were only three evaluable patients, and only one patient received a second cycle of AT13148.

Table 1. Treatment-emergent adverse events.

		Cohort 1 5 mg ($n = 4$)			Cohort 2 10 mg ($n = 5$)			Cohort 3 20 mg ($n = 3$)			Cohort 4 40 mg ($n = 3$)			Cohort 5 80 mg ($n = 4$)			Cohort 6 160 mg ($n = 7$)			Cohort 7 240 mg ($n = 6$)			Cohort 8 300 mg ($n = 5$)				Cohort 9 120-180–240 mg ($n = 7$)			Cohort 10 180 mg ($n = 7$)		
Treatment-emergent adverse event	Total no. subjects ($n = 51$)	1	2	3*	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	1	2	3	1	2	3
Gastrointestinal disorders																																
Diarrhea	7 (13.7%)																1		1									2			3	
Nausea	15 (29.4%)			1												3			1	1		2					4			3	
Vomiting	7 (13.7%)															1			3			1					1			1	
General disorders and administration site conditions																																
Fatigue	9 (17.6%)		1																	1	1						2	2		2		
Metabolism and nutrition disorders																																
Anorexia	7 (13.7%)	1															1				2						2			1		
Nervous system disorders																																
Headache	12 (23.5%)																1	2		2			3							4		
Vascular disorders																																
Flushing	6 (11.8%)																1			2	1		1							1		
Hypotension	20 (39.2%)						1										3	1	1	3	1			3	1	1	2			1		2

*1 2 3 indicate grade of adverse event.

Table 2. Pharmacokinetic parameters of AT13148.

Cycle 1		T_{max} (h)	C_{max} (nmol/L)	HL Lambda z (hours)	AUC_{last} (hour/nmol/L)	*AUC to 48 hours (hour/nmol/L)	AUCINF obs (hour/nmol/L)	Vz F obs (L)	Cl F obs (L/hour)
Cohort 1 5 mg	Mean	5	20	60	400	400	900	1,400	20
	SD	5	15	27	21	210	350	290	11
	% CV	97	70	41	54	54	40	21	60
Cohort 2 10 mg	Mean	6	15	40	300	300	600	1,900	100
	SD	4.2	4.8	30	190	190	460	670	95
	% CV	75	32	85	66	66	77	36	96
Cohort 3 20 mg	Mean	4	30	20	600	600	1,000	2,000	90
	SD	2.8	16	13	440	440	750	660	72
	% CV	66	46	56	70	70	78	32	83
Cohort 4 40 mg	Mean	1.99	120	15	1,900	1,900	2,100	1,100	50
	SD	0.03	21	1	540	540	630	310	17
	% CV	2	18	7	29	29	30	28	33
Cohort 5 80 mg	Mean	3	180	50	5,000	5,000	10,000	1,600	30
	SD	2	69	44	3,000	3,000	16,000	910	22
	% CV	67	38	82	61	61	118	57	67
Cohort 6 160 mg	Mean	5	400	40	12,000	10,000	20,000	2,000	40
	SD	2	290	22	7,900	5,900	16,000	1,300	28
	% CV	41	66	52	64	57	79	72	75
Cohort 7 240 mg	Mean	5	500	40	15,000	13,000	30,000	2,000	50
	SD	2	280	17	9,000	9,000	24,000	930	36
	% CV	40	61	44	63	70	94	48	80
Cohort 8 300 mg	Mean	6.1	700	60	20,000	20,000	40,000	1,400	18
	SD	0.12	130	35	4,100	4,100	10,000	600	4.8
	% CV	2	20	57	21	21	23	42	26
Cohort 10 180 mg	Mean	4	400	40	13,000	9,000	20,000	2,000	50
	SD	2.3	190	21	8,000	4,700	14,000	2,200	59
	% CV	64	50	51	60	52	74	94	110

Note: The pharmacokinetic parameters between 5 and 300 mg in cycle 1. There was considerable variation of AUC and C_{max} at doses of 160 mg and above.

Maximum plasma concentrations in cycle 1 ranged between 7 and 964 nmol/L with mean values of 20 to 700 nmol/L. In cycle 2, plasma concentrations of AT13148 were between 18 and 1,097 nmol/L with mean values of 24 to 712 nmol/L. There was a dose-related increase in the mean C_{max} and AUC $r^2 = 0.97$ and 0.98, respectively (Supplementary Fig. S1); however, a high intrapatient variability was observed at doses of 80 mg and above. In many instances (34 patient profiles of the 55), the terminal $t_{½}$ was not accurately established, which also affected related pharmacokinetic parameters (clearance, volume of distribution, etc.). This was addressed with additional (optional) sampling times over 48 hours (at 72 and 96 hours). The half-life of AT13148 ranged between 15 and 60 hours (**Table 2**). At the tolerable dose of 180 mg, free drug levels or exposure were below those measured in preclinical efficacy experiments.

Pharmacokinetic–hypotension relationships

Because of the ROCK inhibitory activity of the drug, hypotension was regarded as both on-target toxicity (22) and a pharmacodynamic biomarker. The maximum decrease in supine systolic blood pressure and C_{max} or AUC_{last} were studied. Where there was matched data for blood pressure and plasma drug concentrations available, there was a weak linear correlation seen between hypotension and both C_{max} and $AUC_{0\text{-last}}$ ($r^2 = 0.25$ and 0.17, respectively; **Fig. 2**). However, looking at individual cases, of the 8 patients with the most severe hypotension (a fall of greater than 30 mm Hg), 6 patients had high exposure in terms of C_{max} >350 nmol/L and $AUC_{0\text{-last}}$ >1,000 nmol/L/hour. The high incidence of hypotension found at high doses became dose-limiting to the trial and halted recruitment at 300 mg.

Pharmacodynamics

Phosphorylation of GSK3β was quantified in platelet-rich plasma to assess AKT inhibition. At doses 160–300 mg, there was a weak trend in the relationship between the plasma concentration and the p-GSK3β levels ($r^2 = 0.17$; **Fig. 3A**). At the nontolerated dose level of 300 mg, there was a statistically significant reduction of p-GSK3β levels in platelet-rich plasma 2–6 hours after treatment when compared with predose (**Fig. 3B**). We also studied the phosphorylation of cofilin, a known downstream effector of ROCK activity in pre- and posttreatment biopsies at the 180 mg dose level. Of the 8 pre- and posttreatment biopsies conducted, 5 of 8 showed a reduction of p-cofilin in posttreatment biopsies and 3 of 8 showed >50% reduction of p-cofilin (**Fig. 4A**). Myosin II is regulated by ROCK via direct phosphorylation of the regulatory light chain (MLC2). p-MLC2 was assayed using IHC in 5 paired biopsies and there was a reduction in H scores in posttreatment samples in 1 of 5 paired biopsies (**Fig. 4B**).

Clinical efficacy

No complete or partial responses were seen in the study. The median number of cycles of AT13148 on the study was 2 (range 1–10). One patient with non–small cell lung cancer (adenocarcinoma) was on the study for 10 cycles and had been tested and shown not to have an EGFR mutation or ALK rearrangement as part of their standard-of-care.

Discussion

AT13148 is an AGC kinase inhibitor with potent ROCK and AKT inhibitory activity (13). ROCK inhibitors have attracted interest due to their role in tumor invasion, cancer metastasis, and stromal

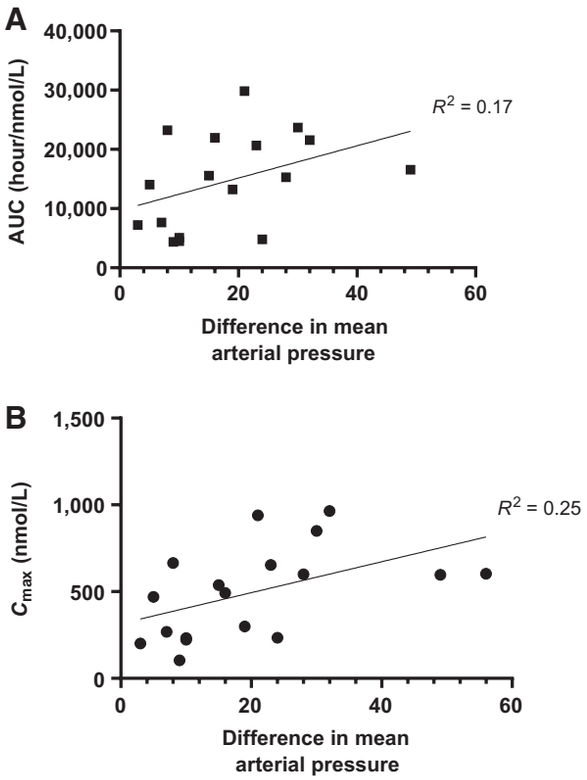

Figure 2.

Relationship of hypotension and pharmacokinetic parameters. The maximum drop in supine systolic blood pressure was measured and correlated with pharmacokinetic profiles of individual patients where matched blood pressure and pharmacokinetic measurements were available at doses 160 mg, 240 mg, and 300 mg. **A,** Relationship between AUC and drop in blood pressure ($r^2 = 0.17$). **B,** Relationship between C_{max} and maximal drop in blood pressure ($r^2 = 0.25$).

remodeling (23, 24). In addition, there are a number of studies demonstrating preclinical antiproliferative activity in melanoma (7), neuroblastoma (9), pancreatic cancer (15), lung cancer, and leukemia (8). AT13148, to our knowledge, is the first dual ROCK-AKT inhibitor that has been evaluated in a clinical trial as an anticancer agent.

Vasodilation caused by ROCK inhibition led to a high number of patients who received AT13148 experiencing hypotension, which was dose-limiting at a dose of 300 mg. Although not dose-limiting, headaches were a common toxicity seen in this study and were also likely to be due to vasodilatation. This property of ROCK inhibitors has led to its use in pulmonary hypertension (25) and in the treatment of cerebral vasospasm following subarachnoid hemorrhage (26). Other dose-limiting toxicities included pneumonitis and skin rash; these have previously been reported in agents such as PI3K, AKT, or mTOR inhibition (2, 5, 20, 27–29). One dose-limiting toxicity of elevated liver enzymes was seen. Elevated liver enzymes have been reported in PI3K pathway inhibitors but it is not clear if this an on-target toxicity (30, 31). Nausea and fatigue were other common toxicities, which were non-specific and have been reported across a wide range of other anticancer drugs. Interestingly, hyperglycemia and nonneutropenic infections, which are common features of PI3K pathway inhibitors, were not commonly seen in this study (32, 33).

The pharmacokinetic profile of AT13148 showed a high degree of variability when administered in the current formulation. This led to considerable overlap between exposure to AT13148 at dose levels of 160 mg and above. This was considered a challenge and risk for further development of the current formulation of the drug considering the narrow therapeutic index.

Interestingly, postural hypotension was seen at dose levels above 80 mg, suggestive of ROCK inhibition. Therefore, we decided to study the relationship of C_{max} and AUC of AT13148 to changes in blood pressure where matched data points were available. This showed a low to moderate correlation. Pharmacodynamic analysis in platelet-rich plasma demonstrated a transient reduction of p-GSK3β levels at the nontolerable dose of 300 mg, and a trend towards reduction of levels

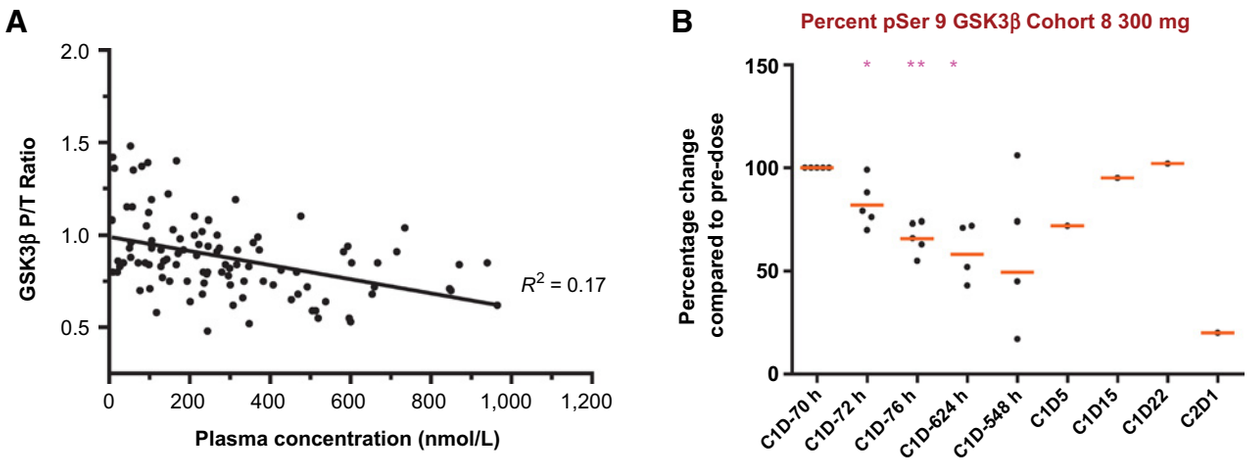

Figure 3.

Pharmacodynamic effects in platelet-rich plasma. Phosphorylation of GSK3β is a downstream event of AKT activation and reduction of p-GSK3β was quantified in platelet-rich plasma before and after treatment with AT13148. **A,** The relationship of the plasma levels of AT13148 and p-GSK3β across the dose levels of 160–300 mg. There was a trend toward reduction of p-GSK3β and increasing plasma levels of AT13148 ($r^2 = 0.17$; $P = 0.0354$). **B,** p-GSK3β normalized to total GSK3β levels were reduced between 2 and 24 hours after treatment of AT3148 at the 300-mg cohort. The asterisk indicates significance in Dunnett test. The changes were not significant at doses lower than 300 mg.

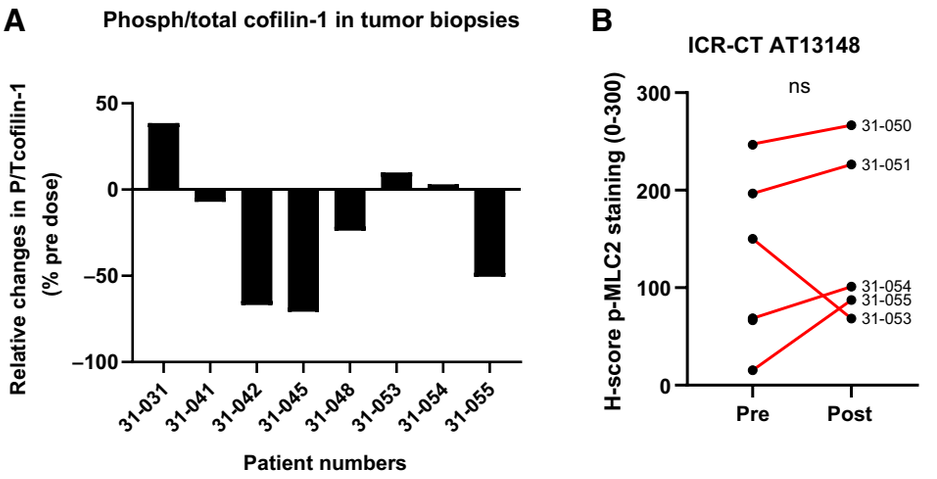

Figure 4.
Pharmacodynamic effects in tumor tissue. Biopsies were done at the dose level of 180 mg thrice a week. Phosphorylation of cofilin normalized to GAPDH at baseline and at 15–28 days of intermittent dosing was assayed using immuno-chemiluminescence assays and results are presented as percentage of predose levels. **A,** A reduction of more than 50% of p-cofilin levels were seen in 3 of 8 patients. **B,** Phosphorylation of MLC2 was measured by IHC in pre- and posttreatment tumor specimens of patients treated with AT13148 showing reduction in phosphorylation in only 1 of 5 samples.

of p-GSK3β between 160 and 300 mg. Reduction of p-GSK3β has been shown to be a reproducible biomarker of AKT inhibition (20, 34) and reduction of p-GSK3β was demonstrated in xenograft models treated with AT13148 showing growth delay (13). Finally, there was a 50% reduction in phosphorylation of cofilin in 3 of 8 patients and reduction in the phosphorylation of MLC2 in only 1 of 5 paired biopsies studied with pre- and posttumor biopsies at the dose level of 180 mg. A reduction of p-MLC2 has been shown to be a biomarker of ROCK inhibition *in vitro* and AT13148 has been shown to inhibit tumor growth in xenograft models. However, measurement of p-MLC2 levels in xenograft tissue that had shown tumor growth delay has not been performed (14, 15), thus, even if a reduction of p-MLC2 was demonstrable in all samples, interpretation of the data would need to be put into this context. The phosphorylation of coflin has been shown to be a biomarker of ROCK inhibition (7), but specific experiments measuring p-cofilin in xenografts of AT13148–treated mice have not been performed and, thus it is difficult to interpret the findings in this study. However, given that changes in both the biomarkers were not seen in a majority of the samples treated at the RP2D of 180 mg thrice a week, these findings were not suggestive of consistent and robust ROCK inhibition in tumor tissue.

There were no clinical responses seen in the study. The reasons for lack of single-agent response could be many. The anticancer activity of ROCK inhibitors is predominantly toward causing a reduction in metastatic spread. This first-in-human study was geared towards defining tolerability and pharmacokinetic–pharmacodynamic relationships, and recruited patients with advanced solid tumors. It would not be possible to study antimetastatic activity of such a drug in these patients: the dose-limiting on-target toxicity of hypotension limited the escalation to biological effective levels of ROCK inhibition in tumor tissue. Furthermore, the hypotension limited the ability to sufficiently inhibit other key AGC kinases such as AKT, which contributed to antiproliferative activity in preclinical models. Prospective sequencing of tumor to enrich cohorts with patients whose tumors had *PIK3CA* or *AKT* mutations would have improved the chances of observing responses but, given that there were no toxicities or consistent biomarker changes suggestive of AKT inhibition, this would have been unlikely. Future research into ROCK inhibitors could consider isoform specific ROCK II inhibitors that may cause reduced hypotension and the possibility of topical applications for selected skin cancers, which could reduce other systemic side effects (18).

The pharmacologic audit trail defines multiple factors involved in go–no–go decisions in drug development (35). The current first-in-human study demonstrated multiple factors that would make further development of this agent challenging, including its toxicity seen, variable pharmacokinetic profile, and inability to reproducibly inhibit AGC kinases in tumor tissue.

AT13148 also brings poly-pharmacology of anticancer drugs into question. It is increasingly found that targeted anticancer drugs inhibit multiple targets (36, 37). Importantly, this has led to drugs, which inhibit multiple targets being licensed for indications that are relevant to one target and not to the other. For example, sorafenib is licensed in renal cancer for its VEGFR inhibitory activity but not in melanoma for its RAF inhibitory activity (38, 39), and crizotinib has been licensed in lung cancer for its ALK inhibitory activity but not as an MET inhibitor (40, 41). AT13148 inhibited multiple AGC kinases and was found to cause cell death and apoptosis when compared with more selective AKT inhibitors *in vitro*. In a biochemical screen, AT13148 was a more potent ROCK inhibitor compared with an AKT inhibitor. The IC_{50} of AT13148 for ROCK I and ROCK II was 6 nmol/L and 4 nmol/L, respectively, when compared with that for AKT1, AKT2, and AKT3 (38 nmol/L, 402 nmol/L, and 50 nmol/L, respectively; ref. 13). The additional putative cytotoxic anticancer effects of ROCK I/II inhibitory activity of AT13148 could not be exploited due to clinical hypotension caused by its activity on normal tissue. The current trial raises a further important issue surrounding cancer drugs that can, by virtue of inhibiting multiple kinases, target the cardiovascular system (e.g., VEGFR or vascular-disrupting agents). In these instances, it could be hypothesized that cells of the vasculature are likely to be exposed to the drug earlier than cells of tumors and possibly at a higher concentration, resulting in cardiovascular adverse events being limiting; for example, the multikinase inhibitor, ilorasertib, targeted the VEGFR and Aurora kinase families resulting in the VEGR-related adverse events being limiting (42). In the case of AT13148, it is difficult to know if the cardiovascular side effects occurred at lower concentrations required for AKT inhibition because the drug inhibits ROCK more potently or because cells in the vascular system are likely to be exposed to such drugs earlier, and to a possibly higher concentration, than cancer cells in the tumor.

This clinical information will be of importance to researchers developing ROCK inhibitors or other AGC kinases as anticancer

drugs and makes the case for the development of specific inhibitors of individual AGC kinases.

Disclosure of Potential Conflicts of Interest

D. Papadatos-Pastos reports personal fees from Roche (consulting), Takeda (consulting), Pfizer (consulting), and Boehringer-Inhelheim (consulting), as well as grants from AstraZeneca outside the submitted work. J. Mateo reports grants and personal fees from AstraZeneca; personal fees from Janssen, MSD, Clovis Oncology, and Roche; and grants from Pfizer outside the submitted work. J. Brown reports employment at AstraZeneca (salary and shareholder). S.A. Decordova reports grants from CRUK (grant number C309.A11566 in support of Clinical PD Biomarker Group Laboratory equipment servicing and maintenance) during the conduct of the study; other from Boston Pharmaceuticals Inc. (research) and Menarini Ricerche (Research) outside the submitted work; and is an employee of The Institute of Cancer Research, which is involved in the development of PI3K, HSP90, HDAC, AKT, ROCK, RAF, CHK1, and HSF1 inhibitors. S. Jueliger reports employment at Astex Therapeutics Ltd. R. Ferraldeschi reports personal fees from Astex Therapeutics (employment) outside the submitted work. V. Sanz-Moreno reports other support from CRUK (agreed to provide lab with a small amount of AT13148 to pursue further future studies). K.E. Swales reports grants from CRUK (grant number C347/A18077 and grant number C309/A11566 in support of post and laboratory equipment servicing and maintenance) during the conduct of the study; other from AstraZeneca (research funding), Bayer (research funding), Boston Pharmaceuticals Inc (research funding), Sierra Oncology (research funding), Menarini Ricerche (research funding), and Merck (research funding) outside the submitted work; and is an employee of The Institute of Cancer Research, which is involved in the development of PI3K, HSP90, HDAC, AKT, ROCK, RAF, CHK1, and HSF1 inhibitors. F.I. Raynaud reports that The Institute of Cancer Research and CRUK would benefit from the development of AT13148. M.D. Garrett reports grants from Cancer Research UK during the conduct of the study; reports grants from Astex Therapeutics (personal fees) and Cancer Research UK outside the submitted work; and is listed as a co-inventor on a patent owned by Astex Therapeutics regarding AT13148 and related compounds. U. Banerji reports other from Institute of Cancer Research (Employee of ICR, which is involved in the development of PI3K, HSP90, HDAC, AKT, ROCK, RAF, CHK1, and HSF1 inhibitors) during the conduct of the study; and reports grants from AstraZeneca [Phase 1 IIT (TAX-TORC)], Onyx [Phase 1 IIT (Onx-0801)], BTG International (additional Funding ONX-0801), Carrick Therapeutics [Phase 1 IIT CT900 (previously ONX-0801)], Chugai Pharma [Phase 1 IIT (RO51226766)], Verastem [Phase 1 IIT (FRAME)], and Chugai Pharma [Phase 1 IIT (FRAME)], as well as personal fees from Astellas (research forum), Novartis (clinical advisory board), Karus Therapeutics (clinical advisory board), Phoenix Solutions (clinical advisory board), Eli Lilly (research forum), Janssen (clinical advisory board), Boehringer-Ingelheim (clinical advisory board), and Bayer (return travel to attend investigator meeting) outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

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