Supplementary Information

Methods

Study Design

The trial implemented standard inclusion/exclusion criteria including age ≥ 18, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, bilirubin < 2, aspartate aminotransferase/alanine aminotransferase (AST/ALT) < 2.5 upper limits of normal (ULN), normal serum creatinine or creatine clearance > 50. Patients were not excluded due to previous treatments, prior stem cell transplantation or other malignancy, if in remission.

Safety

DLTs were defined for both Strata as: 1) any non-hematologic toxicity ≥ Grade 3 attributable to RG7112, except for fatigue, anorexia and alopecia; 2) nausea/vomiting and/or diarrhea would be considered DLT only if they reached ≥ Grade 3 severity despite adequate supportive care measures and 3) Grade 3 AST or ALT toxicity lasting for > 7 days or Grade 4 lasting for any duration of time.

For Stratum A patients, myelosuppression and associated complications are expected during leukemia therapy and were judged to be part of the disease process. Therefore, these complications were not considered dose limiting for Stratum A patients. However, prolonged myelosuppression was considered as a DLT. After a patient was removed from study due to a DLT of uncomplicated hyperuricemia from tumor lysis, the protocol was subsequently amended to exclude isolated laboratory abnormalities associated with tumor lysis as part of the definition of DLT.

For Stratum B patients, the following AEs were considered a DLT: (1) Grade 4 neutropenia lasting at least 7 days; (2) febrile neutropenia: absolute neutrophil count (ANC) < 1.0 x 10^9/L and temperature ≥ 38.5^o C; (3) thrombocytopenia with platelet count ≤ 10 x 10^9/L or any thrombocytopenia requiring platelet transfusion and (4) delay of Cycle 2 - Day 1 treatment for >

14 days due to drug-related toxicity. The DLT criteria for myelosuppression only applied if patient baseline ANC and platelet counts were ≥ 1.5 x 10^9/L and ≥ 100 x 10^9/L, respectively. If the baseline ANC and/or platelet count were low as a result of the involvement of the bone marrow by the disease, then the blood count and DLT criteria as defined for Stratum A were applied.

Supplementary Data 1. Study design	
Stratum A	**Stratum B**
Acute leukemias, including AML (not APL), ALL, CML-BC Inclusion of a “tail” to test efficacy at the MTD	Chronic leukemia, including CLL and sCLL
PO QD dosing $_{x}$ 10 days followed by 18 days off (28-day cycle) Dose escalations, DLT and MTD conducted independently for each stratum	
Dosing changed to BID; at MTD, dose changed to BID flat dose for convenience	

AML, acute myelotic leukemia; APL, acute promyelocytic leukemia; CML-BC, chronic myelogenous leukemia – blast crisis; MTD, maximum tolerated dose; CLL, chronic lymphocytic leukemia; sCLL, small cell lymphocytic leukemia; QD, once-daily; DLT, dose-limiting toxicity; BID, twice-daily

Supplementary Data 2. p53 target genes		
Gene symbol	**Gene name**	**Description**
APAF1	Apoptotic peptide activating factor	Cytoplasmic protein that initiates apoptosis
BAX	Bcl2-associated X protein	Regulated by p53; involved in p53-mediated apoptosis
BBC3/PUMA	Bcl2 binding component 3	Belongs to BH3-only pro-apoptotic subclass and induces mitochondrial outer membrane permeabilization
BTG2	BTG family member 2	Has anti-proliferative properties andregulates the G1/S transition
CDKN1A	Cyclin-dependent kinase inhibitor 1A	Controlled by p53 and mediates p53-dependent G1 arrest
CXCL12	Chemokine (C-X-C motif) ligand 12	Plays a role in tumor growth
CXCR4	Chemokine (C-X-C motif) receptor 4	Overexpressed in tumors
E2F1	E2F transcription factor 1	Mediates cell proliferation and p53-dependent/independent apoptosis
FAS	FAS cell surface death receptor	Forms a death-inducing signaling complex
FDXR	Ferredoxin reductase	Mitochondrial flavoprotein that affects p53-dependent apoptosis
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Used as a housekeeping gene
GDF15/MIC-1	Growth differentiation factor 15	Affects p53 activation and regulates tissue differentiation/maintenance
IL8/CXCL8	Chemokine (C-X-C motif) ligand 8	Chemoattractant with angiogenic functions

MDM2	Human homolog of double minute 2	E3 ubiquitin protein ligase that binds p53
PERP	p53 induced protein PIGFC1	p53 apoptosis effector
PMAIP1/NOXA	PMA induced product 1	Directs the hypoxia response
PTEN	Phosphatase and tensin homolog	Functions as tumor suppressor by negatively regulating the AKT/PKB signaling pathway
TNFRSF10B/DR5	tumor necrosis factor receptor superfamily, member 10b	Receptor for the cytotoxic ligand TNFSF10/TRAIL
TP53	Tumor protein 53	Tumor suppressor protein
TP53I3	Tumor protein p53 inducible protein 3	Induced by p53 and involved in p53-mediated apoptosis
TP53INP1	Tumor protein p53 inducible nuclear protein 1	Damage-dependent induction and induced by p53
XPO1	Exportin 1	Mediates leucin-rich nuclear export signal (NES)-dependent protein transport
ZMAT3	p53 target zinc finger protein	Upregulated by p53 and inhibits tumor cell growth
18S	18S ribosomal RNA	Used as a housekeeping gene

Supplementary Data 3. Evaluable patients treated at the MTD					
Disease	**Age / Sex**	**Status at entry / # prior**	**p53 Status at baseline**	**Response**	**AUC_{ss} (hr*ng)/mL**
AML	48 M	Primary refractory	WT	CR	214,092
AML	70 M	Primary refractory	WT	CR	221,341
AML	25 F	3rd relapse, preceding response ≥ 6 mo., Hx aplastic anemia	WT	CRp	123,900
AML	58 F	Refractory to 1st induction	WT	PR	240,810
AML	69 M	1st relapse, initial response < 6 mo., prior DLBCL	WT	PR	206,109
AML	69 F	Primary refractory, 2 prior regimens	WT	SD	220,040
AML	60 F	2nd relapse, preceding response ≥ 6 mo., Hx breast CA	WT	SD	107,782
AML	63 M	Refractory (2 prior regimens)	WT	SD	226,848
AML	62 F	Refractory (2 prior regimens)	Mutant	SD	314,430
AML	65 F	2nd relapse, preceding response ≥ 6 mo., prior breast CA	WT	SD	236,704
AML	36 M	Refractory NOS, prior MDS	WT	SD	365,078
AML	77 F	1st relapse, initial CR < 6 mo., antecedent MDS	WT	SD	178,671
AML	58 M	2nd relapse, preceding response < 6 mo.	WT	SD	175,170

AML	73 M	1st relapse, initial response ≥ 6 mo.	Mutant	SD	307,322
ALL	22 F	1st relapse, initial response ≥ 6 mo.	WT	SD	NA
ALL	43 F	1st relapse, initial CR < 6 mo.	WT	SD	130303
AML	56 M	Relapse < 6 mo. after MUD BMT, prior rectal CA	WT	PD	78,555
AML	26 M	2nd relapse, preceding response ≥ 6 mo.	WT	PD	58,796
AML	19 M	3rd relapse, preceding response ≤ 6 mo.	Unknown	PD	150,090
AML	60 M	3rd relapse, preceding response ≥ 6 mo.	Mutant	PD	196,265
AML	33 F	FLT3 mutant, refractory relapse after BMT	WT	PD	117,150
AML	73 M	3rd relapse, duration of preceding response unknown	Mutant	PD	NA
ALL	58 M	3rd relapse, preceding response ≥ 6 mo.	WT	PD	39,918
AML	31 F	2nd relapse, initial CR < 6 mo.	WT	PD	153,600
AML	61 M	Relapse > 3 prior regiments, initial CR < 6 mo.	WT	PD	NA

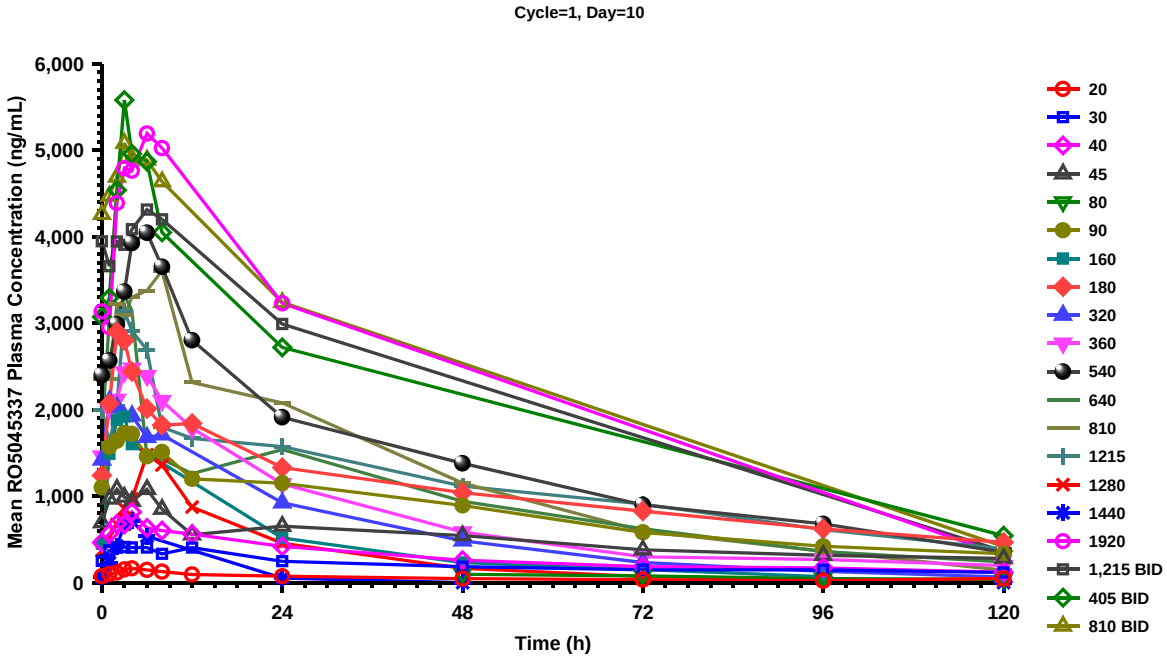
AML	27 M	Biophenotypic, refractory 3rd relapse, preceding response ≥ 6 mo.	Mutant	PD	88,103
AML	63 M	2nd refractory relapse, initial CR < 6 mo.	WT	PD	NA
AML	56 M	Refractory NOS, hx RCC	WT	PD	73,917
AML	29 M	3rd relapse, preceding response < 6 mo.	WT	PD	113,885
AML	66 M	2nd relapse, preceding response ≥ 6 mo.	WT	PD	367,648
AML	72 M	Relapse 1st, initial response ≥ 6 mo., hx MM	Mutant	PD	76,426
AML	72 M	Refractory NOS	WT	PD	N/A
AML	68 M	1st relapse, initial response > 6 mo.	WT	PD	NA

AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; WT, wild type; CR, complete response; CRp, complete response without platelet recovery; PD, progressive disease; SD, stable disease; NA, not available; AUC_{ss}, area under curve at steady state; DLBCL, diffuse large B-cell lymphoma; CA, cancer; RCC, renal cell carcinoma; MM, multiple myeloma; Hx, history; MDS, myelodysplastic syndrome; NOS, refractory anemia; MUD, matched unrelated donor; BMT, bone marrow transplant

Supplementary Data 4. Demographic Data by Cohort			
	Stratum A n = 96	Stratum B n = 20	All N = 116
Sex, n (%)			
Male	52 (54%)	14 (70%)	66 (57%)
Female	44 (46%)	6 (30%)	50 (43%)
Age, years			
Median	60	66	62
Range	19-85	54-79	19-85
Race, n (%)			
Caucasian	79 (82%)	18 (90%)	97 (84%)
Weight, kg			
Mean	71	78	72
SD	15	17	15
Previous regimens			
Mean	3.4	4.7	3.6
SD	2.0	2.9	2.2

Supplementary Data 5. Adverse events > 10% per Stratum			
Stratum A	**Total, n (%)**	**Grade 3, n (%)**	**Grade 4, n (%)**
Nausea	68 (71)	10 (10)	0
Diarrhea	58 (60)	7 (7)	0
Vomiting	39 (41)	6 (6)	0
Febrile neutropenia	29 (30)	22 (23)	2 (2)
Fatigue	28 (29)	5 (5)	0
Abdominal pain	27 (28)	8 (8)	1 (1)
Decreased appetite	27 (28)	2 (2)	0
Hypokalemia	25 (26)	11 (11)	3 (3)
Peripheral edema	23 (24)	0	0
Weight decreased	18 (19)	0	0
Pyrexia	17 (18)	5 (5)	0
Headache	15 (16)	1 (1)	0
Hypomagnesemia	15 (16)	2 (2)	0
Pneumonia	15 (16)	10 (10)	1 (1)
Epitaxis	14 (15)	2 (2)	0
Hypocalcemia	13 (14)	5 (5)	0
Oropharyngeal pain	13 (14)	0	0
Asthenia	12 (13)	0	0
Cough	12 (13)	0	0
Dypnea	12 (13)	0	0
Hypertension	12 (13)	3 (3)	0
Insomnia	12 (13)	0	0
Constipation	11 (11)	1 (1)	0
Hypophosphatemia	11 (11)	4 (4)	1 (1)
Hypotension	11 (11)	3 (3)	0
Stratum B	**Total, n (%)**	**Grade 3, n (%)**	**Grade 4, n (%)**
Nausea	11 (55)	1 (5)	0
Diarrhea	7 (35)	1 (5)	0
Anemia	6 (30)	2 (10)	1 (5)
Fatigue	6 (30)	0	0
Thrombocytopenia	6 (30)	2 (10)	2 (10)
Headache	5 (25)	0	0
Pneumonia	5 (25)	4 (20)	1 (5)
Abdominal pain	4 (20)	2 (10)	0
Arthralgia	4 (20)	0	0
Cough	4 (20)	0	0
Hyperuricemia	3 (15)	0	0
Hypotension	3 (15)	2 (10)	0
Vomiting	3 (15)	0	0

Supplementary Data 6. Day 10 dose-mean PK profiles in patients with leukemia.



The mean plasma concentration of RG7112 (mg/m^2) decreased over time (h) for Stratum A and Stratum B leukemia patients of Cycle 1, Day 10. The doses ranged from 20 mg/m^2 to 1820 mg/m^2 QD and 406 mg/m^2 to 1216 mg/m^2 BID. Each dose is represented as a different color in the plot. QD = once-daily; BID = twice-daily; h = hours.

Supplementary Data 7. Pharmacokinetic parameters								
					CYCLE 1 Day 10			
Stratum	**Dose and Regimen**	**Formulation**	**AUC_{SS}[c]**	**AUC_{0-12}[c]**	**AUC_{0-24}[c]**	**CMax[c]**	**HLife[c]**	**TMax[d]**
A	20 mg/m^2 QD[a]	Crystalline	1597.2 (n=4)	1006.6 (56.5%), n=4	1597.2 (64.4%), n=4	112.3 (48.3%), n=4	58.4 (114.9%), n=4	2.5 (2-4), n=4
A	30 mg/m^2 QD	Crystalline	7859.2 (n=3)	4300.9 (81.4%), n=3	7859.2 (88.4%), n=3	442.7 (67.4%), n=3	61.9 (40.8%), n=3	3.1 (3-4), n=3
A	45 mg/m^2 QD	Crystalline	18842.6 (n=3)	10260.8 (52.7%), n=3	18842.6 (54.9%), n=3	1080.0 (53.1%), n=3	96.2 (45.4%), n=3	2.0 (2-2), n=3
A	90 mg/m^2 QD	Crystalline	31471 (n=1)	17370 (-%), n=1	31471 (-%), n=1	1730 (-%), n=1	52 (-%), n=1	3 (3-3), n=1
A	180 mg/m^2 QD	Crystalline	43824 (n=1)	24682 (-%), n=1	43824 (-%), n=1	2900 (-%), n=1	57 (-%), n=1	2 (2-2), n=1
A	360 mg/m^2 QD	Crystalline	49082.4 (n=2)	27949.6 (82.0%), n=2	49082.4 (84.6%), n=2	3075.0 (78.0%), n=2	22.7 (34.1%), n=2	3.5 (1-6), n=2
A	540 mg/m^2 QD	Crystalline	67635.3 (n=4)	38648.8 (29.8%), n=4	67635.3 (25.0%), n=4	4227.5 (39.9%), n=4	36.3 (39.8%), n=4	4.7 (4-6), n=4
A	810 mg/m^2 QD	Crystalline	67189.8 (n=5)	36991.6 (58.5%), n=5	67189.8 (60.5%), n=5	4074.0 (59.5%), n=5	23.7 (46.0%), n=5	4.0 (3-8), n=5
A	1215 mg/m^2 QD	Crystalline	48023.1 (n=4)	27497.2 (83.4%), n=4	48023.1 (83.6%), n=4	3393.0 (93.0%), n=4	41.0 (43.0%), n=4	4.1 (3-6), n=4
A	405 mg/m^2 BID[b]	Crystalline	47889.4 (n=3)	23944.7 (115.2%), n=3	46137.4 (112.4%), n=3	2518.7 (107.5%), n=3	55.0 (21.7%), n=2	3.0 (1-119), n=3
A	810 mg/m^2 BID	Crystalline	108329.3 (n=7)	54164.6 (49.8%), n=7	100944.3 (49.6%), n=7	5544.3 (52.8%), n=7	36.0 (57.1%), n=7	3.0 (1-6), n=7
A	1215 mg/m^2 BID	Crystalline	93360.8 (n=3)	46680.4 (81.3%), n=3	88065.4 (81.8%), n=3	4611.7 (80.7%), n=3	31.7 (40.0%), n=3	4.0 (2-8), n=3
	1000 mg BID	Amorphous	129711.8 (n=5)	64855.9 (31.5%), n=5	125264.1 (32.7%), n=5	6500.0 (30.1%), n=5	38.8 (30.8%), n=5	4.0 (2-8), n=5
A	1500 mg BID	Amorphous	152244.4 (n=5)	76122.2 (50.2%), n=5	137410.3 (52.9%), n=5	7726.0 (50.1%), n=5	21.8 (34.4%), n=5	3.0 (1-6), n=5
A	1500 mg BID fed	Amorphous	220911.5 (n=6)	110455.7 (50.3%), n=6	211081.2 (51.5%), n=6	10665.0 (51.6%), n=6	39.9 (42.2%), n=5	4.1 (1-6), n=6
B	20 mg/m^2 QD	Crystalline	2752.4 (n=2)	1702.9 (78.1%), n=2	2752.4 (77.8%), n=2	203.2 (76.5%), n=2	31.8 (37.9%), n=2	3.5 (3-4), n=2
B	40 mg/m^2 QD	Crystalline	6397.3 (n=2)	3634.4 (5.0%), n=2	6397.3 (25.0%), n=2	448.0 (10.4%), n=2	32.2 (34.1%), n=2	3.5 (3-4), n=2
B	160 mg/m^2 QD	Crystalline	27242 (n=1)	17062 (-%), n=1	27242 (-%), n=1	1920 (-%), n=1	25 (-%), n=1	3 (3-3), n=1
B	320 mg/m^2 QD	Crystalline	37011 (n=1)	20582 (-%), n=1	37011 (-%), n=1	2100 (-%), n=1	25 (-%), n=1	1 (1-1), n=1
B	1920 mg/m^2 QD	Crystalline	81804.9 (n=3)	42236.8 (102.2%),n =3	81804.9 (111.8%), n=3	4514.3 (92.9%), n=3	20.8 (72.1%), n=3	4.1 (3-8), n=3
B	1920 mg/m^2 QD	Crystalline	89972.3 (n=3)	49820.6 (27.6%), n=3	89972.3 (28.9%), n=3	5646.7 (31.6%), n=3	17.2 (18.9%), n=3	2.3 (2-6), n=3

A	TAIL 1500 mg BID	Amorphous	167325.1 (n=19)	83662.5 (48.0%), n=19	162685.4 (47.0%), n=19	8719.5 (45.9%), n=19	47.1 (53.7%), n=17	3.3 (0-25), n=19
A	MTD (1500 mg BID)	Amorphous	175528.9 (n=30)	87764.5 (49.4%), n=30	168152.0 (49.6%), n=30	8943.0 (47.6%), n=30	41.1 (56.6%), n=27	3.6 (0-25), n=30

AUC_{ss}, area under curve at steady state; MTD, maximum tolerated dose; QD, once-daily; BID, twice-daily; C_{max}, maximum observed plasma concentration; H_{life}, half-life; T_{max}, time to reach maximum concentration

[a] AUC at Steady State for QD patients = AUC 0-24 hr at Cycle 1 Day 10

[b] AUC at Steady State for BID patients = 2 * (AUC 0-12 hr at Cycle 1 Day 10)

[c] Format of Assessment Visit Statistics for PK parameters other than TMax: Mean (CV%), n =

[d] Format of Assessment Visit Statistics for TMax: Median (Minimum – Maximum), n =

Supplementary Data 8. Summary of steady state AUC for AML patients treated at the MTD

	Clinical Activity	**PD**
N	14	11
Mean	224307	134040
Median	221691	113845
Std. Dev.	70093	88069
Std. Err.	18733	26554
Minimum	1077872	58796
Maximum	365078	367648
CV (%)	31	66

Clinical Activity, CR (complete response), CRp (complete response without platelet recovery), PR (partial response) or SD (stable disease); PD, progressive disease; AUC, area under curve; AML, acute myelogenous leukemia; MTD, maximum tolerated dose; CV, coefficient of variation

Supplementary Data 9. Clinical activity for patients with p53 mutations

Portion of Study	Patient (Stratum)	Call	Codon	Codon Change	Exon	Mutant Type	Protein Description	p53 Function	Best Response
Dose Escalation - Blood Only	9701 (B)	C to G	94_2	TCA to TGA	4B	Nonsense	S94STOP	no information	
	8604 (A)	G to A	141_2		5	Missense	C141Y	Non-functional(36)	PD
		C to G	196_1		6	Missense	R196G	Partially functional(36)	
	8607 (A)	A to G			Intron 3	Splice		No information	SD
	9604 (B)	2bp del	209_1-209_2		6	Frameshift		No information	PR
	9605 (B)	A to G			Intron 3	Splice		No information	SD
	9609 (B)	G to T	249_2	AGG to ATG	7	Missense	R249M	Possibly damaging(37, 38)	SD
	9610 (B)	G to A	266_2	GGA to GAA	8	Missense	G266E	Probably damaging(37, 38)	SD
	8806 (A)	A to G	132_2	AAG to AGG	5	Missense	K132R	Non-functional(36)	
	5502 (A)	C to T	282_1	CGG to TGG	8	Missense	R282W	Non-functional(36)	PD
	9303 (B)	A to G	179_2	CAT to CGT	5	Missense	H179R	Probably damaging(37, 38)	PD
		A to G	239_1	AAC to GAC	7	Missense	N239D	Probably damaging(37, 38)	
Amorphous Escalation - Blood & Bone Marrow (when available)	8325 (A)[a]	A to G	240_1	AGT to GGT	7	Missense	S240G	Probably minor impact on DNA binding[c]	SD
	8421 (A)	G to A	175_2	CGC to CAG	5	Missense	R175H	Non-functional(36)	PD[2]
	8821 (A)	G to A	146_2	TGG to TAG	5	Nonsense	W146STOP	No information	PD
	5522 (A)[a]	DELG	323_3-324_1		9	Frame-shift		Probably impact on DNA binding[c]	PD
Tail - Blood and Bone Marrow (when available)	8626 (A)	G to A	248_2	CGG to CAG	7	Missense	R248Q	Significant impact on DNA binding(39)	PD
	8630 (A)	C to G	213_1	CGA to GGA	6	Missense	R213G	Probably damaging(37, 38)	SD
	8635 (A)	A to C			Intron 7	Splice		No information	PD[b]
	8726 (A)	A to G	281_2	GAC to GGC	8	Missense	D281G	Impact on DNA binding[c]	
	8329 (A)	G to T	266_2	GGA to GTA	8	Missense	G266V	Non-functional(36)	

SD, stable disease; PD, progressive disease; PR, partial response

[a] Blood and bone marrow were discordant. Mutation detected in bone marrow but blood was wild type

[b] Patients showed evidence of clinical activity (decreased peripheral blast counts)

[c] Predictions were performed by Roche based on the structure of p53(39)

Supplementary Data 10. RG7112 induces p53-mediated apoptosis in circulating lymphoma cells from a patient with SLL/CLL (810 mg to 1500 mg BID).