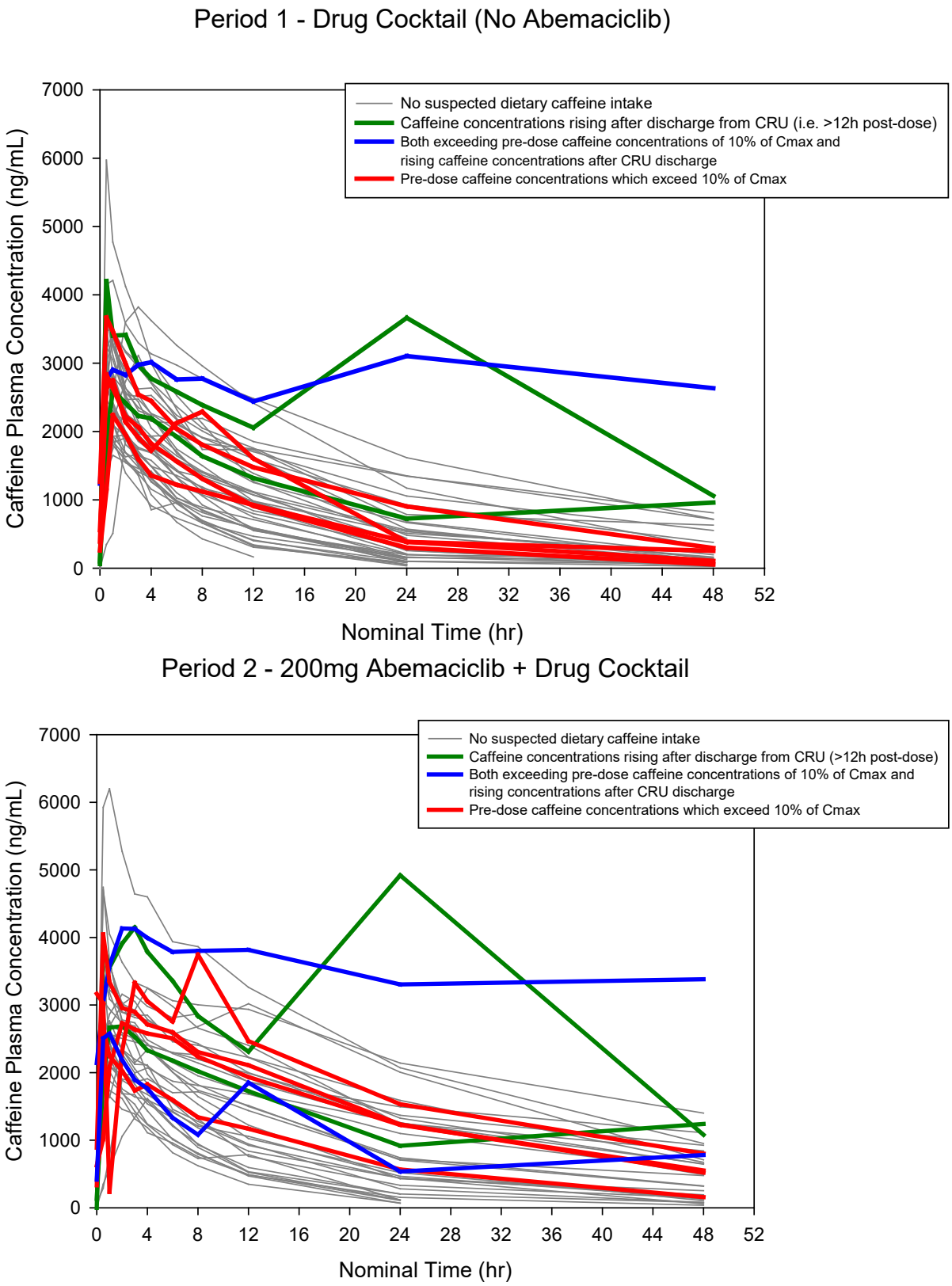
Abemaciclib Does Not Have a Clinically Meaningful Effect on Pharmacokinetics of CYP1A2, CYP2C9, CYP2D6, and CYP3A4 Substrates in Patients with Cancer

P. Kellie Turner, Stephen D. Hall, Sonya C. Chapman, Jessica Rehmel,

Jane Royalty, Yingying Guo, Palaniappan Kulanthaivel

Drug Metabolism and Disposition Manuscript #90092



Supplemental Figure 1. Caffeine Plasma Concentrations in Individuals Who Appear to have Taken Dietary Caffeine in Period 1/2. Individual Plasma Concentration-Time Profiles

of Caffeine After Administration of a CYP Substrate Drug Cocktail Containing 0.2 mg Midazolam (CYP3A4), 10 mg Warfarin (CYP2C19), 30 mg Dextromethorphan (CYP2D6), and 100 mg Caffeine (CYP1A2) Either Alone (Period 1), or In Combination with Abemaciclib After 7 Days of 200 mg Q12H Abemaciclib (Period 2). Line colors denote patients who: have no suspected dietary caffeine intake (black); have concentrations which rise post-discharge from the CRU (>12h post- dose) (green); have pre-concentrations of caffeine which exceed 10% of post-dose C_{max} (red); or have both rising concentrations >12h post-dose and pre-dose concentrations of caffeine which exceed 10% of post-dose C_{max} (blue).

CRU, Clinical Research Unit; CYP, cytochrome P450; C_{max}, maximal steady state plasma concentration.

Supplemental Table 1. Selected details related to the conduct and interpretation of *in vitro* studies to assess the effects of abemaciclib, M2, and M20 on CYP mRNA and activity in cultured human hepatocytes, with selected E_{max} and EC_{50} values.

Test article	Abemaciclib	M2	M20
Hepatocytes (plated)	XenoTech Lot HC3-22; Caucasian female, age 57 years	XenoTech Lot HC10-1; Caucasian female, age 48 years XenoTech Lot HC3-22; Caucasian female, age 57 years XenoTech lot HC5-25; Caucasian male, age 56 years	XenoTech Lot HC10-1; Caucasian female, age 48 years XenoTech Lot HC3-22; Caucasian female, age 57 years XenoTech lot HC5-25; Caucasian male, age 56 years
[µM] tested for cytotoxicity by LDH release assay	Not tested in this study; used 0.1-10 µM (high conc. defined by prior study)	0.05-25 µM	0.1-10 µM
[µM] found cytotoxic initially	At 7.5 and 10 µM, some morphological changes but consistent with healthy cells	Morphological changes: 2.5-5 µM (moderate); 10-25 µM (severe)	Morphological changes: 2.5-5 µM (moderate); 10-25 µM (severe)
Description of initial cytotoxicity	# of vacuoles increased but cells remained cuboidal w/ intact membranes	2.5-5 µM: grainy cytoplasm 10-25 µM: grainy cytoplasm, LDH release, lysed membranes	2.5-5 µM: grainy cytoplasm 7.5-10 µM: grainy cytoplasm, LDH release, lysed membranes

Hepatocyte lot information	1 (prior study included 3 lots) XenoTech Catalog H1500.H15C+ Lot HC3-22	3 XenoTech Catalog H1500.H15C+ Lots HC10-1, HC3-22, and HC5-25	3 XenoTech Catalog H1500.H15C+ Lots HC10-1, HC3-22, and HC5-25
[µM] dosed for induction	0.1-10 µM	0.05, 0.1, 0.25, 0.5, 1, 2.5, 10, 25 µM	0.1, 0.25, 0.5, 1.25, 2.5, 5, 7.5, 10 µM
[µM] not reported due to cytotoxicity	none	10 and 25 µM	5, 7.5, and 10 µM
Description of later cytotoxicity	Not applicable	grainy cytoplasm, LDH release, lysed membranes, little/no enzyme activity, shifted GAPDH mRNA C_T values	grainy cytoplasm, LDH release, lysed membranes, little/no enzyme activity, shifted GAPDH mRNA C_T values
mRNA: induction of primary enzymes	1A2, 2B6, 3A4: no induction	1A2, 2B6, 3A4: no induction	1A2, 2B6, 3A4: no induction
mRNA: downregulation of primary enzymes (≥50% decrease)	1A2: decreases observed E_{max} -0.96-fold, EC_{50} 0.62 µM for HC3-22 2B6: decreases observed	1A2: decreases observed Unable to fit E_{max} and EC_{50} for HC10-1 2B6: decreases (2.5 uM only)	1A2: decreases observed E_{max} -0.63-fold, EC50 0.88 µM for HC10-1 2B6: decreases observed

	3A4: decreases observed E_{max} -0.89-fold, EC50 0.37 μM for HC3-22	3A4: decreases observed E_{max} -0.99-fold, EC50 0.44 μM for HC3-22 E_{max} -0.83-fold, EC50 0.25 μM for HC10-1 E_{max} -0.84-fold, EC50 1.3 μM for HC5-25	3A4: decreases observed E_{max} -0.82-fold, EC50 0.29 μM for HC3-22 E_{max} -1-fold, EC50 0.24 μM for HC10-1 (bottom fixed at -1)
mRNA: induction of secondary enzymes	2C8, 2C9, 2C19, 3A5: no induction 2D6: induction (1.25-5 μM; 2D6 generally not considered inducible); E_{max} 1.68-fold, EC50 0.21 μM for HC3-22	2C8, 2C9, 2C19, 2D6, 3A5: no induction	2C8, 2C9, 2C19, 2D6, 3A5: no induction
mRNA: downregulation of secondary enzymes (≥50% decrease)	2C8: no decreases 2C9: no decreases (44%; <50%) 2C19: no decreases 2D6: decreases (7.5-10 μM) 3A5: decreases observed	2C8: no decreases 2C9: decreases observed 2C19: no decreases 2D6: no decreases 3A5: no decreases	2C8: decreases observed 2C9: decreases observed 2C19: no decreases 2D6: no decreases 3A5: decreases observed

Activity: induction of primary enzymes	1A2, 2B6, 3A4: no induction	1A2, 2B6, 3A4: no induction	1A2, 2B6, 3A4: no induction
Activity: decreases (>50%) for primary enzymes	1A2: decreases observed 2B6: decreases observed 3A4: decreases observed	1A2: decreases (2.5 μM only) 2B6: decreases (2.5 μM only) 3A4: decreases (2.5 μM only)	1A2: decreases observed 2B6: decreases observed 3A4: decreases observed
Test article time-averaged % remaining (0-24 hrs; day 2)	27.5-48.6% remaining	37.0-58.5% remaining	39.3-71.5% remaining
Test article distribution (@24 hr; day 2)	1-16% in medium 84-99% in cell lysate	10-38% in medium 62-90% in cell lysate	5-27% in medium 73-95% in cell lysate

Conc, concentration; C_T, cycle threshold; EC_{50}, half maximal response concentration; E_{max}, maximum extent of induction or downregulation; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LDH, lactate dehydrogenase; M2, N-desethylabemaciclib; M20, hydroxyabemaciclib; mRNA, messenger ribonucleic acid. Where applicable, non-linear regression using a 4-parameter logistic model was used to estimate E_{max}, and EC_{50} (Graphpad Prism version 7, San Diego, CA).

Supplemental Table 2. TaqMan Gene Expression Assays used for RT-qPCR of mRNA isolated from human hepatocytes

Enzyme	Assay ID
GAPDH	Hs99999905_m1
CYP1A2	Hs00167927_m1
CYP2B6	Hs03044634_m1
CYP2C8	Hs00258314_m1
CYP2C9	Hs00426397_m1
CYP2C19	Hs00426380_m1
CYP2D6	Hs00164385_m1
CYP3A4	Hs00604506_m1
CYP3A5	Hs00241417_m1

ID, identification; mRNA, messenger ribonucleic acid; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

Supplemental Table 3. Mass spectrometry conditions used for *in vitro* selective activity assays for CYP1A2, CYP2B6, and CYP3A from the *in vitro* study conducted in primary cultures of human hepatocytes

Enzyme	Substrate	Substrate Concentration (μM)[a]	Internal Standard	Incubation Time	Ionization Mode	Mass Transitions
CYP1A2	Phenacetin	100	Acetaminophen-d_4	45	ESI+	152.1/110.0
CYP2B6	Bupropion	500	Hydroxybuppropion-d_6	45	ESI+	256.1/238.0
CYP3A	Midazolam	30	1'-hydroxymidazolam-d_4	45	ESI+	342.0/323.9

[a]Bupropion and midazolam were co-incubated; equal volumes of supernatant from the phenacetin and bupropion/midazolam incubations were combined for analysis by LC/MS.

ESI, Electrospray ionization in the positive mode.

Supplemental Table 4. Parameters used as inputs for Equations 2 and 3

Parameter	Definition	Value	Source
$f_{u,p}$	Fraction of drug unbound in plasma	0.0557	Measured
C_{max}	Maximal steady-state plasma concentration	0.5 μM	Measured
F_a	Fraction absorbed	0.91	Estimated from human ^{14}C study (Kulanthaivel P et al. 2016)
k_a	Absorption rate constant	0.2 hr^{-1}	PopPK analysis (Tate SC, Sykes AK, Kulanthaivel P et al. 2018)
Dose	Highest approved dose	200 mg	Verzenio Prescribing Information (FDA)
Q_h	Hepatic blood flow	97 L/h	United States Food and Drug Administration 2017

R_b	Blood-to-plasma ratio	0.84	Measured
Q_{en}	Enterocyte blood flow	18 L/h	United States Food and Drug Administration 2017

C_{max}, maximal observed plasma concentration; F_a, fraction of drug absorbed; F_g, fraction of drug escaping metabolism in the gut; f_m, fraction of systemic clearance; $f_{u.p}$, fraction of drug unbound in plasma; k_a, absorption rate constant; R_b, blood-to-plasma ratio; Q_{en}, enterocyte blood flow**;** Q_h, hepatic blood flow.

Supplemental Table 5. Changes in CYP mRNA and activity measured in cultured hepatocytes following 2 days of treatment with abemaciclib, M2, and M20, presented as percent change from 0.1% DMSO control.

Concentration (uM)	0.05	0.1	0.25	0.50	1.0	1.25	2.5	5.0	7.5	10	25
Abemaciclib_CYP1A2_mRNA_HC3-22		-31	-20	-42		-58	-70	-74	-78	-79	
Abemaciclib_CYP1A2_Activity_HC3-22		-22	-23	-36		-46	-51	-53	-57	-55	
Abemaciclib_CYP2B6_mRNA_HC3-22		-14	-10	-37		-48	-57	-57	-47	-49	
Abemaciclib_CYP2B6_Activity_HC3-22		-23	-23	-42		-49	-55	-56	-66	-62	
Abemaciclib_CYP3A4_mRNA_HC3-22		-22	-29	-55		-80	-83		-87	-87	
Abemaciclib_CYP3A4_Activity_HC3-22		-16	-17	-34		-49	-55	-53	-61	-58	
Abemaciclib_CYP2C8_mRNA_HC3-22		-4	2	-5		-13	-8	6	16	20	
Abemaciclib_CYP2C9_mRNA_HC3-22		1	-8	-12		-32	-44	-39	-34	-33	
Abemaciclib_CYP2C19_mRNA_HC3-22		8	6	3		5	15	38	43	42	
Abemaciclib_CYP2D6_mRNA_HC3-22		51	90	104		187	124	172	-79	-48	
Abemaciclib_CYP3A5_mRNA_HC3-22		1	-4	-12		-25	-37	non-detect	-51	-44	
M2_CYP1A2_mRNA_HC10-1	-63	-61	-65	-71	-75		-91			cytotoxic	cytotoxic
M2_CYP1A2_mRNA_HC3-22	-7	-9	-39	-32	-46		-71			cytotoxic	cytotoxic
M2_CYP1A2_mRNA_HC5-25	15	23	37	30	43		17			cytotoxic	cytotoxic
M2_CYP2B6_mRNA_HC10-1	15	10	-7	-31	-49		-68			cytotoxic	cytotoxic
M2_CYP2B6_mRNA_HC3-22	-8	-6	-31	-35	-50		-58			cytotoxic	cytotoxic
M2_CYP2B6_mRNA_HC5-25	-5			-13	-25		-58			cytotoxic	cytotoxic
M2_CYP1A2_Activity_HC10-1	7	3	1	-7	-12		-50			cytotoxic	cytotoxic
M2_CYP1A2_Activity_HC3-22	-8	-13	-25	-25	-32		-45			cytotoxic	cytotoxic
M2_CYP1A2_Activity_HC5-25	-13	-17	-21	-27	-32		-47			cytotoxic	cytotoxic
M2_CYP2B6_Activity_HC10-1	25	14	9	-9	-19		-61			cytotoxic	cytotoxic
M2_CYP2B6_Activity_HC3-22	-11	-10	-26	-25	-36		-48			cytotoxic	cytotoxic
M2_CYP2B6_Activity_HC5-25	-12	-18	-26	-41	-45		-65			cytotoxic	cytotoxic
M2_CYP3A4_mRNA_HC10-1	30	-5	-35	-64	-72		-87			cytotoxic	cytotoxic
M2_CYP3A4_mRNA_HC3-22	-5	-9	-35	-53	-69		-89			cytotoxic	cytotoxic
M2_CYP3A4_mRNA_HC5-25	24	12	5	-14	-23		-57			cytotoxic	cytotoxic

M2_CYP3A4_Activity_HC10-1	21	9	3	-15	-24		-63			cytotoxic	cytotoxic
M2_CYP3A4_Activity_HC3-22	-7	3	-20	-26	-37		-51			cytotoxic	cytotoxic
M2_CYP3A4_Activity_HC5-25	-0.2	-10	-10	-26	-31		-44			cytotoxic	cytotoxic
M2_CYP2C8_mRNA_HC10-1	7	-1	-7	-20	-33		-46			cytotoxic	cytotoxic
M2_CYP2C8_mRNA_HC3-22	-16	-23	-24	-24	-27		-13			cytotoxic	cytotoxic
M2_CYP2C8_mRNA_HC5-25	-4	6	-7	-16	-20		-21			cytotoxic	cytotoxic
M2_CYP2C9_mRNA_HC10-1	17	-1	-4	-33	-53		non-detect			cytotoxic	cytotoxic
M2_CYP2C9_mRNA_HC3-22	14	11	6	-5	-17		-54			cytotoxic	cytotoxic
M2_CYP2C9_mRNA_HC5-25	13	1	1	-9	-8		-26			cytotoxic	cytotoxic
M2_CYP2C19_mRNA_HC10-1	-8	-17	-11	-9	-16		non-detect			cytotoxic	cytotoxic
M2_CYP2C19_mRNA_HC3-22	-6	-11	-8	-15	-12		-20			cytotoxic	cytotoxic
M2_CYP2C19_mRNA_HC5-25	18	7	16	7	9		10			cytotoxic	cytotoxic
M2_CYP2D6_mRNA_HC10-1	-15	-15	-11	-8	19		7			cytotoxic	cytotoxic
M2_CYP2D6_mRNA_HC3-22	6	2	4	2	13		-6			cytotoxic	cytotoxic
M2_CYP2D6_mRNA_HC5-25	7	7	28	28	48		34			cytotoxic	cytotoxic
M2_CYP3A5_mRNA_HC10-1	6	3	20	-7	-16		non-detect			cytotoxic	cytotoxic
M2_CYP3A5_mRNA_HC3-22	0	-6	-1	-21	-22		-26			cytotoxic	cytotoxic
M2_CYP3A5_mRNA_HC5-25	16	19	30	23	33		41			cytotoxic	cytotoxic
M20_CYP1A2_mRNA_HC10-1		-4	-21	-10		-56	-63	cytotoxic	cytotoxic	cytotoxic	
M20_CYP1A2_mRNA_HC3-22		39	10	-8		1	-28	cytotoxic	cytotoxic	cytotoxic	
M20_CYP1A2_mRNA_HC5-25		4	-10	6		22	41	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_mRNA_HC10-1		-14	-27	-23		-58	-51	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_mRNA_HC3-22		14	17	-1		-13	-22	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_mRNA_HC5-25		-7	-19	-25		-46	-58	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A4_mRNA_HC10-1		-30	-49	-65		-89	-90	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A4_mRNA_HC3-22		21	-38	-53		-79	-85	cytotoxic	cytotoxic	cytotoxic	

M20_CYP3A4_mRNA_HC5-25		0	-8	-8		-4	-42	cytotoxic	cytotoxic	cytotoxic	
M20_CYP1A2_Activity_HC10-1		-7	-14	-18		-39	-56	cytotoxic	cytotoxic	cytotoxic	
M20_CYP1A2_Activity_HC3-22		2	-10	-24		-28	-39	cytotoxic	cytotoxic	cytotoxic	
M20_CYP1A2_Activity_HC5-25		-12	-15	-22		-24	-34	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_Activity_HC10-1		-13	-24	-34		-53	-69	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_Activity_HC3-22		8	-37	-41		-49	non-detect	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2B6_Activity_HC5-25		-16	-17	-39		-54	-66	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A4_Activity_HC10-1		-13	-20	-32		-56	-65	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A4_Activity_HC3-22		3	-17	-25		-38	-45	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A4_Activity_HC5-25		-12	-19	-20		-42	-49	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C8_mRNA_HC10-1		-23	-40	-25		-50	-32	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C8_mRNA_HC3-22		10	51	0		27	20	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C8_mRNA_HC5-25		-10	-29	-29		-50	-58	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C9_mRNA_HC10-1		-15	-35	-40		-67	-65	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C9_mRNA_HC3-22		-11	-10	-29		-43	-48	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C9_mRNA_HC5-25		12	-3	2		-4	-16	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C19_mRNA_HC10-1		-4	-15	-6		10	26	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C19_mRNA_HC3-22		4	0	13		14	11	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2C19_mRNA_HC5-25		-2	-4	5		47	49	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2D6_mRNA_HC10-1		7	-1	17		-10	2	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2D6_mRNA_HC3-22		16	3	34		47	4	cytotoxic	cytotoxic	cytotoxic	
M20_CYP2D6_mRNA_HC5-25		6	12	26		41	39	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A5_mRNA_HC10-1		-9	-22	-20		-67	-70	cytotoxic	cytotoxic	cytotoxic	

M20_CYP3A5_mRNA_HC3-22		-11	-24	-31		-52	-61	cytotoxic	cytotoxic	cytotoxic	
M20_CYP3A5_mRNA_HC5-25		-4	4	11		8	39				

Cultured hepatocytes were incubated for 48 hours with abemaciclib, M2, and M20, at which time mRNA levels for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 and CYP-selective activities for CYP1A2 (phenacetin O-deethylation to acetaminophen), CYP2B6 (bupropion hydroxylation), and CYP3A4 (midazolam 1'-hydroxylation) were measured. Values are presented as mean of 3 replicates. Blank cells = concentration not tested; non-detect = no amplification of mRNA; cytotoxic = concentration judged cytotoxic. Data are reported only for non-cytotoxic concentrations as determined by LDH release, increases in cycle threshold (CT) values for GAPDH, and/or morphological observations.