Preclinical ADME Evaluation of GDC-0068, a novel, ATP-competitive Akt Inhibitor

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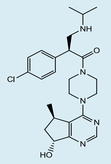
INTRODUCTION

GDC-0068 is a potent, highly selective small molecule inhibitor of all three isoforms of the serine/threonine kinase Akt, and is being developed by Genentech as a treatment for a broad range of human cancers. The objectives of this study were to assess the preclinical ADME characteristics of GDC-0068 and its potential as a human drug candidate. Plasma protein binding, blood-plasma partitioning, metabolism, reaction phenotyping and induction were determined *in vitro*. The preclinical pharmacokinetic properties of GDC-0068 were evaluated following single IV and PO dose administration to mouse, rat, dog and monkey.

Figure 1: Chemical structure of GDC-0068.



MATERIALS & METHODS

In Vitro

Plasma Protein Binding

The plasma protein binding of 0.1, 1, 10, and 40 μM GDC-0068 (a combination of [^{14}C]GDC-0068 and unlabeled test article) was assessed from preclinical species and human in a 96-well equilibrium dialysis block for 5 hrs at 37°C (95% humidity with 5% CO_2) with gentle mixing. Post-dialysis plasma samples and buffer samples were analyzed on a liquid scintillation counter.

Blood-Plasma Partitioning

The blood–plasma partitioning of 0.1, 1, 10, and 40 μM GDC-0068 (a combination of [^{14}C]GDC-0068 and unlabeled test article) was assessed from preclinical species and human. The blood samples were incubated at 37°C for 1 hr in a shaking water bath. Radioactivity in whole blood and plasma was quantitated by liquid scintillation counting.

Metabolite Identification

Rat liver microsomes (RLM), cynomolgus monkey liver microsomes (CLM), and human liver microsomes (HLM) were incubated with 10 μM of [^{14}C]GDC-0068 for 1 hr in the presence of an NADPH-regenerating system. Samples were analyzed by LC/MS coupled to a radiometric detector.

Reaction Phenotyping

HLM (0.5 mg/mL) incubations containing the inhibitors furafylline, 1-aminobenzotriazole (ABT), or troleandomycin (TAO) were pre-incubated for 15 min at 37°C with NADPH (1 mM) as well as HLM. HLM incubations using other chemical inhibitors were pre-incubated for 5 minutes at 37°C with NADPH (1 mM). The reactions were initiated with the addition of GDC-0068 (1 mM). rCYP (40 pmol/mL) and NADPH (1 mM) were pre-incubated for 5 min at 37°C. The reaction was initiated with the addition of GDC-0068 (1 μM). Samples from the HLM and rCYP incubations were collected at the 0- and 60-min timepoints and analyzed by LC/MS/MS.

Induction

Cryopreserved human hepatocytes from three male donors were used. 0.5 x 10^6 million cells per well were plated in each well of a 96-well, collagen coated plate. The plate was incubated at 37°C in 5% CO_2 with saturating humidity. The cells were dosed on the third day with 0.1, 1, 5, 10, 25 and 50 μM of GDC-0068 or 0.1% DMSO as vehicle control and rifampicin 25 μM (CYP3A4/5), phenobarbital 1000 μM (CYP2B6) and omeprazole 50 μM (CYP1A2) as positive controls. Cells were treated for 48 hrs with medium changed every 24hrs. Enzyme activities were assessed on day 5, using probe substrates testosterone 100 μM (CYP3A4/5), bupropion 500 μM (CYP2B6) or phenacetin 100 μM (CYP1A2) . After the activity assay, the cells were washed and lysed with lysis mixture provided in the Quantigene® Plex Assay Kit. Samples were stored in the -80^{0}C freezer until use. A custom Quantigene® Plex panel kit was used to deteremine mRNA levels.

In Vivo

PK Studies

PK studies were performed in female CD-1 mice, female nu-nu mice, male Sprague-Dawley rats, male Beagle dogs and male Cynomolgus monkeys. Animals were given single oral (PO) doses of GDC-0068 at 3.125 to 150 mg/kg (mice), 5 mg/kg (rats), 2 mg/kg (dogs) or 3 to 25 mg/kg (monkeys) and single IV doses at 1 to 30 mg/kg (mice), 5 to 100 mg/kg (rats), 1 mg/kg (dogs) or 1 to 10 mg/kg (monkeys). The IV dose formulations were prepared in saline. The PO dose formulations were prepared in sterile water or MCT. For all PK studies GDC-0068 was dosed as the dihydrochloride salt.

RESULTS AND DISCUSSION

In Vitro

Plasma Protein Binding and Blood-Plasma Partitioning

Table 1: Mean plasma protein binding and blood-plasma ratios of GDC-0068.

Species	Plasma Protein Binding: % Bound [^{14}C]GDC-0068	Blood Plasma Paritioning: Blood-Plasma Ratio [^{14}C]GDC-0068
Mouse	51.1 - 63.0	1.20 - 1.46
Rat	48.7 - 61.0	1.42 - 1.56
Rabbit	30.8 - 35.1	1.53 - 1.65
Dog	65.3 - 90.1	0.800 - 1.19
Monkey	42.1 - 47.6	1.43 - 1.59
Human	34.5 - 39.0	1.34 - 1.64

· Plasma protein binding of GDC-0068 was low (~30% to 60%) in mouse, rat, rabbit, monkey and human plasma, and low to moderate (~65% to 90%) in dog plasma. A concentration dependency was observed in mouse, rat and dog.
· Blood-plasma ratios ranged from 0.80 to 1.65, indicating that GDC-0068 did not preferentially distribute to red blood cells.

Metabolite Identification

Figure 2: Proposed metabolic pathways for [^{14}C]GDC-0068 in liver microsomes (Note: *denotes position of ^{14}C-label).

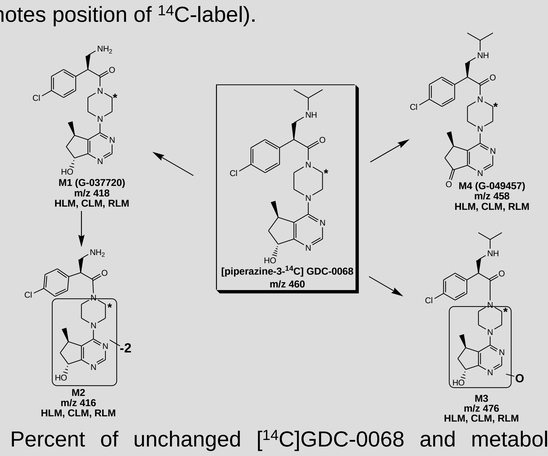


Table 2: Percent of unchanged [^{14}C]GDC-0068 and metabolites in liver microsomes.

Analyte	$[MH]^+$ (*m/z*)	RT (min)	RLMs (% TRA)	CLMs (%TRA)	HLMs (% TRA)	Biotransformation Pathway
[^{14}C]GDC-0068	460	20.8	96.9	42.9	76.0	Parent
M1 (G-037720)	418	13.1	<1.5	14.1	8.9	*N*-dealkylation
M2	416	26.0	<1.5	5.5	<1.5	Oxidation/Desaturation
M3	476	28.0	<1.5	8.1	<1.5	Oxidation
M4 (G-049457)	458	30.7	2.4	27.8	13.0	Oxidation

· The main metabolic pathways for GDC-0068 observed *in vitro* in RLM, CLM and HLM were oxidations and *N*-dealkylation. No human-specific metabolites were detected.
· The preliminary human metabolite profiling of GDC-0068 in urine and plasma samples is presented in poster P73.

Reaction Phenotyping

Figure 3: Graph representing % GDC-0068 remaining at the end of 60 minutes incubation with various CYP inhibitors.

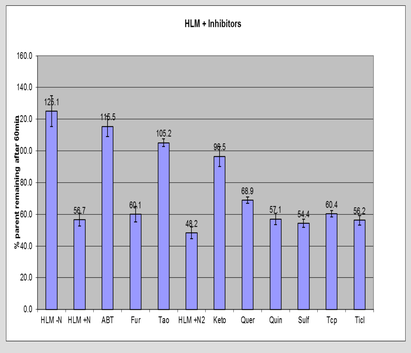


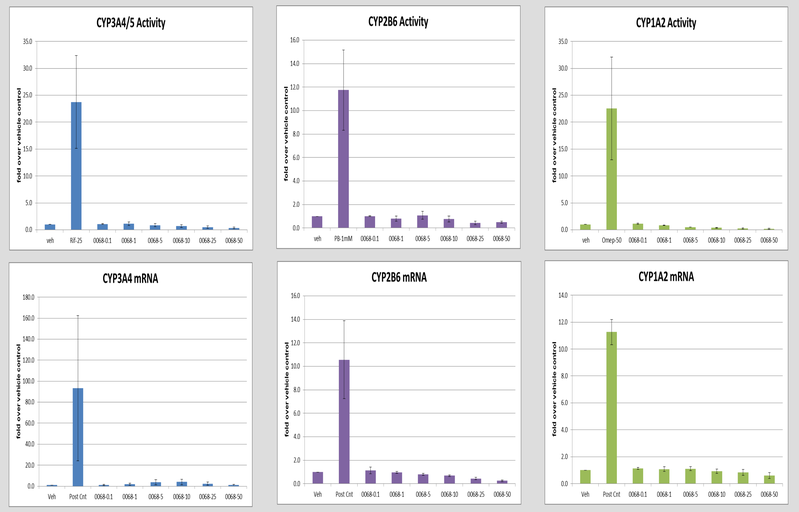
Table 3: Table representing % GDC-0068 remaining and formation of metabolites detected at the end of 60 minutes incubation with recombinant CYPs.

Isoform	Parent average (± SD)	M1 average (± SD)	M3 average (± SD)	M4 average (± SD)
rCYP1A2	98.8 (12.6)			
rCYP2A6	103 (5.33)			
rCYP2B6	102 (3.96)			
rCYP2C18	99.7 (8.87)			
rCYP2C19	95.8 (3.92)			
rCYP2C8	97.1 (4.18)			
rCYP2C9	92 (2.98)			
rCYP2D6	82.8 (1.04)	0.374 (0.0331)	100 (1.70)	6.97 (0.328)
rCYP2E1	102 (0.663)			
rCYP3A4	59.6 (3.03)	100 (8.52)	54.4 (7.67)	100 (11.3)
rCYP3A5	98 (2.53)	4.67 (0.438)	3.45 (0.263)	30.4 (1.92)

· CYP3A4/5 were the major contributors to the formation of M1 and CYP3A4/5 and CYP2D6 to the formation of M3 and M4 in HLM.

Induction

Figure 4: Graphs representing CYP3A4/5, CYP2B6 and CYP1A2 activity and mRNA levels after dosing cells for 48 hrs with GDC-0068 compared to vehicle control and positive control.



· GDC-0068 was not an inducer of CYP1A2, CYP2B6 or CYP3A4/5.

In Vivo

PK Studies

Table 4: Mean PK parameters of GDC-0068 after IV administration.

Species	IV Dose (mg/kg)	CL (mL/min/kg)	$t_{1/2}$ (hr)	V_{ss} (L/kg)
CD-1 Mice	1	70.9	7.47	22.2
Nude Mice	1	88.9	1.52	5.67
	3	86.4	3.84	7.15
	10	72.0	2.26	4.77
	30	48.0	2.71	4.52
Sprague-Dawley Rats	5	83.1± 20.1	3.11±1.42	14.3±3.72
	10	45.7±17.9	3.89±1.66	9.19±0.811
	20	50.3±7.71	3.25±0.657	9.80±0.787
	30	25.5±3.75	3.22±0.243	6.80±0.775
	100	26.4±6.25	8.51±2.82	12.8±1.56
Beagle Dogs	1	17.1±3.50	6.91±1.00	4.35±1.60
Cynomolgus Monkeys	1	43.0±5.52	21.4±6.30	31.3±2.90
	3	21.4±5.85	17.0±2.17	9.49±3.49
	10	25.5 ±8.73	7.98±2.90	8.90±3.35

· The clearance of GDC-0068 decreased with increasing dose and was moderate to high in mice, rats, and monkeys. In dogs, clearance was moderate.
· The Vss was high as expected for a basic amine and tended to decrease with increasing dose.

Table 5: Mean PK parameters of GDC-0068 after PO administration.

Species	PO Dose (mg/kg)	AUC_{inf} (ng•hr/mL)	F (%)
CD-1 Mice	5	300	25.5
Nude Mice	3.125	145	ND
	12.5	1150	ND
	25	4100	ND
	50	15100	ND
	150	43400	ND
Sprague-Dawley Rats	5	489 ±224	47.1 ±21.5
Beagle Dogs	2	521 ±329	26.7 ±16.9
Cynomolgus Monkeys	3	228±62.6	19.3±5.43
	10	2310±351	ND
	25	5260±1560	ND

· Oral bioavailability after a single PO dose of GDC-0068 in mice and rats at 5 mg/kg, in dogs at 2 mg/kg, and in monkeys at 3 mg/kg was 25.5%, 47.1%, 26.7% and 19.3%, respectively.
· AUC estimates after PO dose of GDC-0068 increased in a non-dose proportional manner in mice and monkeys.

CONCLUSIONS

Overall, GDC-0068 exhibited a favorable preclinical profile and was advanced as the lead molecule. GDC-0068 is currently in Phase Ib human clinical trials with encouraging preliminary results.

ACKNOWLEDGMENTS

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