Poster number P139

Elimination of [^{14}C]-LY3023414 by Aldehyde Oxidase and CYP Enzymes in Humans Following Oral Administration

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ABSTRACT

The class I phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin (PI3K/AKT/mTOR) pathway regulates the cell cycle and is altered in cancer cell growth and survival.[1] LY3023414 is an orally available, potent, selective small molecule dual kinase inhibitor of this pathway[1] investigated as a potential treatment for patients with prostate cancer. The pharmacokinetics and disposition of [14C]-LY3023414 were investigated in healthy subjects (N=5) following oral administration of a single dose of unlabeled and radiolabeled LY3023414 corresponding to a total LY3023414 dose of 150 mg with approximately 100 µCi of radioactivity. Plasma samples were analyzed for LY3023414 using a validated liquid chromatography with tandem mass spectrometry method. Whole blood, plasma, urine, and fecal concentrations of radioactivity were determined using liquid scintillation counting techniques. Pharmacokinetic parameter estimates for whole blood and plasma total radioactivity, and plasma LY3023414 were calculated by standard noncompartmental methods. The enzymes responsible for the LY3023414 metabolism were determined using recombinant cytochrome P450 (CYP) enzymes, human liver microsomes and cytosol in the presence and absence of a probe inhibitor. The enzyme kinetic parameter values were determined by detection of metabolite formation in human liver microsomes and cytosol. LY3023414 was rapidly absorbed; the median time to maximum observed concentration postdose was 0.5 hours and the mean half-life was 2.59 hours. The overall recovery of radioactivity over 120 hours was 96.1%, corresponding to 71.5% and 24.6 % of the dose excreted in feces and in urine, respectively, indicating that LY3023414 is eliminated completely from the body following an oral dose. LY3023414 was cleared primarily by metabolism with only 5% of the dose being excreted as parent drug. The major metabolic clearance pathways involved were aromatic hydroxylation of the quinoline moiety (M2), *N*-demethylation (M5), and quinoline oxidation with *N*-demethylation (M12) (Figure 1). LY3023414 exposure accounted for approximately 46% of total plasma radioactivity based on area under the concentration versus time curve from zero to infinity, indicating significant amounts of metabolites circulating. Parent LY3023414 and metabolites, M2, M5, and M12, accounted for 62%, 23%, 3%, and 9% of the circulating radioactivity, respectively, in plasma over 12 hours postdose. In vitro studies using human liver microsomes and cytosol confirmed M2 and M5 were formed through aldehyde oxidase (AO)- and CYP-mediated pathways, respectively. An $f_{m,AO}$ of 0.46 and an $f_{m,CYP}$ of 0.54 were calculated from kinetic parameters determined for the formation of M2 and M5. M12 was formed from M2 and M5 through CYP- and AO-mediated pathways, respectively. The drug-related adverse events with single-dose LY3023414 included dysgeusia, oral paraesthesia, dizziness, nausea, and diarrhea; all were mild in severity and resolved without treatment. In conclusion, LY3023414 was absorbed rapidly and eliminated completely after oral dosing, with metabolism as the predominant route of elimination in humans. Both AO and CYP enzymes were responsible for the metabolic clearance of LY3023414 with the non-CYP enzymes mediating approximately half of the clearance of the drug.

BACKGROUND

- The class I phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin (PI3K/AKT/mTOR) pathway regulates the cell cycle and is altered in cancer cell growth and survival[1]
- LY3023414 is an orally available, potent, selective, small-molecule, dual-kinase inhibitor of this pathway[1] investigated as a potential treatment for patients with prostate cancer

OBJECTIVES

- To determine the pharmacokinetics (PK) and disposition of [^{14}C]-LY3023414 following oral administration of a single dose of unlabeled and radiolabeled LY3023414
- To identify the enzymes responsible for LY3023414 metabolism
- To estimate the fraction of LY3023414 metabolized through aldehyde oxidase (AO) and CYP-mediated pathways by determination of the enzyme kinetics in vitro

METHODS

Elimination Study in Humans and Sample Analysis

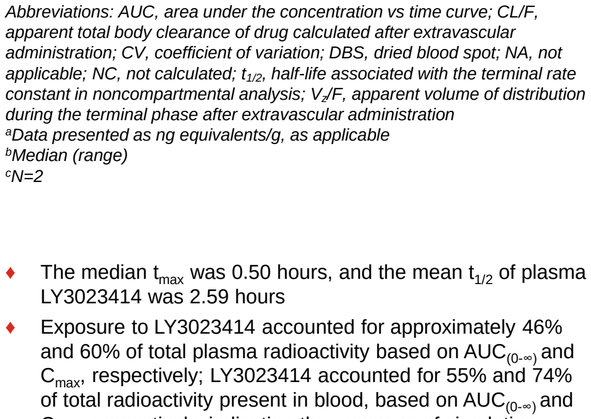
- **Study Design.** This was a single-center, open-label study evaluating the disposition of radioactivity and LY3023414 in healthy male (N=1) and female (N=4) subjects (mean age, 46.4 years; body mass index, 21.8-29.6 kg/m^2) following oral administration of a single 150 mg (~100 µCi) dose of [^{14}C]-LY3023414
- **Analysis of Radioactivity.** Whole blood, plasma, urine, and fecal concentrations of radioactivity were determined using liquid scintillation counting techniques; expired air was collected for analysis of $^{14}CO_2$
- **Analysis of LY3023414 in Plasma and Dried Blood Spot (DBS) Collection.** Plasma and DBS samples obtained during this study were analyzed for LY3023414 using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods
- **Biosynthesis and Analysis of Metabolites.** Metabolites M2 and M4 were obtained via microbial biosynthesis, and metabolite M12 was obtained from incubation with liver S9; All three metabolites were prepared and isolated at Hypha Discovery, UK. Parent compound and metabolites were identified by accurate mass LC-MS/MS and NMR
- **PK Analysis.** PK parameter estimates were calculated by standard noncompartmental methods of analysis
- **Safety.** Adverse events were presented for all causality and those related to the study drug

Determination of the Fraction of LY3023414 Metabolized Through Aldehyde Oxidase (AO)- and Cytochrome P450 (CYP)-Mediated Pathways

- The intrinsic clearance of LY3023414 was determined in human liver cytosol (HLC) and human liver microsomes (HLM) using a metabolite formation approach; metabolite formation quantified in incubations with HLC and HLM without additional nicotinamide adenine dinucleotide phosphate (NADPH) was attributed to AO-mediated pathways; metabolite formation quantified in incubations with HLM in the presence of NADPH was attributed to CYP-mediated pathways
- The fraction of LY3023414 metabolized through each pathway in liver, $f_{m,AO}$ and $f_{m,CYP}$, was determined by calculating the liver clearance ($CL_{int,liver}$) through each pathway as a fraction of the total liver clearance through both pathways

RESULTS

Figure 1. Mean Concentration of LY3023414 in Plasma and DBS and Total Radioactivity in Plasma and Whole Blood Following Oral Administration of Single 150-mg Dose of [^{14}C]-LY3023414



Abbreviation: DBS, dried blood spot

- LY3023414 was rapidly absorbed, followed by a biphasic decline
- For DBS LY3023414, the concentration-time profiles were, in general, similar to plasma LY3023414

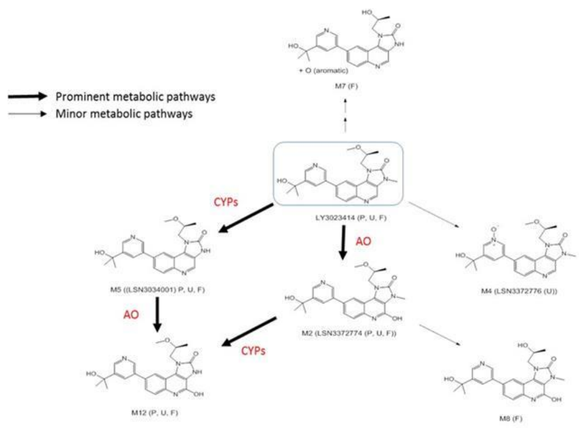
Table 1. LY3023414 PK Parameters in Plasma and DBS and Total Radioactivity in Plasma and Whole Blood Following Single 150-mg Dose of [^{14}C]-LY3023414

	Geometric Mean (CV%)			
Parameter	Plasma LY3023414 (N=4)	DBS LY3023414 (N=4)	Plasma Total Radio-activity[a] (N=4)	Whole Blood Total Radio-activity[a] (N=4)
$AUC_{(0-12)}$, ng*hr/mL	2120 (31)	2190 (27)	4620 (24)	3770 (23)
$AUC_{(0-tlast)}$, ng*hr/mL	2130 (32)	2200 (27)	4450 (26)	3700 (28)
$AUC_{(0-\infty)}$, ng*hr/mL	2140 (31)	2210 (27)	4690 (24)	4000 (28)
C_{max}, ng/mL	1050 (40)	1040 (40)	1740 (40)	1410 (33)
t_{max}, h[b]	0.50 (0.50-0.50)	0.50 (0.50-0.50)	0.50 (0.50-0.50)	0.50 (0.50-0.50)
$t_{1/2}$, h	2.59 (1.85-4.81)	2.23 (1.72-2.49)	2.02 (1.88-2.22)	NC[c] (1.99-2.82)
CL/F, L/h	70.1 (31)	67.8 (27)	NA	NA
V_z/F, L	262 (15)	218 (22)	NA	NA

Abbreviations: AUC, area under the concentration vs time curve; CL/F, apparent total body clearance of drug calculated after extravascular administration; CV, coefficient of variation; DBS, dried blood spot; NA, not applicable; NC, not calculated; $t_{1/2}$, half-life associated with the terminal rate constant in noncompartmental analysis; V_z/F, apparent volume of distribution during the terminal phase after extravascular administration
[a]Data presented as ng equivalents/g, as applicable
[b]Median (range)
[c]N=2

- The median t_{max} was 0.50 hours, and the mean $t_{1/2}$ of plasma LY3023414 was 2.59 hours
- Exposure to LY3023414 accounted for approximately 46% and 60% of total plasma radioactivity based on $AUC_{(0-\infty)}$ and C_{max}, respectively; LY3023414 accounted for 55% and 74% of total radioactivity present in blood, based on $AUC_{(0-\infty)}$ and C_{max}, respectively, indicating the presence of circulating metabolites

Figure 2. Proposed Metabolic Pathways With Circulating and Excreted LY3023414 Metabolites



- Radioprofiling detected a total of 6 metabolites across plasma, urine, and feces
- The major metabolic clearance pathways involved were aromatic hydroxylation of the quinoline moiety (M2), N-demethylation (M5), and quinoline oxidation with N-demethylation (M12)

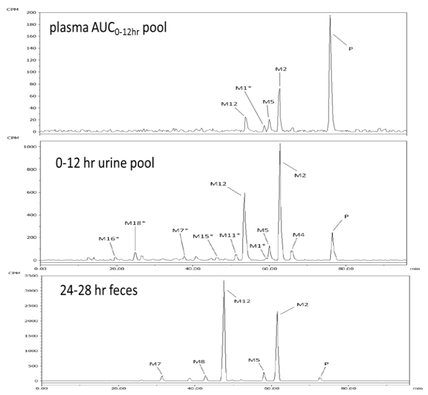
Table 2. Distribution of LY3023414 and Its Metabolites in Plasma, Urine, and Feces

Peak	Mean % Radioactivity in AUC_{0-12hr} Pooled Plasma	Mean % Dose in Urine	Mean % Dose in Feces	Mean Total % Dose in Excreta
LY3023414	62.4	3.1	1.8	4.9
M2	23.0	8.8	27.3	36.1
M4	NR	0.9	0.0	0.9
M5	2.7	1.2	2.6	3.8
M7	NR	0.0	0.9	0.9
M8	NR	0.0	1.0	1.0
M12	8.7	6.1	31.5	37.6
Overall recovered, mean±SD	NA	24.6±5.5[a]	71.5±3.37[a]	96.1±3.14

Abbreviations: AUC, area under the concentration vs time curve; NR, not reported
[a]A small amount of radioactivity remains unidentified but is accounted for the overall recovery
Blood and/or plasma concentrations were below the limit of quantification

- The overall recovery of radioactivity over 120 hours was 96.1%, corresponding to 71.5% and 24.6% of the dose excreted in feces and in urine, respectively, indicating that LY3023414 is eliminated completely from the body following an oral dose
- No appreciable radioactivity was detected in expired air

Figure 3. Distribution of Metabolites in Plasma, Urine, and Feces



Peaks labeled with * are below the set quantitation limits

Abbreviation: AUC, area under the concentration vs time curve

- In AUC_{0-12hr} pooled plasma, parent drug accounted for a mean of 62% of the total radioactivity, whereas metabolites M2 and M12 accounted for 23% and 9% of the radioactivity, respectively
- Metabolites M2 and M12 were the predominant drug-related components observed in urine and feces, accounting for 9% and 6% of the dose in urine and 27% and 32% in feces, respectively

Table 3. Enzyme Kinetics of AO- and CYP-Mediated LY3023414 Metabolism

Substrate of Incubation	Metabolite of Detection	Matrix	With(+)/ Without (-) NADPH Added	V_{max}, pmol/ mg/min, Mean (SD)	K_m, µM, Mean (SD)	$CL_{int,tested\ matrix}$, µl/min/ mg	$CL_{int,liver}$, µl/min/ g
LY3023414	M5	HLM	+	61.6 (2.4)	8.8 (1.2)	7.00	280.0
LY3023414	M5	HLM	-	ND			
LY3023414	M5	HLC	-	ND			
LY3023414	M2	HLM	+	2.1 (0.2)	20.3 (5.2)	0.10	4.1
LY3023414	M2	HLC	-	36.4 (0.5)	12.7 (0.5)	2.87	232.2
LY3023414	M2	HLM	-	3.5 (0.04)	26.8 (0.9)	0.13	5.2
M2	M12	HLM	+	31.1 (1.6)	72.4 (6.9)	0.43	17.2
M2	M12	HLM	-	ND			
M5	M12	HLM	+	17.2 (0.6)	22.6 (2.1)	0.76	30.5
M5	M12	HLC	-	122.5 (3.1)	5.5 (0.5)	22.27	1804.1

Abbreviations: AO, aldehyde oxidase; CYP, cytochrome P450; HLC, human liver cytosol; HLM, human liver microsomes; NADPH, nicotinamide adenine dinucleotide phosphate
$CL_{int,tested\ matrix}$=V_{max}/K_m: matrix-specific intrinsic clearance
$CL_{int,liver}$=$CL_{int,tested\ matrix}$ x scaling factor: liver-specific intrinsic clearance
Scaling factor of HLM=40 mg of microsomal protein per g of liver; scaling factor of HLC=81 mg of cytosolic protein per g of liver

- Studies using HLM and HLC indicated M2 and M5 were formed through AO- and CYP-mediated pathways, respectively; M12 was formed from M2 mediated by CYP and from M5 mediated by AO pathways
- The fractions of LY3023414 metabolized through AO- and CYP-mediated pathways was described by $f_{m,AO}$ and $f_{m,CYP}$ values, respectively; an $f_{m,AO}$ of 0.46 and an $f_{m,CYP}$ of 0.54 were calculated from kinetic parameters determined for the formation of M2 and M5, respectively

Adverse Events

- All adverse events (AEs) reported in the study were mild and resolved without treatment, except for non-drug-related myalgia, which was treated with ibuprofen
- Drug-related AEs occurring in ≥2 subjects were dysgeusia, oral paresthesia, dizziness, diarrhea, and nausea

CONCLUSIONS

- LY3023414 was absorbed rapidly and eliminated completely after oral dosing, with metabolism as the predominant clearance route
- After oral dosing, radioactivity was recovered in feces (71.5%) and in urine (24.6%)
- AO and CYP enzymes were responsible for the metabolic clearance of LY3023414, with the non-CYP enzymes mediating approximately half the clearance of the drug

Reference:

1. Smith MC, et al. *Mol Cancer Ther* 2016;15(10):2344-56.