SUPPLEMENTAL INFORMATION

A. SUPPLEMENTAL TABLES

Supplemental Table S1. ***In vitro*** **efficacy of MK-8353 in tumor cell line panel.** See Materials and Methods for experimental details. For reference, the previously reported EC_{50} for SCH772984 are also reported. *Abbreviations*: NSCLC, non-small cell lung cancer; CNS, central nervous system; WT, wild type.

Cell Line	Type	SCH 772984 EC_{50} (nM)	MK-8353, EC_{50} (nM)	*BRAF*	*RAS*
Malme-3M	Melanoma	10	21	*V600E*	*WT*
WM-266-4	Melanoma	20	26	*V600D*	*WT*
UACC-62	Melanoma	30	51	*V600E*	*WT*
SK-MEL-1	Melanoma	37	27	*V600E*	*WT*
M14	Melanoma	47	175	*V600E*	*WT*
WM-115	Melanoma	60	223	*V600D*	*WT*
HT-144	Melanoma	60	172	*V600E*	*WT*
SK-MEL-28	Melanoma	85	96	*V600E*	*WT*
LOX	Melanoma	100	91	*V600E*	*WT*
SK-MEL-3	Melanoma	118	266	*V600E*	*WT*
RPMI-7951	Melanoma	344	1,268	*V600E*	*WT*
A-2058	Melanoma	360	994	*V600E*	*WT*
SK-MEL-2	Melanoma	34	141	*WT*	*NRASQ61R*
CHL-1	Melanoma	1,460	>3,000	*WT*	*WT*
Colo-205	Colon	36	19	*V600E*	*WT*
HT-29	Colon	50	42	*V600E*	*WT*
LoVo	Colon	47	40	*WT*	*KRASG13D*
HCT 116	Colon	128	153	*WT*	*KRASG13D*
NCI-H292	Lung	90	130	*WT*	*WT*
A-549	NSCLC	326	230	*WT*	*KRASG12S*
A-427	NSCLC	1,433	88	*WT*	*KRASG12D*
8505C	Thyroid	50	210	*V600E*	*WT*
BHT-101	Thyroid	300	265	*V600E*	*WT*
SW-626	Ovarian	33	108	*WT*	*KRASG12V*
OVCAR-5	Ovarian	208	419	*WT*	*KRASG13D*
IGROV-1	Ovarian	146	262	*WT*	*WT*
A2780	Ovarian	143	376	*WT*	*WT*
A-204	Rhabdomyosarcoma	3,001	4,200	*WT*	*WT*
A-673	Rhabdomyosarcoma	3,001	>3,000	*V600E*	*WT*
Hs 746T	Gastric	1,000	846	*WT*	-
MCF7	Breast	1,001	3,000	*WT*	*WT*
BT-474	Breast	3,001	>3,000	*WT*	*WT*
DBTRG-05MG	CNS	3,001	839	*V600E*	*WT*
PC3	Prostate	3,001	>3,000	*WT*	*WT*
A-498	Renal	3,001	>3,000	*WT*	*WT*

Supplemental Table S2. **Response rate to MK-8353, 60mg/kg, oral gavage bid, in tumor xenograft models.** *Abbreviations*: TGI, tumor growth inhibition.

Xenograft Model	Cancer Type	Mutation	Response Rate
LOX	Melanoma	*BRAF*	100% regression
SK-MEL-28	Melanoma	*BRAF*	53% regression
A375-SM	Melanoma	*BRAF*	53% regression
UACC-62	Melanoma	*BRAF/PTEN*	100% TGI
SK-MEL-5	Melanoma	*BRAF*	56% TGI
A-2058	Melanoma	*BRAF/PTEN*	53% TGI
IPC-298	Melanoma	*NRAS*	28% regression
SK-MEL-30	Melanoma	*NRAS*	97% TGI
Colo-205	Colon	*BRAF*	40% regression
HT-29	Colon	*BRAF*	81% TGI
LoVo	Colon	*KRAS*	82% TGI
SW-403	Colon	*KRAS*	81% TGI
SNU-C2B	Colon	*KRAS*	63% TGI
HCT 116	Colon	*KRAS/PI3K*	89% TGI
SW-948	Colon	*KRAS/PI3K*	80% TGI
MIA PaCa-2	Pancreas	*KRAS*	51% regression
AsPC-1	Pancreas	*KRAS*	61% TGI
Calu-6	NSCLC	*KRAS*	38% regression

Supplemental Table S3. Pharmacokinetic parameters of MK-8353 in mice. Female athymic nude mice received by oral gavage (PO) or intravenously (IV), via a tail vein injection, a single dose of MK-8353, administered as amorphous batch in 20% 2-hydroxypropyl-β-cyclodextrin (HPBCD). Blood samples were collected by cardiac puncture after euthanasia with carbon dioxide at 9 timepoints (5 min, 15 min, 30 min, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours; 3 mice per timepoint) in heparinized syringes and collection tubes. Blood was then centrifuged and plasma was collected to determine MK-8353 levels using the assay and statistical methods described in the Methods section. *Abbreviations*: mpk, mg per kilogram; AUC, area under curve; C_{max}, peak plasma concentration; T_{max}, time from drug administration to reach the maximum plasma concentration; %F, fraction of an orally administered drug that reaches systemic circulation (oral bioavailability); Vd [ss], volume of distribution at steady state.

Route	**IV**	**PO**						
Dose (mpk)	10	10	30	40	50	60	100	200
$AUC_{0\text{-}24\ hours}$ (μM*hour)	6.4	2.6	15.3	30	39	55	121	154.6
Oral C_{max} (μM)		1.3	4.6	6	7	10	17	19.8
T_{max} (hours)		0.5	1	2	0.5	1	1	1
T1/2 (hours)	1.8							
% F		41	80	>100	>100	86	>100	100
Clearance (ml/min/kg)	38							
$Vd_{[ss]}$ (L/kg)	3.3							

Supplemental Table S4. Repeated-dose toxicity studies with MK-8353. Abbreviations: NOAEL, no-observed-adverse-effect level; GLP, good laboratory practice.

Species/Strain	Dose range (mg/kg, PO, BID)	Duration	Noteworthy Findings	NOAEL	GLP
Exploratory Male Rats	0-100	2 weeks	Scabs, lymph node enlargement	50	N
Rats	0-80	1 month	Scabs, skin inflammation, lymphadenopathy, lymphoid hyperplasia,	10	Y
Exploratory Male Beagle Dogs	0-450	7 days	At high doses (≥150mg/kg) decreased activity, vomiting, decreased body weight, erythema of the GI tract,	50	N
Exploratory Male Beagle Dogs	0-60	2 weeks	At high doses (≥30 mg/kg) anorexia, weight loss, ↓ phosphor-ERK staining in skin punch biopsy.	≥60	N
Exploratory Male Beagle Dogs	0-120	1 month	At 120 mg/kg, anorexia, weight loss, increase in AST/ALT/Total bilirubin, bile duct inflammation and bile pigment in canaliculi	<60	N
Beagle Dogs	0-120	1 month on, on month off	At 120 mg/kg, anorexia, weight loss, dehydration. At 60 and 120 mg/kg reversible small intestinal necrosis, portal inflammation, multifocal liver necrosis and arterial necrosis	30	Y

Supplemental Table S5. **Pharmacokinetic Parameters of MK-8353 Following Single-Dose Oral Administration of MK-8353 in Normal Healthy Volunteers** (P07652 study). The 400-mg dose cohort included two parts plus a cohort that received proton pump inhibitors (PPI). Data are presented as: [1]back-transformed least squares with mean and 95% confidence intervals from mixed effects model performed on natural log-transformed values; [2]median (minimum, maximum); [3]harmonic mean (pseudo standard deviation). Highlighted in grey are the dose cohorts that correspond to a biologically effective dose as predicted by the animal models. *Abbreviations*: C12, concentration of drug in plasma at 12 hours; rMSE, square root of conditional mean squared (residual error) from the linear mixed effect model.

PK parameter	10 mg	20 mg	40 mg	80 mg	150 mg	300 mg	400 mg (Cohort 1)	400 mg (Cohort 2)	400 mg + PPI	80 mg + food	rMSE
AUC_{0-24} (μM/L*hr)[1]	0.6 (0.5, 0.9)	1.0 (0.7, 1.4)	2.6 (1.9, 3.6)	4.2 (3.1, 5.8)	11.9 (8.7, 16.3)	15.9 (11.5, 21.9)	27.5 (20.0, 37.8)	18.4 (13.4, 25.4)	24.1 (17.6, 33.2)	5.7 (4.2, 7.9)	0.281
AUC_{0-12} (μM/L*hr)[1]	0.6 (0.4, 0.8)	0.9 (0.6, 1.2)	2.3 (1.7, 3.2)	3.7 (2.7, 5.2)	10.4 (7.6, 14.3)	12.6 (9.1, 17.4)	21.1 (15.3, 29.1)	14.2 (10.3, 19.6)	19.1 (13.9, 26,3)	4.8 (3.5, 6.7)	0.299
C_{max} (μM/L)[1]	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)	0.5 (0.3, 0.6)	0.8 (0.5, 1.0)	1.9 (1.4, 2.6)	2.0 (1.4, 2.7)	3.2 (2.3, 4.5)	2.2 (1.6, 3.1)	2.8 (2.0, 3.8)	1.0 (0.7, 1.4)	0.306
C_{12} (nM/L)[1]	12 (8, 18)	19 (12, 29)	41.0 (27, 62)	74 (48, 114)	207 (136, 315)	403 (262, 620)	729 (478, 111)	482 (313, 740)	662 (434, 1010)	143 (93,219)	0.326
Tmax (hr)[2]	2.0 (1.0, 3.0)	2.0 (2.0, 3.0)	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	1.5 (1.0, 6.0)	3.0 (1.0, 4.0)	2.5 (1.0, 3.0)	3.0 (2.0, 4.0)	3.5 (2.0, 6.0)	
Apparent $t_{1/2}$ (hr)[3]	4.2 (2.0)	5.0 (2.4)	5.6 (0.8)	8.3 (4.0)	6.9 (1.7)	8.9 (2.7)	7.4 (1.6)	8.8 (2.9)	8.1 (2.3)	6.3 (1.1)	

Supplemental Table S6. Geometric Means (geometric CV%) of Pharmacokinetic Parameters of MK-8353 Following Administration of Single and Multiple Oral Doses to Subjects with Advanced Tumors.

Day	Dose		C_{max}	T_{max}[a]	$AUC_{0-\infty}$	$t_{½}$	AUC_{0-12}	R $AUC_{0-12hours}$[b]
		N	(μM)	(hours)	(μM*hours)	(hour)	(μM*hours)	
1	100	2	3.12 (NA)	2.5 (1-4)	31.6 (NA)	7.24 (NA)	21 (NA)	—
1	200	3	3 (62.1)	4 (3-24)	21.9 (65.4)	5.51 (6.08)	20.6 (65.9)	—
1	300	4	4.43 (40.7)	3.5 (2-24)	45.6 (43.6)	8.67 (38.2)	30.3 (26)	—
1	350	3	4.17 (46.2)	2 (2-3)	46.4 (118)	7.83 (49.7)	28.8 (69.7)	—
1	400*	6	5.55 (67.4)	3.5 (2-8)	91.1 (144)	14.1 (46.6)	42.9 (81.8)	—
1	800	6	7.84 (36.5)	5 (3-10)	124 (67.3)	10.3 (22.1)	62.3 (44.1)	—
15	100	1	3.06 (NA)	4 (4-4)	—	—	25.9 (NA)	1.55 (NA)
15	200	3	6.23 (79.3)	2 (1-3)	—	—	48.9 (95)	2.37 (51.6)
15	300	4	8.63 (36.6)	3 (2-3)	—	—	83.6 (41.6)	2.76 (24.9)
15	350	3	4.69 (39.8)	1 (1-2)	—	—	33.1 (75.1)	1.15 (20.5)
15	400	5	10.1 (109)	4 (2-8)	—	—	69.3 (200)	1.86 (172)

a: Median (Min-Max)

NA: Not applicable, Sample size<3

*: At 400 mg dose level, a patient (#1004) had part of GI and bile duct removed by surgical procedure, which affected the absorption of MK-8353; data from this subject was excluded from the PK data analysis

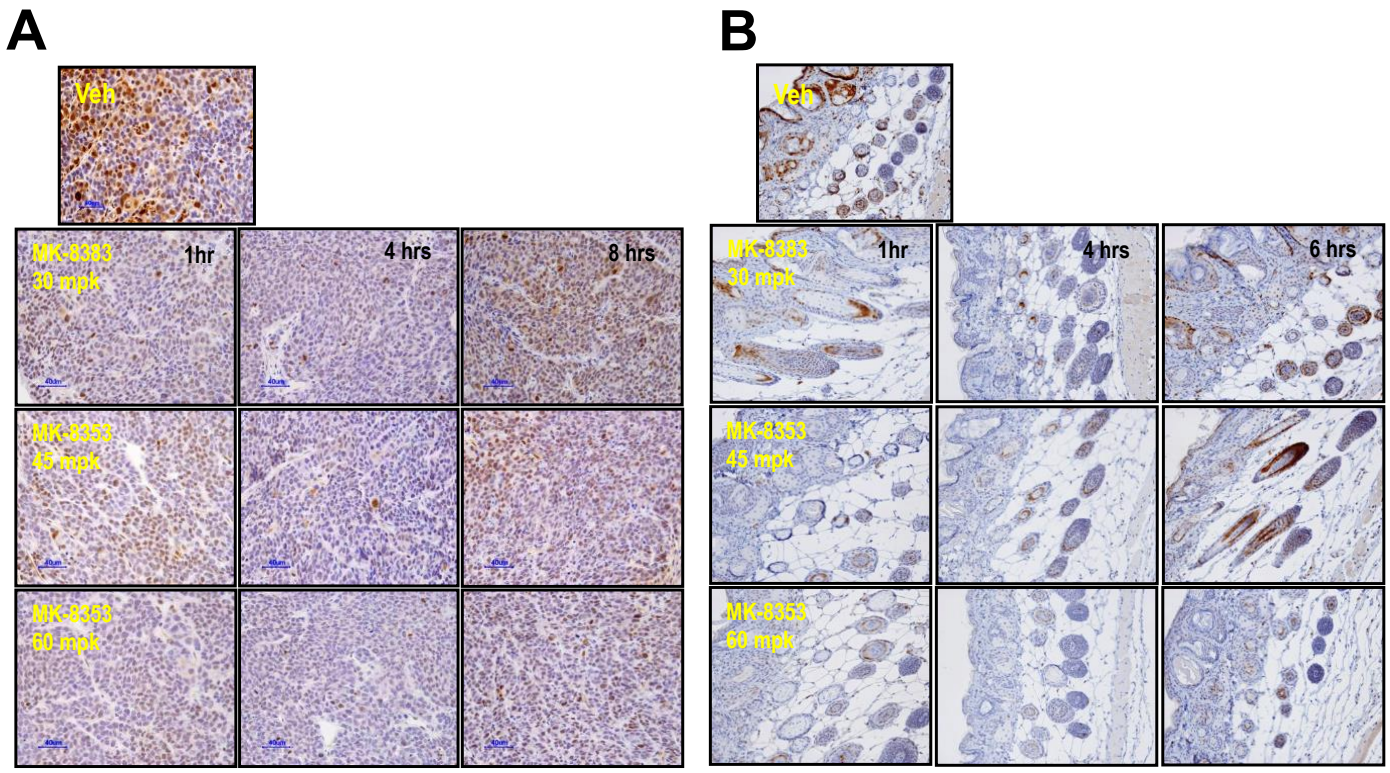
b: Calculated as the ratio of AUC_{0-12} hours after multiple dose to single dose

Supplemental Table S7. Adjusted Scores for pERK expression in skin biopsies by immunohistochemistry for subjects with at least one on-treatment sample from the MK-8353-001 study. Mean and standard deviations (SD) are shown for each sample.

Subject #	MK-8353 Cohort	Timepoint	Mean±SD	% Inhibition
1002	100 mg	Screening	84.3 ± 6.0	
		Day 8	129.9 ± 1.7	-52.8
		Day 15	83.4 ± 4.2	0.0
1003	200 mg	Screening	90.3 ± 4.0	
		Day 8	110.1 ± 6.0	-21.9
		Day 15	70.2 ± 10.1	36.3
2005	200 mg	Screening	228.1 ± 21.7	
		Day 8	98.4 ± 17.6	56.8
		Day 15	48.8 ± 17.6	78.6
2007	300 mg	Screening	121.5 ± 1.2	
		Day 8	149.8 ± 4.6	-23.3
		Day 15	124.2 ± 18.0	-2.2
2008	300 mg	Screening	130.0 ± 5.7	
		Day 8	114.8 ± 0.2	11.6
		Day 15	103.2 ± 7.4	20.6
2009	300 mg	Screening	78.9 ± 8.9	
		Day 8	43.4 ± 7.9	44.9
		Day 15	43.3 ± 7.3	45.1
2010	300 mg	Screening	75.8 ± 5.0	
		Day 8	95.6 ± 1.4	-26.1
		Day 15	56.3 ± 34.3	25.8
2011	350 mg	Screening	102.7 ± 11.4	
		Day 8	58.5 ± 2.8	43.0
		Day 15	100.3 ± 7.6	2.4
2012	350 mg	Screening	91.7 ± 15.4	
		Day 8	106.1 ± 12.8	-15.7
		Day 15	99.9 ± 8.2	-9.0
2015	350 mg	Screening	151.9 ± 2.5	
		Day 8	62.8 ± 14.7	13.2
		Day 15	46.9 ± 9.0	35.2
1004	400 mg	Screening	84.9 ± 0.6	
		Day 8	106.1 ± 3.7	-25.0
		Day 15	108.2 ± 1.7	-1.9
2002	400 mg	Screening	143.9 ± 13.4	
		Day 8	89.5 ± 7.4	37.8
		Day 15	50.2 ± 3.6	65.1
2003	400 mg	Screening	102.8 ± 3.1	
		Day 8	20.4 ± 1.9	80.2
2004	400 mg	Screening	113.2 ± 2.9	
		Day 8	146.4 ± 1.1	-29.4
2013	400 mg	Screening	97.4 ± 5.4	
		Day 8	94.0 ± 11.0	3.5
2014	400 mg	Screening	72.3 ± 2.5	
		Day 8	62.8 ± 14.7	13.2
		Day 15	46.9 ± 9.0	35.2
2016	400 mg	Screening	165.2 ± 9.9	
		Day 8	95.4 ± 1.9	42.3
1007	800 mg	Screening	99.5 ± 11.8	
		Day 8	105.8 ± 0.1	-6.29

B. SUPPLEMENTAL FIGURES

Supplementary Figure S1. Effect of MK-8353 in pERK in tumor tissues from Colo-205 tumor xenografts (A) and same nude mouse normal skin (B). See **Figure 2c** and **2d** for details. Representative tissue sections from corresponding tissues that were collected during various timepoints following a single dose of MK-8353 were immunohistochemically stained with an antibody against pERK as described in Materials and Methods. Each image is a representative picture from a total of 3 animals dosed. Please note that pERK signal is low early as 1 hour following administration but increases at an earlier timepoint in normal tissues compared to tumor tissues for a given MK-8353 (Student's *t*-test).



Supplemental Figure S2. *In vitro* sensitivities and clinical response for subject 2009. *A.* M435 and M428 cells were treated with 0-10 μM of SCH772984 alone, trametinib alone, always at 1:10 concentration compared to SCH772984 (0-1 μM) or concurrent trametinib and MK-8353 (combo), in duplicates, while a constant amount of DMSO was kept in all conditions. Following incubation for 120 hours, cell viability was determined using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI), as previously described (Wong et al. Mol Cancer 2014). **B.** Clinical activity following 4 weeks of treatment with MK-8353.