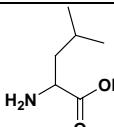
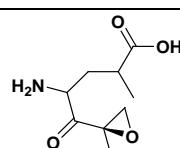
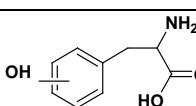
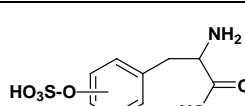
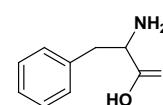
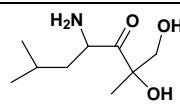


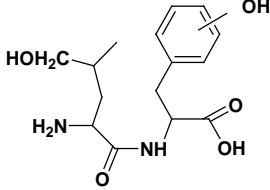
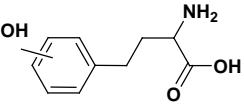
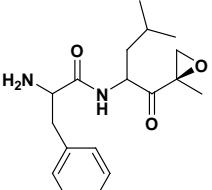
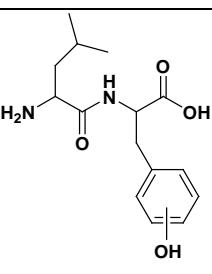
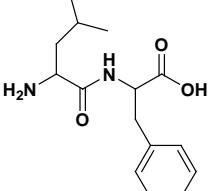
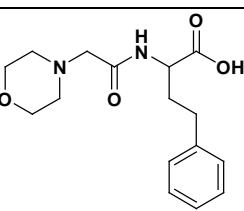
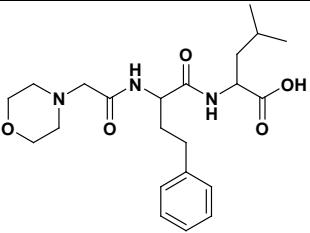
Clinical Pharmacokinetics, Metabolism, and Drug-Drug Interaction of Carfilzomib

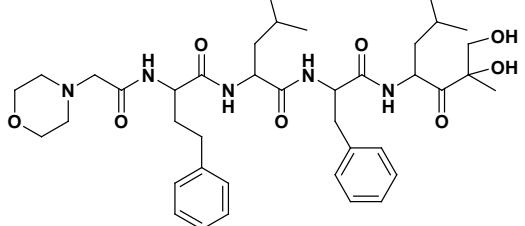
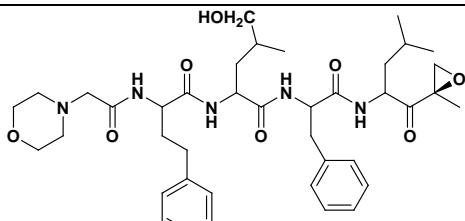
Authors: Jinfu Yang, Zhengping Wang, Christopher Kirk, Ying Fang, Melissa Alsina, Ashraf Badros, Kyriakos Papadopoulos, Alvin Wong, Tina Woo, Darrin Bomba, Jin Li, Jeffrey R. Infante.

Drug Metabolism & Disposition**Supplementary Materials**

Supplemental Table 1. Carfilzomib and its metabolites identified from patient urine (U) and plasma (P) samples.

Metabolite Code	Proposed Structure	MW (Da)	Source
M1		131	P, U
M2		201	P, U
M5		181	P, U
M6		261	U
M7		165	P, U
M8		189	U

M9		310	P, U
M10		195	U
M11		318	P, U
M12		294	U
M13		278	P
M14		306	P, U
M15		419	P, U

M16		737	P, U
M17		735	U

Supplemental Table 2. Effect of cytochrome P450 (CYP) inhibitors on the disappearance rate of carfilzomib in cryopreserved human hepatocytes

CYP	Inhibitor	Inhibition of Probe	Mean Carfilzomib Metabolism	
			Substrate Metabolism	
			Rate (\pm SD)	
			(% Inhibition)	(min$^{-1}$)
				Without
				Inhibitor
				With
				Inhibitor
1A2	Furafylline	Phenacetin O-deethylation	0.015	0.016
	(30 μ M)	(79)	(0.0003)	(0.002)
2C8	Montelukast	Amodiaquine N-deethylation	0.018	0.014
	(30 μ M)	(83)	(0.001)	(0.001)
2C9	Sulfaphenazole	4'-hydroxy-diclofenac	0.0097	0.0073
	(10 μ M)	(75)	(0.0015)	(0.0012)
2C19	(+)-N-3-benzyl nirvanol	S-mephenytoin 4'-hydroxylation	0.013	0.016
	(10 μ M)	(78)	(0.0004)	(0.002)
2D6	Quinidine	Dextromethorphan O-demethylation	0.0093	0.013
	(10 μ M)	(97)	(0.0001)	(0.0001)
3A	Ketoconazole	Midazolam 1'-hydroxylation	0.010	0.012
	(10 μ M)	(91)	(0.002)	(0.002)

Supplemental Table 3. Pharmacokinetic parameters of carfilzomib metabolites M14, M15, and M16 in patients with multiple myeloma and varying degrees of renal dysfunction receiving carfilzomib at 15 mg/m²

PK parameter		C _{max} (ng/mL)	t _{max} (hr)	AUC _{last} (hr·ng·mL)	AUC _{inf} (hr·ng/mL)	AUC _{ratio} (Metabolite/CFZ)	t _{1/2} (hr)
Normal renal function (N=8)	M14	90.6 (20.0)	0.54 (0.28, 1.17)	262 (33)	281 (35)	1.20	1.53 (1.20, 3.50)
	M15	14.4 (23.6)	0.31(0.28, 0.58)	24.2 (30.1)	25.9 (28.4)	0.11	1.47 (1.13, 1.78)
	M16	147 (24)	0.12 (0.08, 0.15)	64.6 (28.4)	66.2 (26.7)	0.28	0.64 (0.43, 1.03)
Mild impairment (N=9)	M14	94.9 (17.6)	0.62 (0.28, 1.07)	263 (36)	287 (33)	1.19	1.39 (1.01, 3.89)
	M15	15.2 (22.8)	0.32 (0.08, 0.53)	23.0 (24.1)	24.7 (21.6)	0.10	1.42 (1.01, 1.72)
	M16	131 (37)	0.12 (0.00, 0.17)	57.0 (30.9)	57.8 (30.2)	0.24	0.54 (0.38, 1.08)
Moderate impairment (N=5)	M14	122 (43)	1.03 (0.53, 1.08)	605 (65)	609 (72)	4.20	3.61 (1.64, 4.44)
	M15	16.2 (18.2)	0.28 (0.28, 0.58)	34.6 (27.8)	36.9 (31.1)	0.25	1.79 (1.40, 2.01)
	M16	134 (31)	0.12 (0.08, 0.17)	62.6 (51.8)	64.2 (49.7)	0.44	0.53 (0.38, 1.08)
Severe impairment (N=5)	M14	148 (18)	1.53 (1.00, 1.85)	799 (34)	906 (34)	5.27	3.66 (2.41, 4.57)
	M15	15.7 (51.9)	0.35 (0.28, 0.50)	36.9 (55.5)	40.9 (64.2)	0.24	1.80 (1.59, 1.99)
	M16	144 (56)	0.10 (0.00, 0.16)	69.3 (34.9)	70.6 (34.1)	0.41	0.84 (0.33, 0.986)
Chronic dialysis (N=8)	M14	158 (33)	1.31 (1.03, 2.03)	1854 (42)	1904 (48)	9.87	4.94 (3.24, 6.72)
	M15	16.9 (34.4)	0.50 (0.28, 0.53)	43.1 (84.2)	44.9 (87.6)	0.23	1.75 (1.11, 5.26)

DMD #47662

M16	106 (39)	0.12 (0.07, 0.28)	53.2 (50.8)	50.7 (44.4)	0.26	0.67 (0.48, 1.32)
-----	----------	-------------------	-------------	-------------	------	-------------------

C_{\max} , AUC_{last} , and AUC_{inf} : geometric mean (CV%)

t_{\max} and $t_{1/2}$: median (min, max)

Supplemental Table 4. Recovery of carfilzomib, M14, and M15 (Mean \pm S.D.) in urine samples from patients with multiple myeloma and varying degrees of renal dysfunction receiving carfilzomib at 15 mg/m²

Urinary PK parameters	Analyte	Normal renal function (N=8)	Mild impairment (N=8)	Moderate impairment (N=7)	Severe Impairment (N=5)
Ae ₀₋₅ (mg·eq)	Carfilzomib	0.157 \pm 0.104	0.118 \pm 0.0813	0.0497 \pm 0.0315	0.0589 \pm 0.0228
	M14	8.92 \pm 4.57	5.69 \pm 1.14	4.36 \pm 2.22	3.30 \pm 0.543
	M15	0.552 \pm 0.347	0.327 \pm 0.0655	0.195 \pm 0.109	0.128 \pm 0.0514
Ae ₅₋₂₄ (mg·eq)	Carfilzomib	0.00	0.00	0.00	0.000568 \pm 0.00127
	M14	1.63 \pm 0.750	1.04 \pm 0.753	2.39 \pm 2.49	1.89 \pm 0.902
	M15	0.0606 \pm 0.0270	0.0505 \pm 0.0256	0.0437 \pm 0.0401	0.0259 \pm 0.00533
TAe (mg·eq)	Carfilzomib	0.157 \pm 0.104	0.118 \pm 0.0813	0.0497 \pm 0.0315	0.0595 \pm 0.0232
	M14	10.5 \pm 4.32	6.72 \pm 1.65	6.75 \pm 2.42	5.19 \pm 1.10
	M15	0.613 \pm 0.361	0.378 \pm 0.0790	0.239 \pm 0.111	0.154 \pm 0.0543
Fe (%)	Carfilzomib	0.490 \pm 0.316	0.429 \pm 0.271	0.160 \pm 0.101	0.226 \pm 0.0921
	M14	33.1 \pm 13.1	25.0 \pm 4.81	21.7 \pm 7.59	19.2 \pm 4.36
	M15	1.93 \pm 1.12	1.42 \pm 0.314	0.776 \pm 0.387	0.578 \pm 0.230

Ae₀₋₅ = amount excreted over 0 to 5 hr collection interval; Ae₅₋₂₄ = amount excreted over 5 to 24 hr collection interval; TAe = total amount of drug excreted over 24 hr collection interval; Fe = fraction of drug excreted in urine

Supplemental Table 5. Least squares mean ratios, 90% geometric confidence intervals (CIs), and intrapatient coefficients of variance (CV) for midazolam pharmacokinetic parameters

Statistical Analysis	Treatment Comparisons	Ratio (%)	90% Geometric CI	Intra-Patient
				CV (%)
C_{\max}		99.0	82.9–118.2	31.1
AUC_{last}	Day 1 vs Day -7	95.0	84.6–106.7	19.6
AUC_{inf}		95.2	84.7–107.2	20.3
C_{\max}		98.1	80.1–120.3	31.1
AUC_{0-12}	Day 16 vs Day -7	113.1	96.8–132.1	21.4
AUC_{inf}		108.2	94.1–124.4	20.3

AUC_{last} = area under the concentration-time curve from hour 0 to the last measurable plasma concentration; AUC_{inf} = AUC from time zero to infinity (extrapolated); C_{\max} = maximum plasma concentration

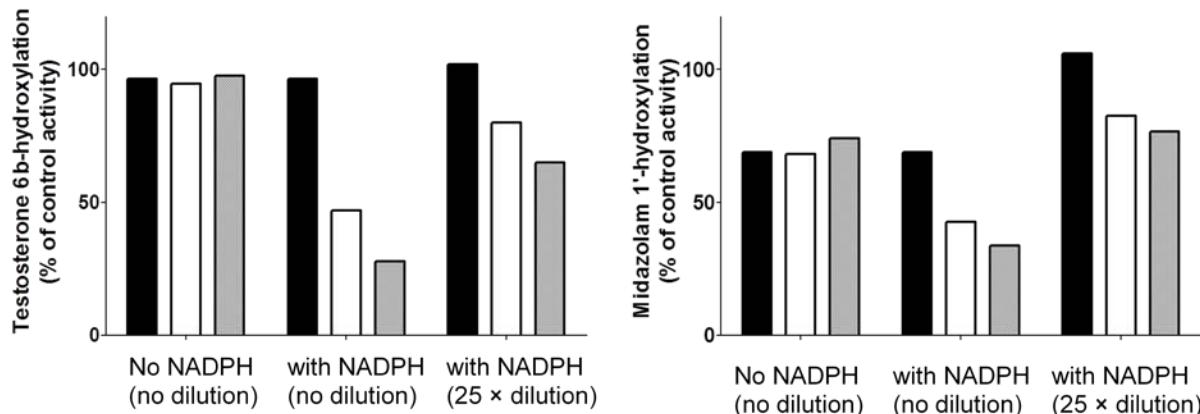
Supplemental Table 6. Serious adverse events occurring in 5 patients during the first cycle of co-administration of midazolam and carfilzomib

Event	Carfilzomib treatment, mg/m ²	Duration, days	Severity, NCI-CTCAE Grade	Relationship to carfilzomib	Action taken to midazolam	Relationship to midazolam	Action taken	Outcome
Urinary tract infection	27	7	2	None	Dose delayed	None	Dose delayed	Resolved
Hypertension	27	5	3	None	Discontinued	None	Discontinued	Stabilized
Pneumonia	27	6	3	None	Discontinued	None	Discontinued	Resolved
Hematuria	27	6	1	None	Discontinued	None	Discontinued	Resolved
Pneumonia	27	6	3	None	Discontinued	None	Discontinued	Resolved
Generalized pain	27	6	3	None	Discontinued	None	Discontinued	Resolved
Hypotension	27	6	3	Possible	Discontinued	Possible	Discontinued	Resolved
Acute renal failure	27	4	3	Possible	Discontinued	None	None	Resolved

Supplemental figure legends

Supplemental Figure 1. Time-dependent inhibition of carfilzomib on CYP3A requires NADPH and is resistant to dilution.

Cafilzomib concentrations were 3 μ M and 1 μ M for probe substrates testosterone and midazolam, respectively. Black bar, no preincubation; white bar, 15 minute preincubation; cross-hatched bar, 30 minute preincubation.



Supplemental Figure 2. Plasma concentration (mean + SD) over time profile of carfilzomib at 27 mg/m².

Carfilzomib (27 mg/m²) was administered via a 2–10 minute infusion to patients (N=18) with solid tumors on Days 1, 2, 8, 9, 15, and 16. Midazolam at 2 mg was administered orally immediately following carfilzomib administration on Days 1 and 16. Plasma samples from Day 1 were analyzed to determine the concentration of carfilzomib.

