SUPPLEMENTARY MATERIAL

Methods

Pharmacokinetics

Samples were centrifuged at 1000g at 4ºC for 10min, and plasma was stored at -80ºC until analysis. Rigosertib plasma concentrations were determined using a validated LC-MS-MS method, as described previously with modifications (46). PK parameters were calculated from rigosertib concentration-time data using standard non-compartmental methods as implemented in WinNonlin (Pharsight Corp., Cary, NC). The area under the plasma concentration-time curve (AUC) value was calculated to the last quantifiable sample (AUC_{last}) by use of the linear trapezoidal rule. The AUC values were extrapolated to infinity (AUC_{∞}) by dividing the last quantifiable concentration by the terminal disposition rate constant (λz), which was determined from the slope of the terminal phase of the concentration-time profile. The terminal half-life ($T_{1/2}$) was calculated as 0.693 divided by λz. A t-test was used for the comparison of parameters on days 1 and 21 of the first cycle and day 15 of the second cycle. The *a priori* level of significance was $p< 0.05$. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, IL).

Sample concentrations in human urine were measured by a validated LC-MS/MS method with a calibration range of 25.0 to 37500 ng/mL for ON 01910 and 1.00 to 1500 ng/mL for ON 01500. Urine samples (100 µL) were extracted by acetonitrile protein precipitation and then separated by BDS C18 column (HyperClone, 5 µm, 100 x 2.0 mm) under gradient elution. Sciex API-4000 LC/MS/MS system (Applied Biosystems) was operated in positive mode with multiple reaction monitoring (MRM) using ion transitions at m/z as follows: ON 01910: 452>194; ON 01500:394>136 and internal standard Temazepam: 301>255.

Bioavailability studies were carried out in a parallel phase 1 trial for patients with MDS and were not performed here (Komrokji *et al*, submitted).

SNaPshot® Assay

The Colorado Molecular Correlates (CMOCO) Laboratory SNaPshot® panel tests for 68 discrete mutational loci, 8 of which have been validated for treatment guidance in non-small cell lung carcinoma. Briefly, after nucleic acid is extracted, target regions of the 15 tested genes are PCR amplified with multiplex primer sets. After phophatase and exonuclease treatment to remove unincorporated dNTS and primers, the PCR products are then used as a template for labeled single base extension using matched probe sets. The resulting SNaPshot® products are analyzed by capillary electrophoresis and examined for the presence of mutations. Determination of the presence or absence of mutation is carried out by manual evaluation of the data through GeneMapper® software (Applied Biosystems), using parameters defined in the CMOCO laboratory.

Exome sequencing

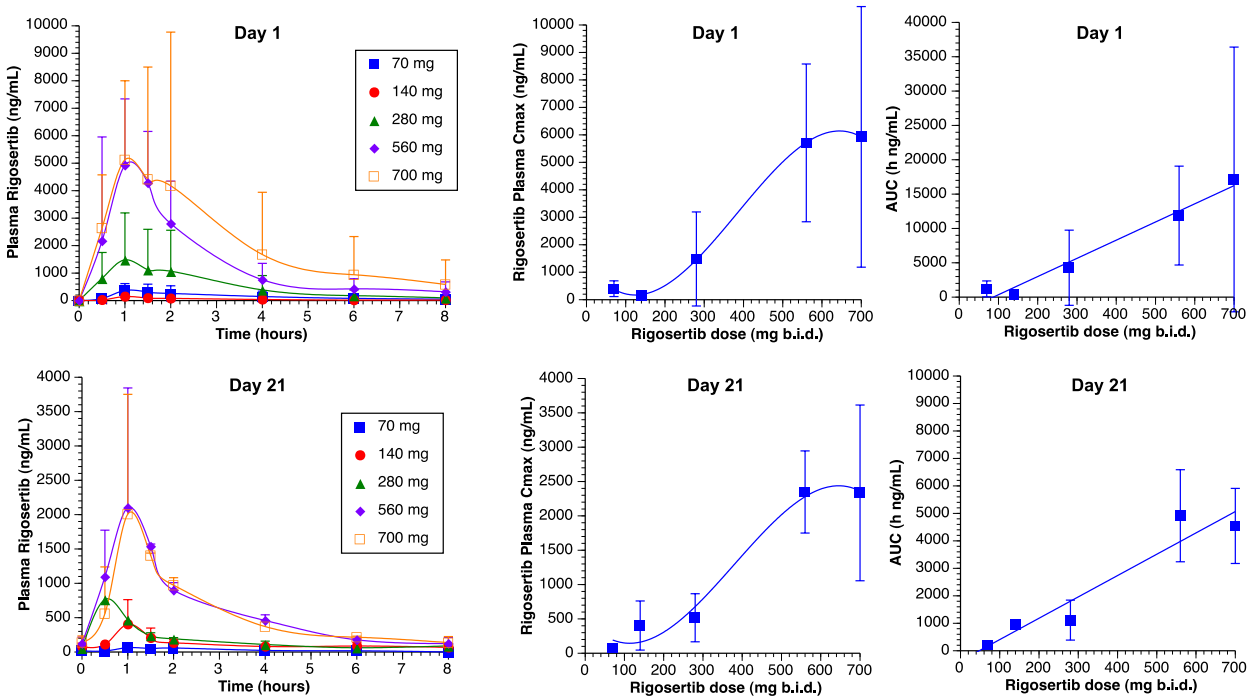
On average 161 million 100 bp paired-end reads were obtained per sample at an average coverage of 75x (Supplemental Table 6). Due to an error in the sequencing, the second read of each pair was trimmed to 70bp. The reads were mapped against the human genome (hg19) using Bowtie2 (version 2.0.5)(47). On average 98% of the reads aligned to the human genome one or more times and on average 80% of the reads aligned exactly once. Reads aligning to multiple locations or with a mapping quality score less than 30 were excluded from the analysis. Variants were identified using Samtools/Bcftools (version 0.1.18) and filtered according to the following criteria. Variants were excluded if the total depth was less than 20, the total depth was more than 2000, had fewer

than 5 alternate bases, variant quality score less than 20, or if the alternate base was not found on both strands (e.g. strand specific)(48). Common variants were excluded by comparing the variants obtained by Exome-seq to the variants found in a previous RNA-seq study.(33) Variants common to more than 70% of the samples were not of interest to this study. The potential impact of each variant on protein function was inferred using PolyPhen2 (49). Variants predicted to be damaging were used in the subsequent analysis.

SUPPLEMENTAL FIGURE LEGENDS

Figure 1. Pharmacokinetics of oral rigosertib. Following a single oral dose of rigosertib, the plasma concentration-time profile exhibited a rapid distribution phase followed by a slower elimination phase. Rigosertib C_{max} and $AUC_{0-\infty}$ were linear and dose-proportional over the dose range of 70-700mg (right hand panels). C_{max} was significantly lower at steady-state on day 21 than on day 1.

Figure 1.


Day 1
Plasma Rigosertib (ng/mL)
Time (hours)
70 mg
140 mg
280 mg
560 mg
700 mg
Day 1
Rigosertib Plasma Cmax (ng/mL)
Rigosertib dose (mg b.i.d.)
Day 1
AUC (h ng/mL)
Rigosertib dose (mg b.i.d.)
Day 21
Plasma Rigosertib (ng/mL)
Time (hours)
70 mg
140 mg
280 mg
560 mg
700 mg
Day 21
Rigosertib Plasma Cmax (ng/mL)
Rigosertib dose (mg b.i.d.)
Day 21
AUC (h ng/mL)
Rigosertib dose (mg b.i.d.)

SUPPLEMENTAL TABLES

Table 1. Patient characteristics and treatment responses

	PID	Dose	Sex	Age	Tumor type	TOS	Best response	DOR
D	1	70	M	56	Transitional cell carcinoma	4	PD	
O	3	70	F	49	Ovarian carcinoma	42	SD	36
S	4	70	F	81	Ovarian carcinoma	18	SD	12
E	5	140	F	62	Pancreatic neuroendocrine	30	SD	24
	6	140	F	57	Vulvovaginal melanoma	12	PD	
E	8	280	F	57	Carcinoid tumor	25	SD	20
S	9	280	M	59	Salivary gland carcinoma	1	NE	
C	10	280	M	50	HNSCC	101^{+}	CR	96+
A	11	560	M	61	Hepatocellular carcinoma	6	PD	
L	12	560	F	50	Adenoid cystic nasopharyngeal	27	SD	22
A	13	560	F	63	Colorectal carcinoma	6	PD	
T	14	700	M	53	Renal cell carcinoma	6	PD	
I	16	700	M	71	Renal cell carcinoma	29	SD	23
O	17	700/560	M	60	HNSCC	11	PD	
N	18	700/560	M	63	GIST	5	PD	
	19	700/560	M	51	Renal cell carcinoma	6	PD	
	21*	700	F	60	Breast carcinoma	1	NE	
	22	700/560	M	46	HNSCC	12	PD	

	24	560	M	72	Esophageal adenocarcinoma	8	PD	
E	26	560	M	57	Esophageal adenocarcinoma	6	PD	
X	27	560	F	72	Uterine carcinosarcoma	*6*	PD	
P	28	560/280/140	F	63	Ovarian carcinoma	10	PD	
A	30	560	F	45	Breast carcinoma	3	PD	
N	31	560	M	39	Craniopharyngioma	18	SD	12
S	32	560	F	54	Adrenocortical carcinoma	4	PD	
I	33	560	F	68	Colorectal carcinoma	12	PD	
O	34	560	F	40	Cervical adenocarcinoma	5	PD	
N	35	560	M	54	HNSCC	50	PR	40
	36	560	M	60	Esophageal adenocarcinoma	4	PD	
C	37	560	M	64	Colorectal carcinoma	10	PD	
O	38	560	M	50	Colorectal carcinoma	6	PD	
H	39	560	F	69	Uterine adenocarcinoma	6	PD	
O	40	560	M	69	Esophageal adenocarcinoma	6	PD	
R	41	560	F	63	SCC of the vulva	12	PD	
T	43	560	M	47	HNSCC	10	PD	
	44	560	F	64	Colorectal carcinoma	5	PD	
	45	560	F	54	Ovarian carcinoma	5	PD	
	46	560	F	77	Hepatocellular carcinoma	22	SD	15
	47	560	F	68	HNSCC	5	PD	
	48	560	M	74	Colorectal carcinoma	5	PD	

	50	560	F	65	Colorectal carcinoma	6	PD	
	51	560	M	28	Colorectal carcinoma	6	PD	
	52	560	M	79	Leiomyosarcoma	5	PD	
	53	560	M	56	Colorectal carcinoma	6	PD	
	54	560	M	58	Prostate carcinoma	5	PD	
	55	560	F	56	Lung SCC	8	PD	
	56	560	F	49	Colorectal carcinoma	7	PD	
	57	560	F	20	Osteosarcoma	6	PD	

*This patient was taken off study 2 days after treatment initiation because of PD, and replaced with a seventh patient in the 700mg cohort. Abbreviations: PID: Patient identifier; TOS: Time on study (weeks); PR: Partial response; SD: Stable disease; PD: Progressive disease; DOR: Duration of response (weeks).

Table 2. Grade ≥2 adverse events possibly, probably or definitely related to rigosertib treatment

PID	Dose (mg BID)	Grade	Toxicity	Onset (week)	Hold	Reduce
01-03	70	2	Hematuria	42	No	No
		3	Pelvic pain	42	No	No
01-05	140	2	Cystitis	22	No	Yes
		2	Micturition urgency	22	No	No
01-12	560	2	Dysuria	8	No	No
01-13	560	2	Hypertonic bladder	5	No	No
01-14	700	2	Dysuria	1	No	No
		2	Hematuria	3	Yes	Yes
		3	Hyponatremia	6	No	No
01-16	700	2	Dysuria	26	Yes	Yes
		2	Abdominal discomfort	3	Yes	No
		2	Abdominal distension	3	Yes	No
		2	Nausea	4	Yes	No
		2	Micturition urgency	26	Yes	Yes
		2	Micturition frequency	26	Yes	Yes
01-17	700	2	Abdominal pain	2	No	Yes
01-18	700	2	Dysuria	1	Yes	Yes
		3	Hematuria*	2	Yes	Yes
01-19	700	2	Micturition urgency	1	Yes	Yes
		2	Dysuria	1	Yes	Yes
01-27	560	2	Cystitis	3	Yes	No

01-28	560	2	Dysuria	4	Yes	No
		2	Pelvic pain	5	No	No
		3	Dysuria	6	Yes	Yes
		2	Cystitis	4	No	Yes
01-32	560	3	Dysuria**	3	Yes	No
		3	Hematuria**	3	Yes	No
01-35	560	2	Dysuria	14	Yes	No
01-37	560	2	Dysuria	6	No	Yes
01-38	560	2	Dysuria	3	Yes	No
01-40	560	2	Dysuria	4	Yes	Yes***
01-45	560	2	Dysuria	6	No	No
01-46	560	2	Dysuria	12	No	Yes
01-47	560	2	Urinary hesitancy	2	No	Yes
		3	Fatigue	3	No	Yes
		2	Fatigue	4	No	No
01-50	560	2	Dysuria	5	No	No
01-51	560	2	Dysuria	2	Yes	Yes
01-53	560	2	Dysuria	4	Yes	Yes***
01-54	560	2	Dysuria	2	Yes	No
		2	Micturition frequency	2	Yes	No
		2	Micturition urgency	2	Yes	No
01-56	560	2	Dysuria	2	Yes	No
		2	Cystitis	2	Yes	No

*: DLT (Grade 3 hematuria week 2); **: DLT (Grade 3 hematuria and dysuria week 3); ***: Treatment discontinued because of toxicity.

Table 3. Serious Adverse Events

PID	Dose (mg)	Grade	Serious Adverse Event	Relationship to rigosertib
01-01	70	3	Bile duct obstruction	None (disease progression)
01-01	70	2	Alanine aminotransferase increased	None (disease progression)
01-01	70	2	Aspartate aminotransferase increased	None (disease progression)
01-01	70	3	Pain	None (disease progression)
01-02	70	3	Bilateral pulmonary embolism	None (disease progression)
01-06	140	3	Abdominal pain and dehydration	None (disease progression)
01-09	280	5	Pleural effusion	None (disease progression)
01-24	560	3	Pneumonia	None
01-24	560	3	Sepsis	None
01-27	560	3	Pyelonephritis	None
01-27	560	5	Death	None (disease progression)
01-30	560	3	Nausea	None
01-30	560	3	Small intestinal obstruction	None (disease progression)
01-32	560	3	Dyspnea	None (disease progression)
01-34	560	3	Hepatic hemorrhage	None (disease progression)
01-39	560	3	Rectal hemorrhage	None
01-52	560	3	Weakness	Possible
01-52	560	3	Pancreatitis	None

01-18	700	3	Abdominal pain, Dehydration	None (disease progression)
01-18	700	5	Death	None (disease progression)
01-21	700	3	Renal failure	None (disease progression)

Table 4. Summary of mutations and gene copy number changes

PID	**Site**	***TP53***	***PIK3CA***	***KRAS***	***NRAS***	**Other**	***PTEN* copy#**	***PIK3CA* copy#**
3	Ovarian		34G>T					
8	Carcinoid							
10	HNSCC	743G>A					LLG	HLG
11	Hepatocellular							
12	Adenoid cystic							
14	Renal cell							
17	HNSCC						HLL	HLG
19	Renal cell*							
21	Breast		3140G>A					
24	Esophageal							
26	Esophageal							
27	Uterine		1637A>C					
28	Ovarian							
30	Breast							
32	Adrenocortical							

33	Colorectal	733G>A			34G>T	*APC* 4666 ins		
35	HNSCC				181C>A		HLL	D
36	Esophageal							
37	Colorectal							
39	Uterine		3140A>G					
40	Esophageal							
41	SCC vulva							
43	HNSCC					*AKT* 49G>A	D	LLL
44	Colorectal	524G>A						
45	Ovarian			35G>A				
48	Colorectal		1633G>A	35G>A				
50	Colorectal			34G>A				
51	Colorectal		1624G>A	35G>A				
53	Colorectal			35G>A				
55	Lung SCC	524G>A					D	HLG

*: Tissue sample rejected. Gene copy number analysis by FISH was performed only on 5 SCC samples: HLG: High level copy number gain; HLL: High level copy number loss; LLG: Low level copy number loss; LLL: Low level copy number loss; D: Disomy

Table 5. Validated exome variants seen exclusively in sensitive and resistant squamous cell carcinomas.

Gene	Description	Chromosome	Position	Nucleotide change	Amino-acid change
Non-synonymous SNPs present in both sensitive tumors but in none of the resistant tumors					
A2ML1	Alpha-2-macroglobulin-like 1	12	9009820	G>A	R1122W
ABCC11	ATP-binding cassette, sub-family C (CFTR/MRP), member 11	16	48242379	G>A	T546M
ABCC11	ATP-binding cassette, sub-family C (CFTR/MRP), member 11	16	48204078	T>A	N1277Y
ACIN1	Apoptotic chromatin condensation inducer 1	14	23564437	T>C	N20S
FAT1	FAT tumor suppressor homolog 1 (Drosophila)	4	187630590	G>A	A131V
FREM1	FRAS1 related extracellular matrix 1	9	14819370	G>T	S803Y
KIAA1683	KIAA1683	19	18376518	T>A	Q611L
ROBO3	Roundabout, axon	11	12474236	G>A	

	guidance receptor, homolog 3 (Drosophila)		5		R416H
ROS1	C-ros oncogene 1, receptor tyrosine kinase	6	117725448	T>A	T145P
SLC26A8	Solute carrier family 26, member 8	15	85448875	C>A	I148V
TIGD2	Tigger transposable element derived 2	4	90035549	A>G	H475R
ZC3H4	Zinc finger CCCH-type containing 4	19	47570343	G>A	A1061V
Non-synonymous SNPs present in all resistant tumors but in neither of the two sensitive tumors					
A2ML1	Alpha-2-macroglobulin-like 1	12	9013755	C>T	C970Y
ADAMTSL 1	ADAMTS-like 1	9	18777196	A>C	E990A
CDH23	Cadherin-related 23	10	73492079	A>G	N1351D
HCLS1	Hematopoietic cell-specific Lyn substrate 1	3	121351338	C>T	E361K
PDE4C	Phosphodiesterase 4C, cAMP-specific	19	18329784	C>T	R344Q

Table 6. Exome Sequencing Coverage

PID	Number of Reads	Aligned one or more times	Aligned once	Mean Fold Coverage
01-10	216,099,716	98.10%	79.20%	88 x
01-17	225,139,870	98.80%	81.80%	92 x
01-22	172,639,390	97.20%	79.60%	79 x
01-35	244,163,170	97.70%	77.70%	95 x
01-43	18,433,134	96%	76.70%	33 x
01-55	91,803,484	98.80%	83.90%	61 x