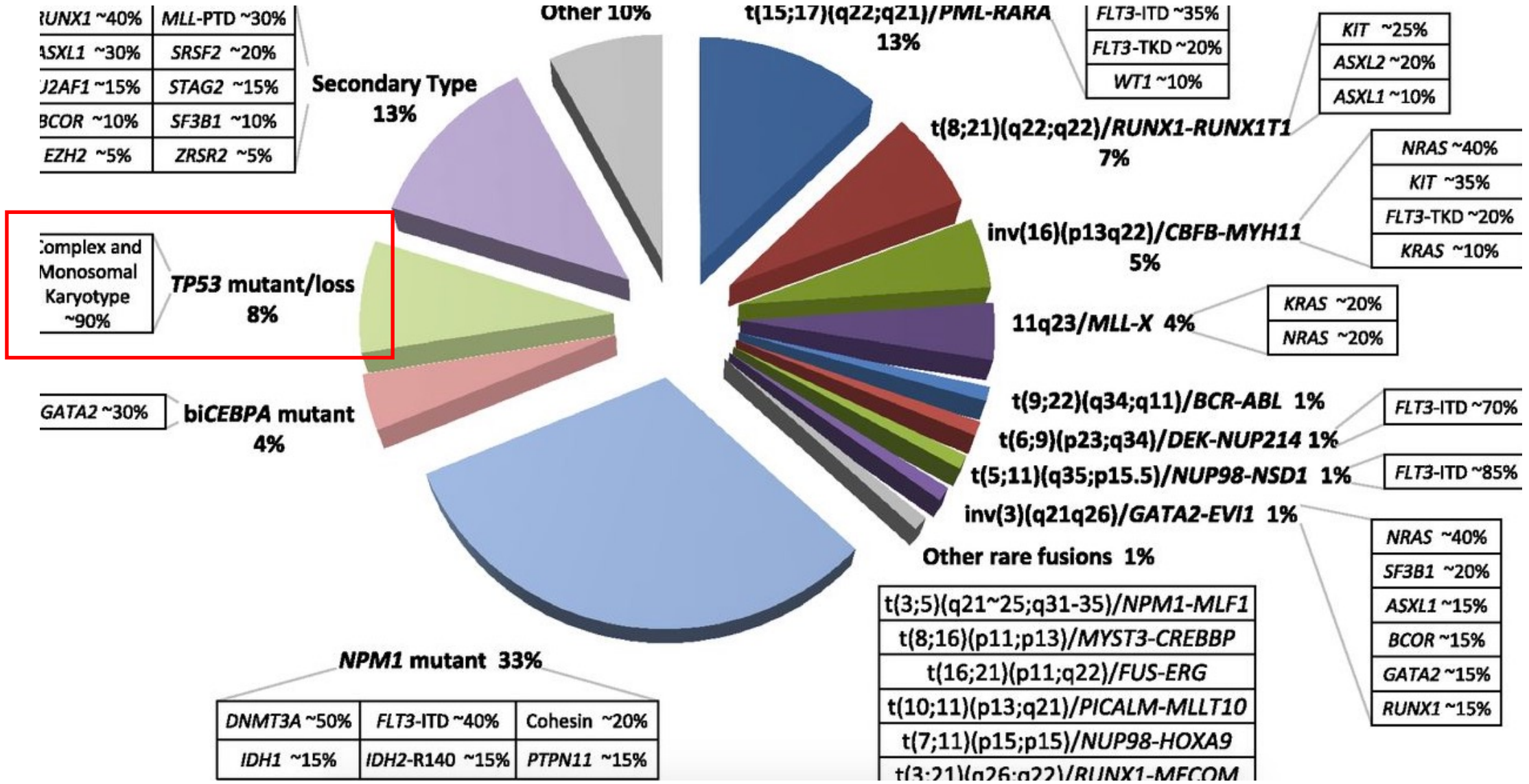
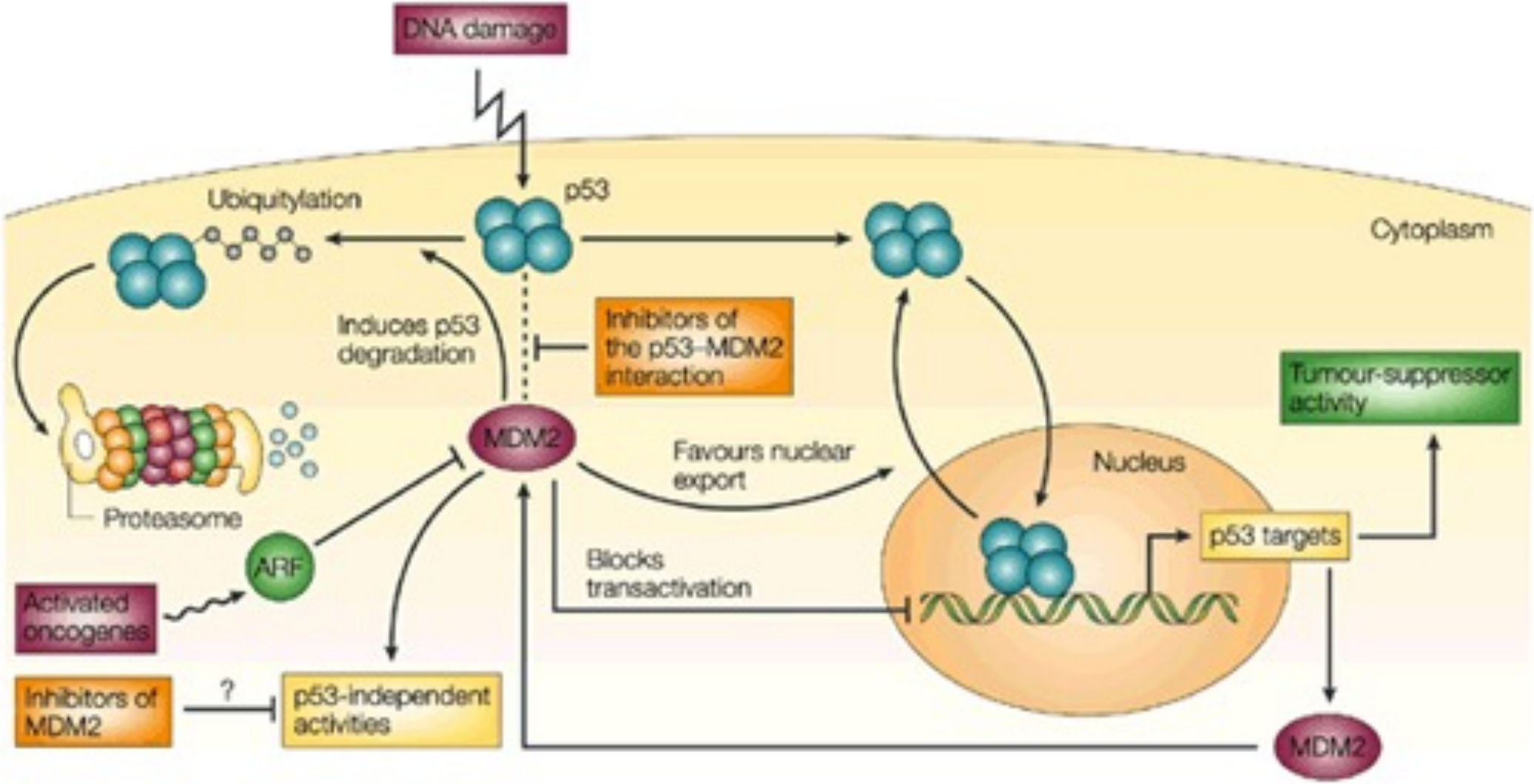
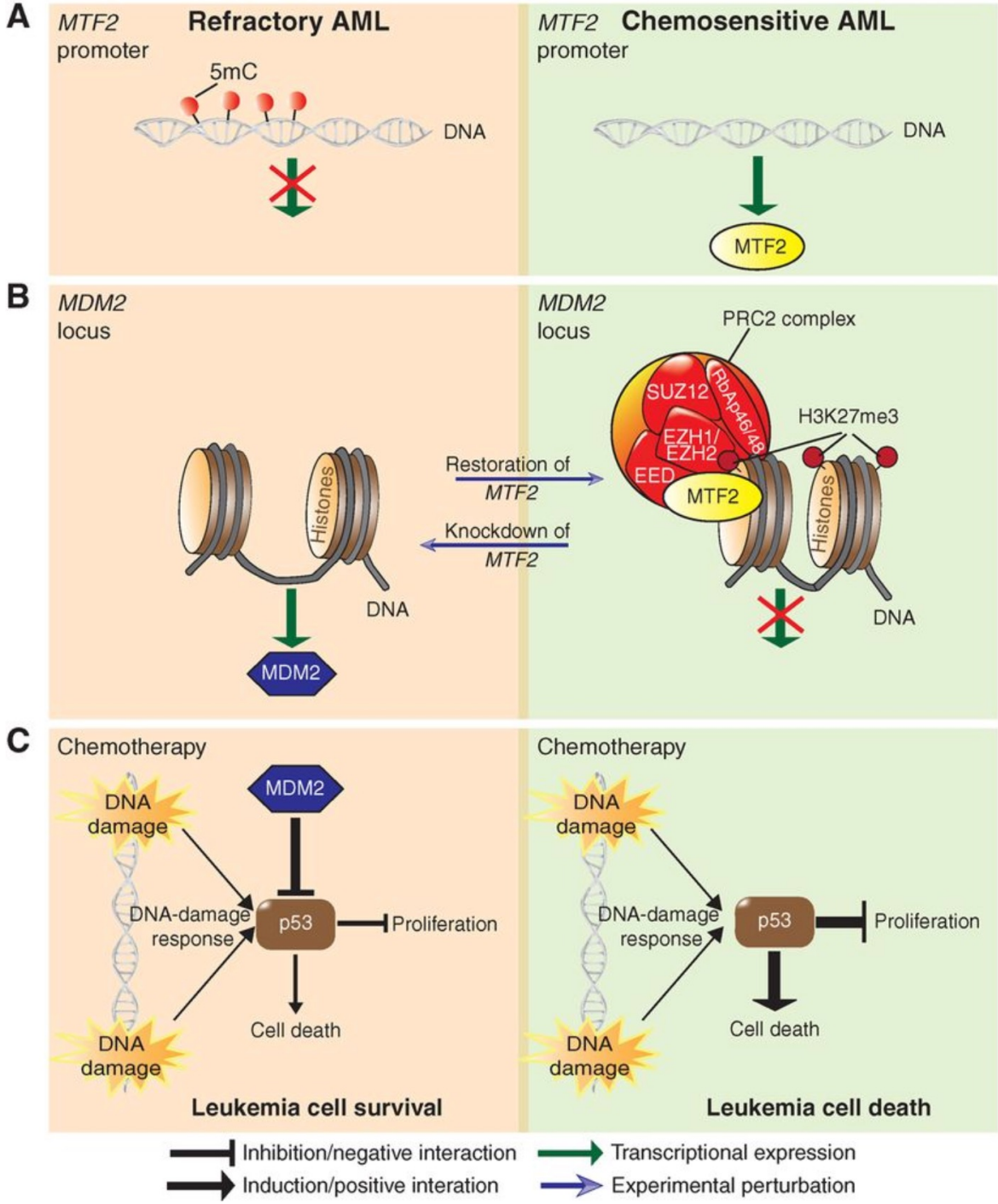
Kevin Kelly, MD, PhD
Acute Myeloid Leukemia

Targeting p53: MDM2 Inhibitors

TP53 mutations are rare in AML



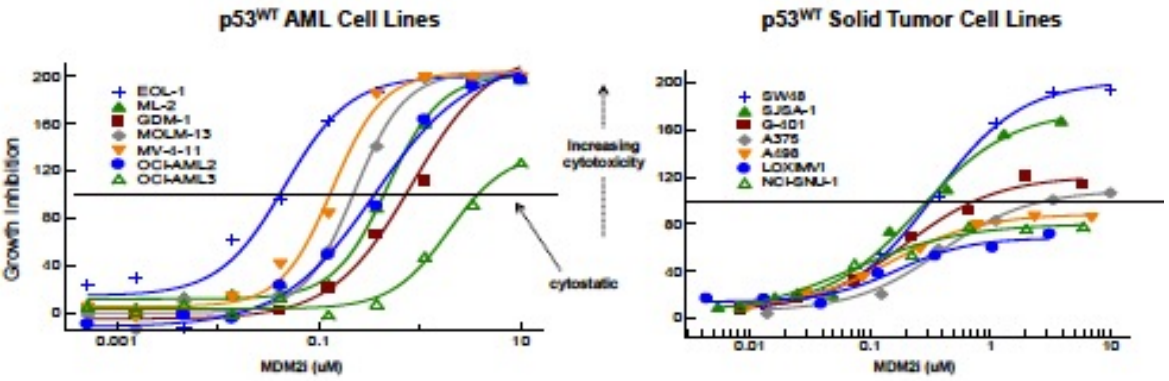

DNA damage
p53
Ubiquitylation
Cytoplasm
Induces p53 degradation
Inhibitors of the p53–MDM2 interaction
Tumour-suppressor activity
MDM2
Favours nuclear export
Nucleus
Proteasome
p53 targets
ARF
Blocks transactivation
Activated oncogenes
Inhibitors of MDM2
?
p53-independent activities
MDM2


A
MTF2 promoter
Refractory AML
5mC
DNA
MTF2 promoter
Chemosensitive AML
DNA
MTF2
B
MDM2 locus
Histones
DNA
MDM2
Restoration of MTF2
Knockdown of MTF2
MDM2 locus
PRC2 complex
SUZ12
RbAp46/48
EZH1/ EZH2
EED
MTF2
H3K27me3
Histones
DNA
C
Chemotherapy
DNA damage
MDM2
DNA-damage response
p53
Proliferation
Cell death
DNA damage
Leukemia cell survival
Chemotherapy
DNA damage
DNA-damage response
p53
Proliferation
Cell death
DNA damage
Leukemia cell death
Inhibition/negative interaction
Induction/positive interation
Transcriptional expression
Experimental perturbation

MDM2 Inhibitors

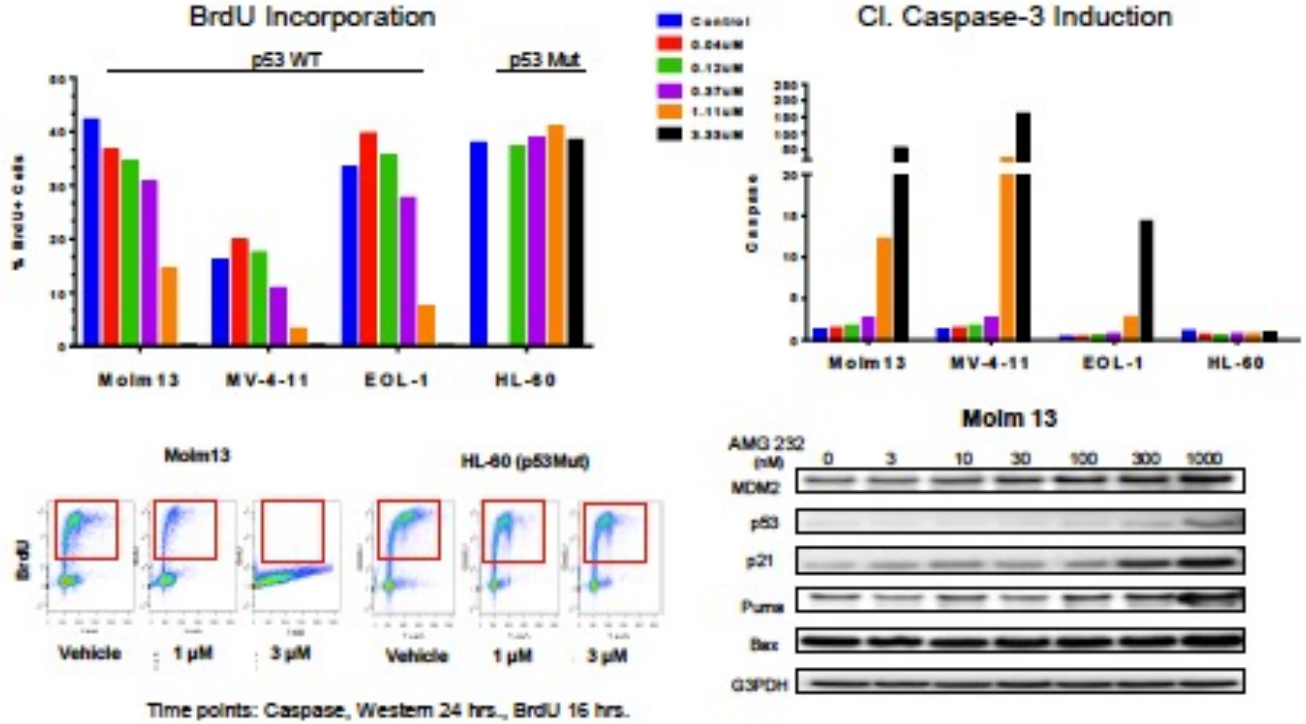
	Biacore K_D		Cell Proliferation (EdU)	
	IC_{50} (nM)	Potency Shift from AMG 232	IC_{50} (nM)	Potency Shift from AMG 232
NVP-CGM097	---	---	931	-102.3
RG7112	2.9	-64.4	590.2	-64.9
SAR299155	2.7	-60	214.2	-23.5
RG7388	0.15	-3.3	45.4	-5.0
KRT- 232	**0.045**	---	**9.1**	---
DS-3032b	----	---	103	-10.0
MK8242	0.08 (HDM2 p53) FRET IC50		73	-8.0

MDM2 Inhibition Induces Cytotoxicity to AML Cell Lines *in vitro*



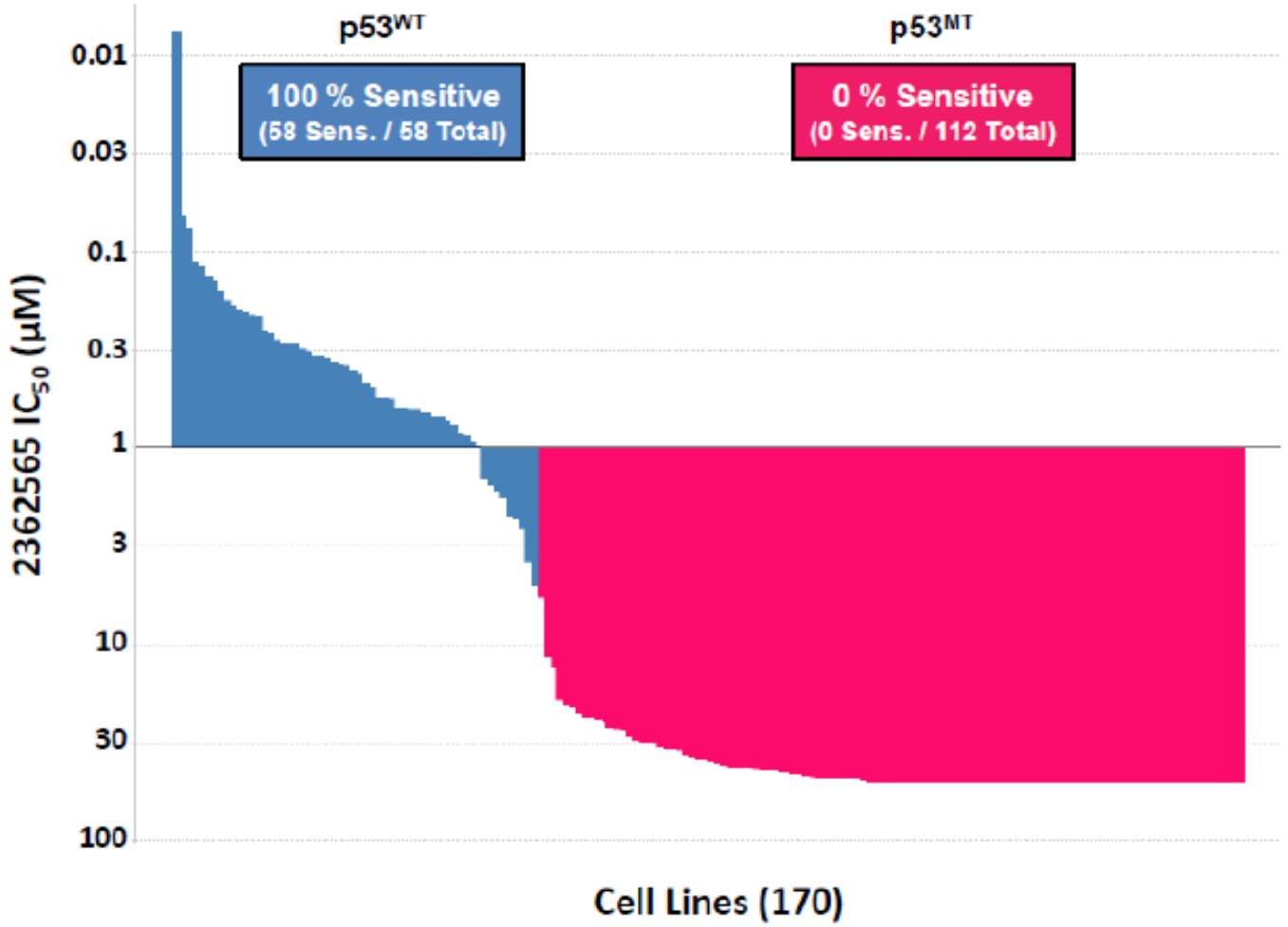
Tumor cells were treated with DMSO control or MDM2I for 72 hours. ATP quantification was used to determine cell viability at time zero and at 72 hours. Growth inhibition was measured on a 200-point scale where 0 = uninhibited cell growth, 100 = cell stasis, 200 = complete cell killing.

AMG 232 Inhibits the Cell Cycle and Induces Apoptosis in p53 WT AML Cell Lines



Time points: Caspase, Western 24 hrs., BrdU 16 hrs.

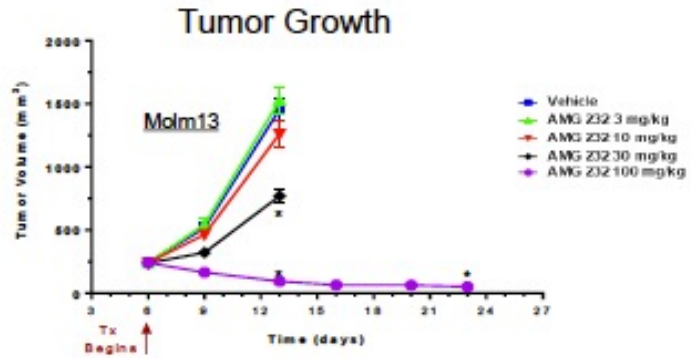
Figure 5-1. The Primary Determinant of MDM2 Inhibitor Sensitivity – p53 Mutational Status

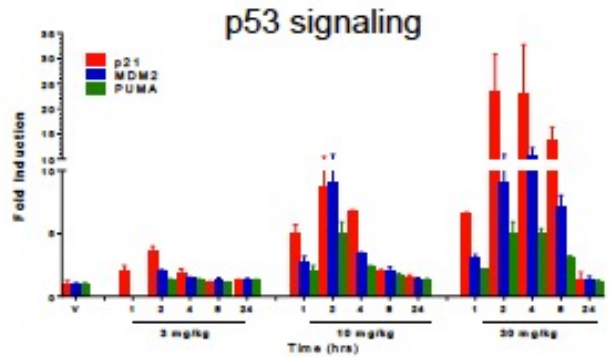


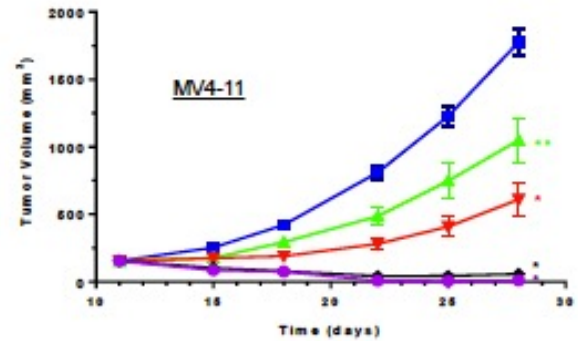
MDM2 inhibitor 2362565 IC_{50} values in a curated set of 170 Ricerca Oncopanel cell lines, colored by p53 status (WT = blue, MT = red). Mutation data from Amgen and Cosmic databases; redundant, misannotated, virally inactivated, and mutationally heterozygous cell lines are excluded; cell lines with low proliferation, high CV, and poor IC_{50} curve-fitting are excluded; sensitivity cutoff of 5.5 µM IC_{50} transit

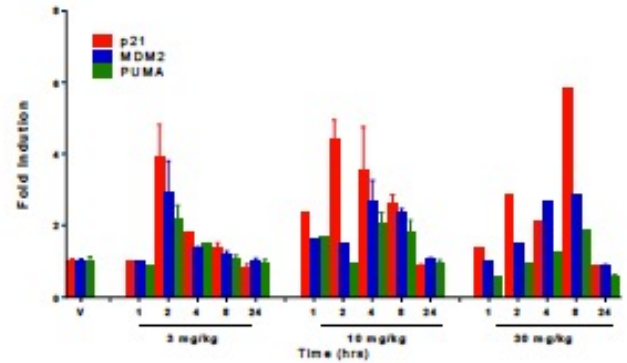
Source: Study R20120148

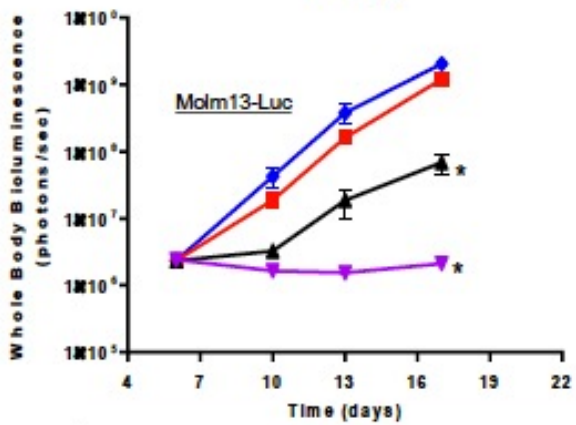
AMG 232 Regresses AML Tumors and Induces p21, MDM2 and PUMA Expression *in vivo*

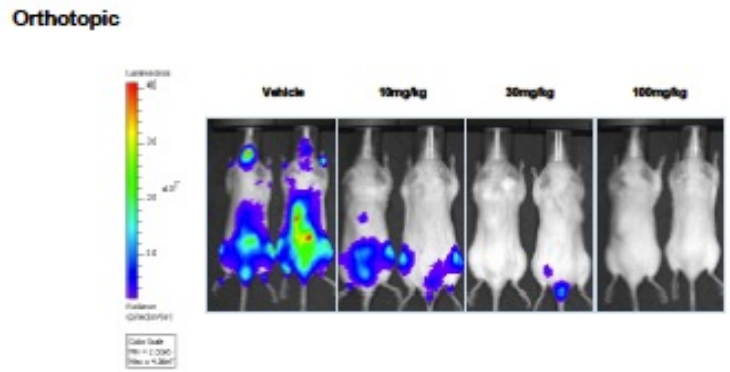










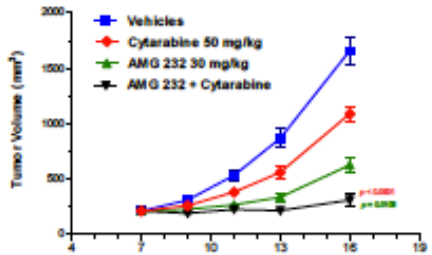


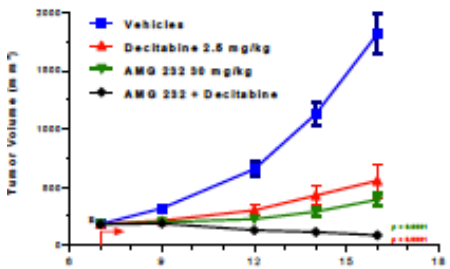
Each xenograft study shows the effect of AMG 232 treatment on tumor growth over time (N=10/group). Daily oral treatment of AMG 232 or vehicle began when tumors reached approximately 200mm^3 (subcutaneous) or 2.5x10^6 photons/sec luminescence signal (orthotopic). Data represent mean ± SEM. *p<0.0001 , **p<0.001 for tumor growth inhibition, by RMANOVA followed by Dunnett's post-hoc test.

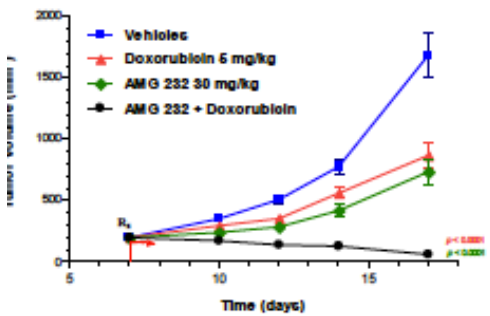
AMG 232 Combined with Cytotoxic Agents Enhances Suppression of AML Tumor Growth

	MCF7 (Breast)	RKO (Colon)	KS-1 (GBM)	A427 (NSCLC)	SJSA-1 (Sarcoma)	SW982 (Sarcoma)	MKN45 (Stomach)	NCI-SNU-1 (Stomach)	EOL-1 (AML)	MOLM-13 (AML)	HT-29 (Colon)	PC-3 (Prostate)
AMG 232 x Cisplatin				0.70							0.19	0.48
AMG 232 x Oxaliplatin		0.85									0.08	0.18
AMG 232 x Doxorubicin	4.63								4.37	8.63	1.14	2.14
AMG 232 x Etoposide					2.16	9.05	2.86	1.88			1.92	0.52
AMG 232 x Irinotecan		1.69									0.95	0.67
AMG 232 x Temozolomide			2.61								0.04	0.18
AMG 232 x Cytarabine									6.90	8.70	0.56	1.33
AMG 232 x Decitabine									12.48	14.67	0.07	0.86

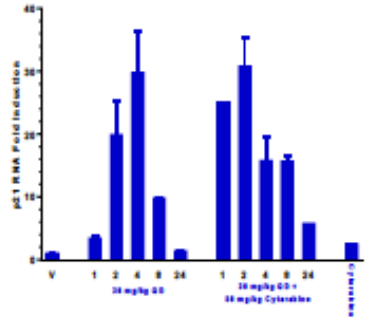
Molm13 Model

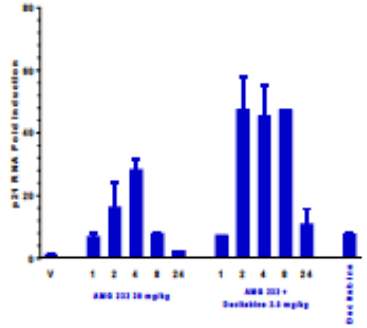


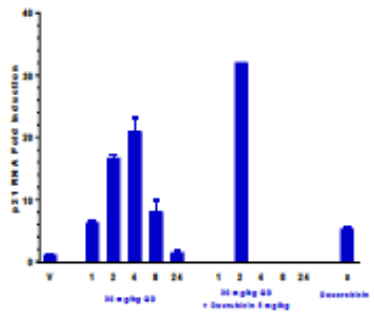




P21 induction

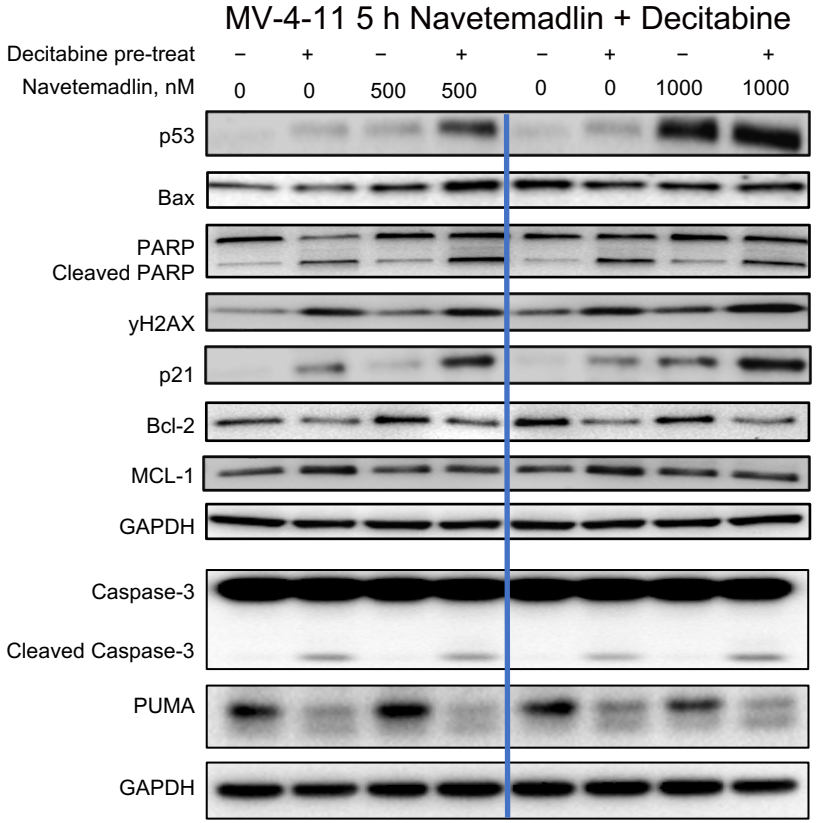


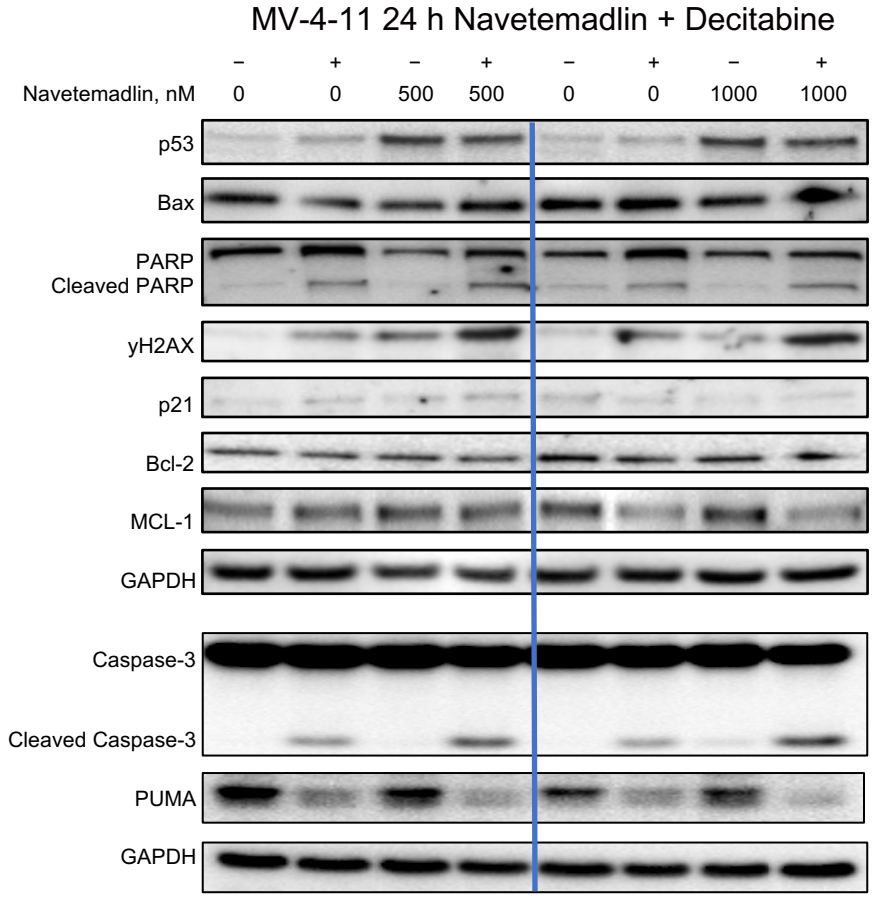




AMG 232 and the MEK inhibitor (PD-0325901) were dosed orally once per day. Cytarabine was dosed 5 times/week, decitabine 3 times/week, and doxorubicin once/week. Data represent mean tumor volume ± SEM (n=10/group). *p<0.0001 for comparison of combination treatments to the single agents . Right panels for chemo studies show the effect of treatment on p21 mRNA induction in tumors taken at the end of the study (at 1, 2, 4, 8, and 24 hrs. post AMG 232 treatment. N=2/time point).

DAC and KRT- 232 Combine to induce DNA Damage and Apoptosis



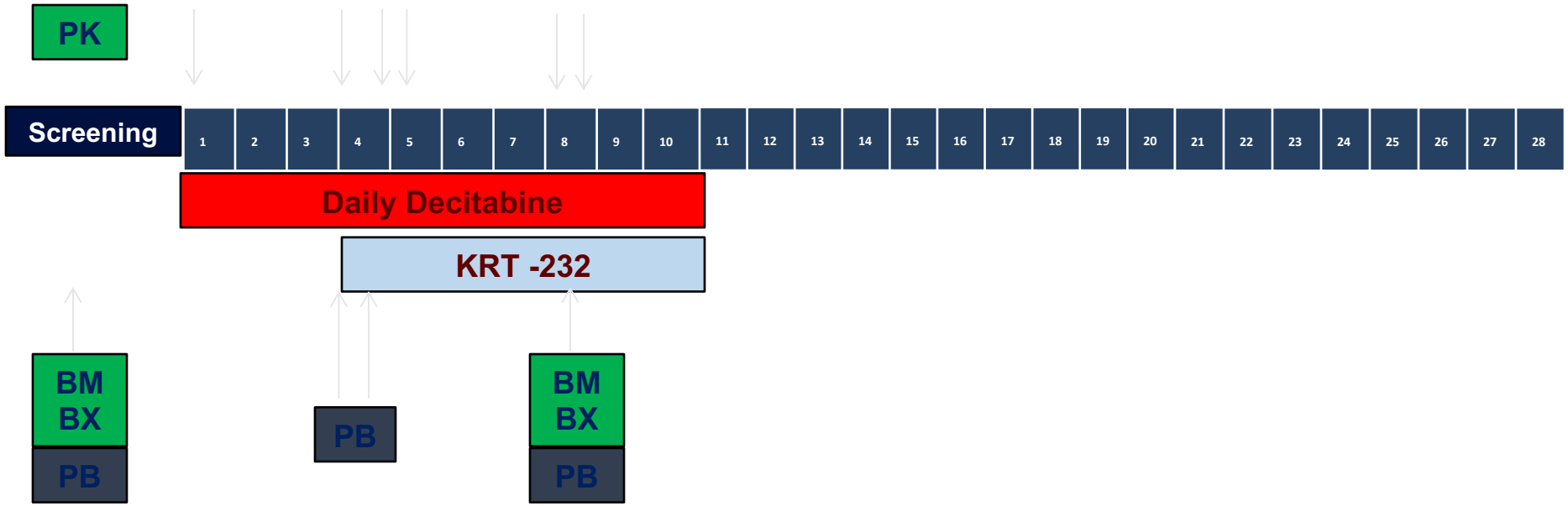


Phase 1B Study of KRT-232 in Combination with Decitabine in Acute Myeloid Leukemia

Objectives: Current Version

- **Exploratory Objectives:**To evaluate the Response Rate (RR) and Progression Free Survival (PFS) of KRT-232 and decitabine in AML.
- (b) To evaluate potential predictive biomarkers of response to KRT-232 and decitabine in AML.
- (c) To evaluate the pharmacodynamic (PD) effects of KRT-232 and decitabine in AML Blasts.
- (d) To determine the variability of decitabine incorporation into genomic DNA and correlate with systemic pharmacokinetics and exposure-response relationships.

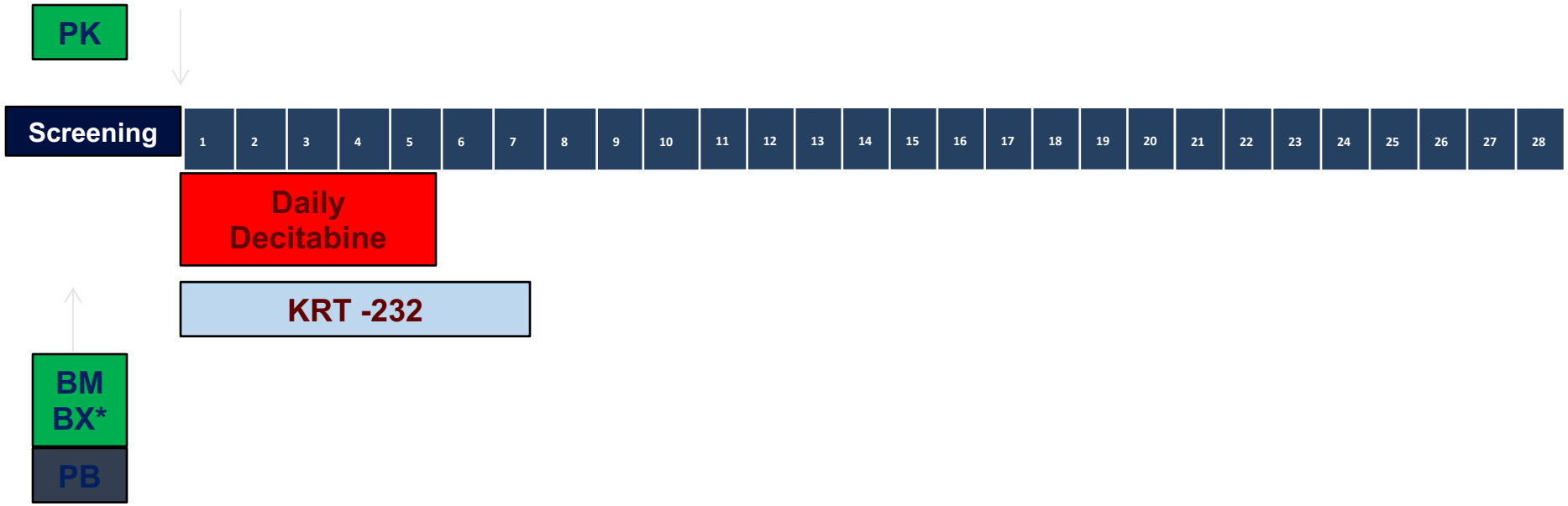
Current Protocol: Induction



Decitabine: 20 mg/m^2/day IV for 10 days per cycle on Days 1 to 10
KRT-232: **PO** Days 4 to 10

BM Bx = Bone Marrow Biopsy; PB = Peripheral Blood AML Blasts;
PK= Pharmacokinetic Sample

Current Protocol: Maintenance



Decitabine: 20 mg/m^2/day IV for 5 days per cycle on Days 1 to 5
KRT-232: **PO** Days 1 to 7

BM Bx = Bone Marrow Biopsy; PB = Peripheral Blood AML Blasts;
PK= Pharmacokinetic Sample; * Until CR

Current Protocol- Phase 1b Dose Escalation

Dose Level	Dose*	
	AMG-232 (mg/day)	Decitabine (mg/m^2/day)
Level -1	60 Day 4 to 10	20 Day 1 to 10
Level 1	60 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 2	90 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 3	120 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 4	180 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 5	240 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 6	300 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 7 (OLD- Not used)	360 Day 4 to 10 and 18 to 24	20 Day 1 to 10
Level 7 (New)	360 Day 4-10 only	20 Day 1 to 10

Phase 1B Study of KRT-232 /Decitabine in AML, #10075. Results

- 30 patients enrolled
- 6 Patients replaced for DLT evaluation
- All patients have relapse refractory AML
- 27 patients with prior HMA treatment
- No DLTs
- 6 Patients have been enrolled at new dose level 7 (highest dose escalation).

Phase 1B Study of KRT-232 /Decitabine in AML, #10075. Patient Characteristics

Characteristic	All (N=30)
Age, median (range) years	64 (23-82)
Sex	
Male	15 (31%)
Female	15 (69%)
Race/ethnicity, N (%)	
Non-Hispanic White	14 (47%)
Hispanic	9 (30%)
Asian	3 (10%)
African American	2 (7%)
Unknown	2 (7%)
ECOG performance status, N (%)	
0	12 (40%)
1	12 (40%)
2	6 (20%)
Lines of Prior Chemo	
Median (range)	4 (0 - 12)
Pts w/ Prior Decitabine	11 (37%)
Pts w/ Prior Azacitidine	12 (40%)
Pts w/ Prior Decitabine and Azacitidine	4 (13%)
Prior Therapy, N (%)	
No	1 (3%)
Yes	29 (97%)
BMT	3 (10%)
Chemo, Multi Agents Systemic	28 (93%)
Chemo, NOS	4 (13%)
Chemo, Single Agent Systemic	23 (77%)
Immunotherapy	2 (7%)
Prior Therapy (NOS)	4 (13%)

Phase 1B Study of KRT-232 /Decitabine in AML, #10075 Clinical Responses

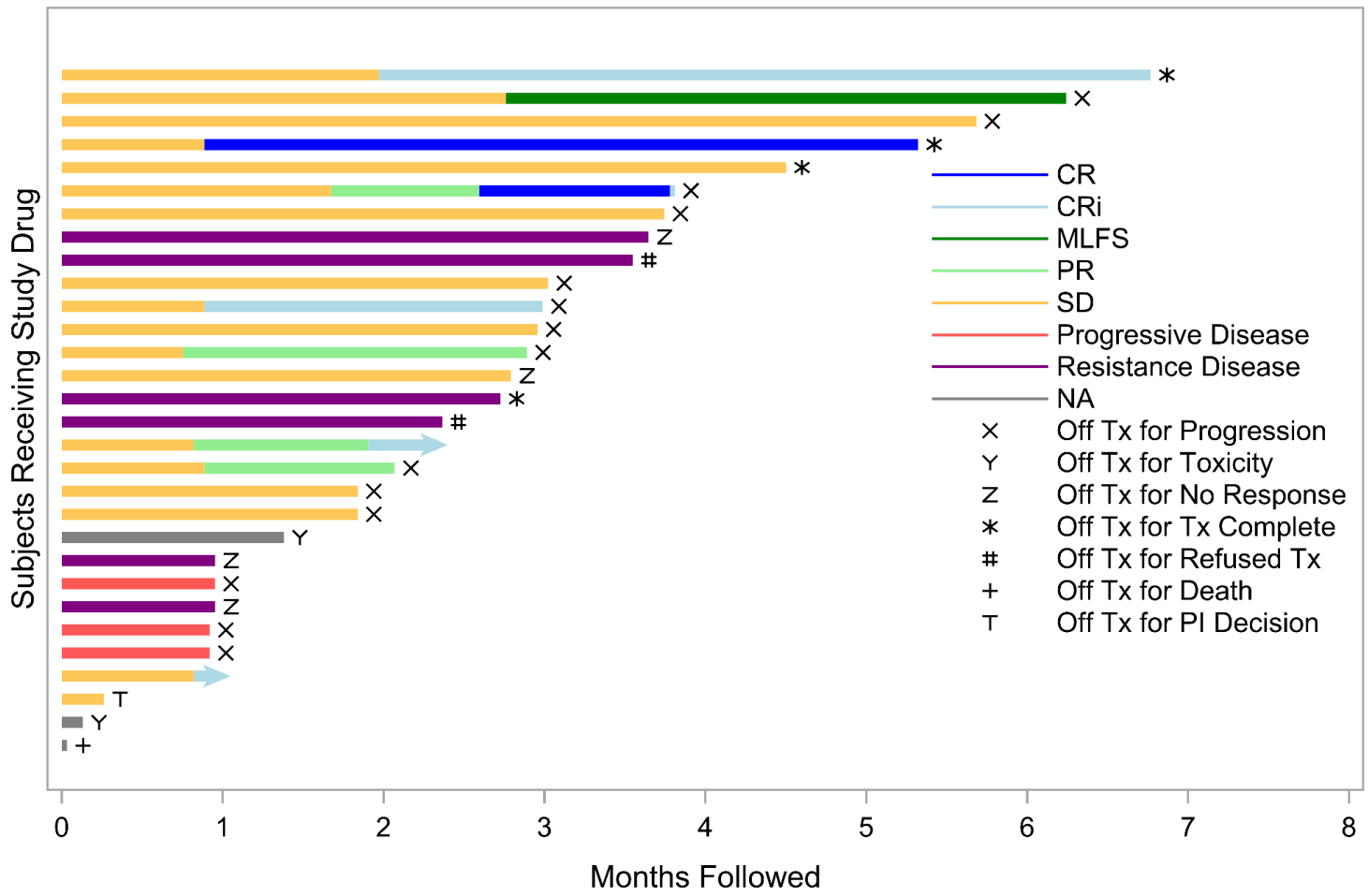
Best Overall Response*, n (%)	RR AML (n = 30)
ORR	7 (23)
CR	2 (7)
CRi	4 (13)
MLFS	1(3)

4 of 12 patients with prior Azacitidine responded (CRi-3, MLFS-1).

Responding Patient Characteristics

Patient	*Cohort*	*Gender*	*ECOG*	*Age @ Enrollment*	*Race*	*Response*	*Prior Decitabine*	*Prior Azacitidine*	*Prior Therapies*	*# Prior Therapies*
CA189-0001	TAC1	Male	0	80	Not Reported	CRi	No	Yes	Azacitidine	1
CA011-0004	TAC1	Male	0	66	Hispanic	CRi	No	No	Induction 7+3; Intrathecal chemotherapy; Midostaurin	3
MD017-0006	TAC2	Male	0	44	Hispanic	CR	No	No	Dauno-AraC-Cladrabine; BMT; 6MP; AraC zoloft; HIDAC consolidation; Dauno-AraC-Cladrabine	6
MD017-0014	TAC4	Female	1	71	AA	MLFS	No	Yes	CD33 BITE (AMV 564); azacitidine, venetoclax; cytarabine, anthracyclin	3
CA011-0029	TAC6	Male	1	57	Hispanic	CR	No	No	Bone Marrow transplant; HiDAC; 5+2; 7+3(cytarabine/idarub)	4
MD017-0034	TAC7	Male	2	80	White	CRi	No	Yes	Cytarabine; Azacitidine & Venetoclax	2
MD017-0035	TAC7	Female	1	26	White	CRi	No	Yes	Azacitidine; induction with 7+3; consolidative HiDAc; BMT; CLAG-M; Aza/ Ventoclax	6

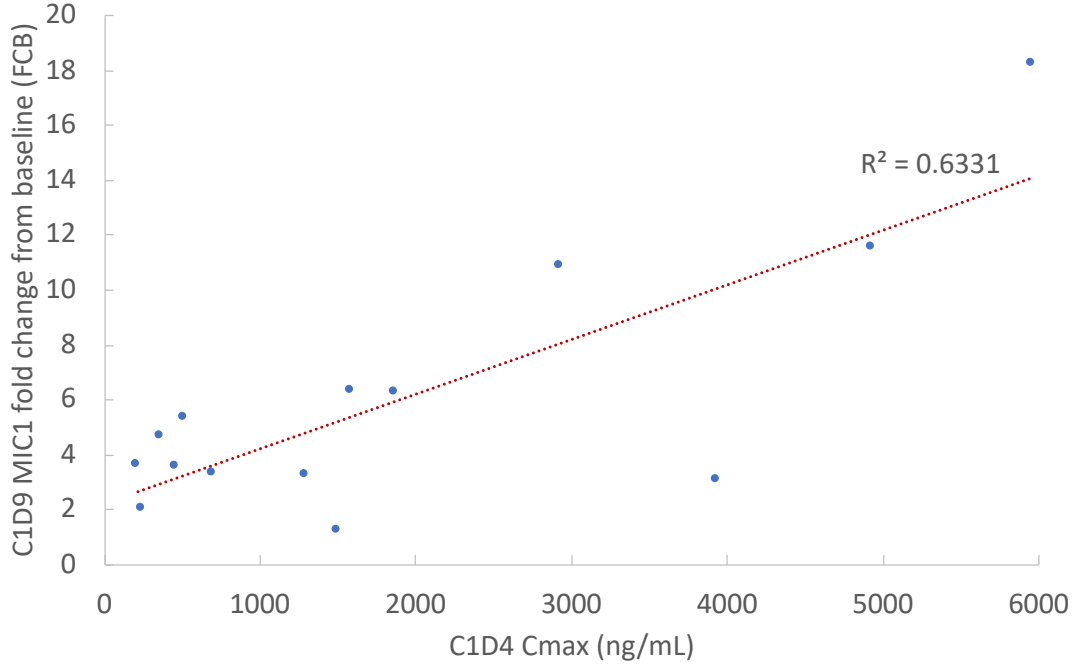
Best response and time to progression for individual patients



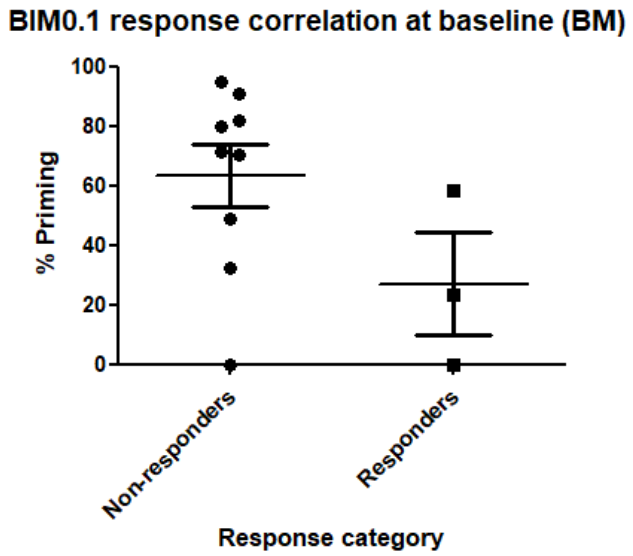
Each bar represents one subject in the study.

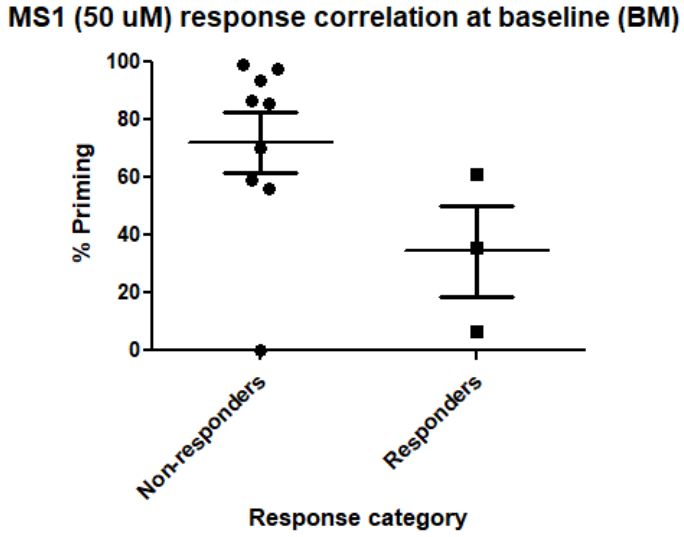
MIC1 (Marker of P53 signaling) increased with KRT-232 exposure

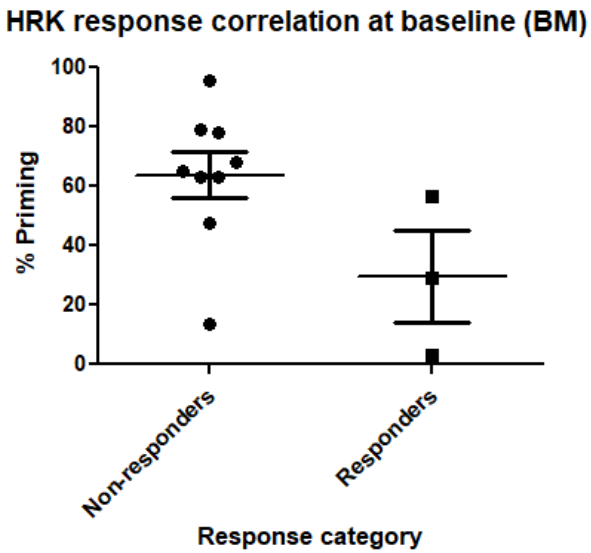
Dose level	PK Period	T_{max} (h)	C_{max} (ng/mL)	AUC_{24h} (h*ng/mL)	Cl/F (L/h)	V_z/F (L)	$T_{1/2}$ (h)
60 mg	With Decitabine	1.0 (1.0-3.0, 5)	726 ± 457 (5)	5726 ± 4647 (4)	8.0 (1)	65.7 (1)	5.7, 20.3 (2)
60 mg	Alone	3.0 (3.0-8.0, 4)	1176 ± 1064 (4)	8675 ± 4695 (3)	7.4 (1)	98.4 (1)	9.9 (1)
90 mg	With Decitabine	3.0 (1.0-5.1, 3)	459 ± 260 (3)	1525 (1)	NR	NR	NR
90 mg	Alone	1.0 (1.0-1.1, 3)	773 ± 503 (3)	2683, 4166 (2)	21.4 (1)	170 (1)	5.5 (1)
120 mg	With Decitabine	1.1 (1.1-4.9, 3)	536 ± 91 (3)	5037 ± 1681 (3)	NR	NR	16.6 (1)
120 mg	Alone	1.0 (0.9-3.0, 3)	655 ± 196 (3)	2763, 5208 (2)	NR	NR	NR
180 mg	With Decitabine	3.0 (2.0-3.1, 3)	2757 ± 1175 (3)	12893 ± 7041 (3)	8.9 (1)	65.7 (1)	5.1 (1)
180 mg	Alone	4.4 (3.0-5.3, 3)	1831 ± 1771 (3)	19493 ± 19133 (3)	4.3 (1)	36.8 (1)	5.9 (1)
240 mg	With Decitabine	1.0, 3.0 (2)	1870, 2930 (2)	NR	NR	NR	NR
240 mg	Alone	3.0, 3.0 (2)	1270, 1810 (2)	3922, 8445 (2)	28.1 (1)	456 (1)	11.3 (1)
300 mg	With Decitabine	3.0 (3.0-3.3, 3)	2897 ± 1801 (3)	15110 ± 7062 (3)	NR	NR	NR
300 mg	Alone	1.0, 3.0 (2)	1270, 1990 (2)	10942, 18095 (2)	27.4 (1)	500 (1)	12.7 (1)
360 mg	With Decitabine	3.0 (0.7-3.0, 3)	7887 ± 5316 (3)	36257 (1)	9.9 (1)	44.7 (1)	3.1 (1)



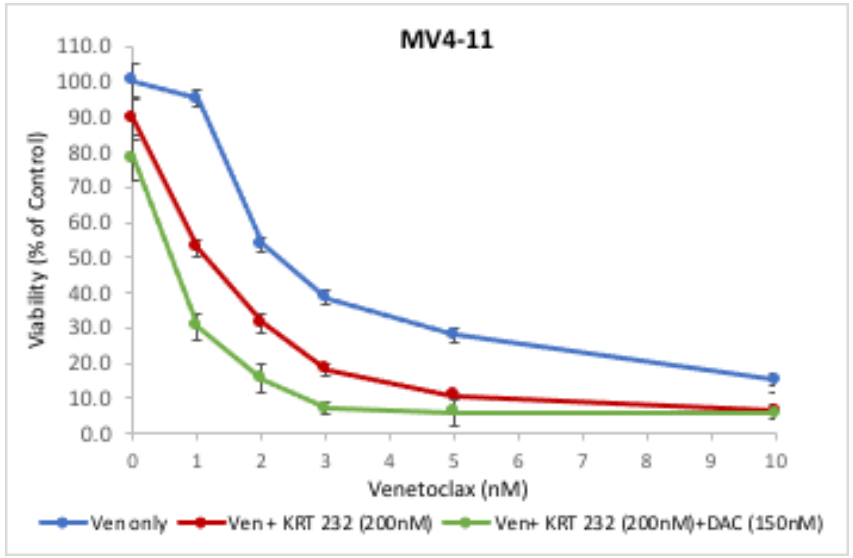
BH3 Primed AML Cells Predicts Resistance to HMA/KRT-232

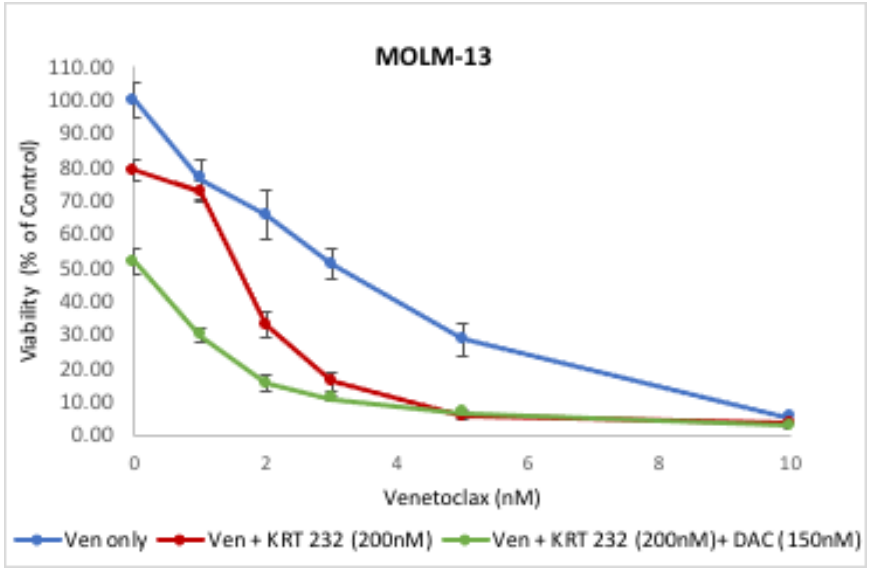






The combination of KRT-232, Venetoclax, and Decitabine induces significantly greater Growth inhibition than either agent alone.

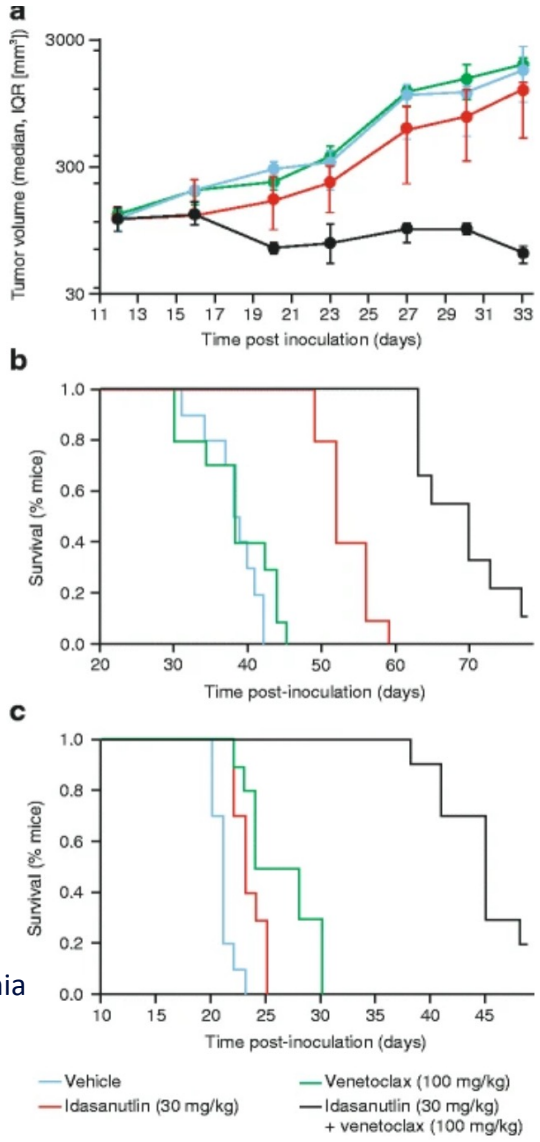




MTT assay (72h) of MOLM-13 and MV4-11 AML cell lines treated with indicated doses of Venetoclax, DAC and KRT-232.

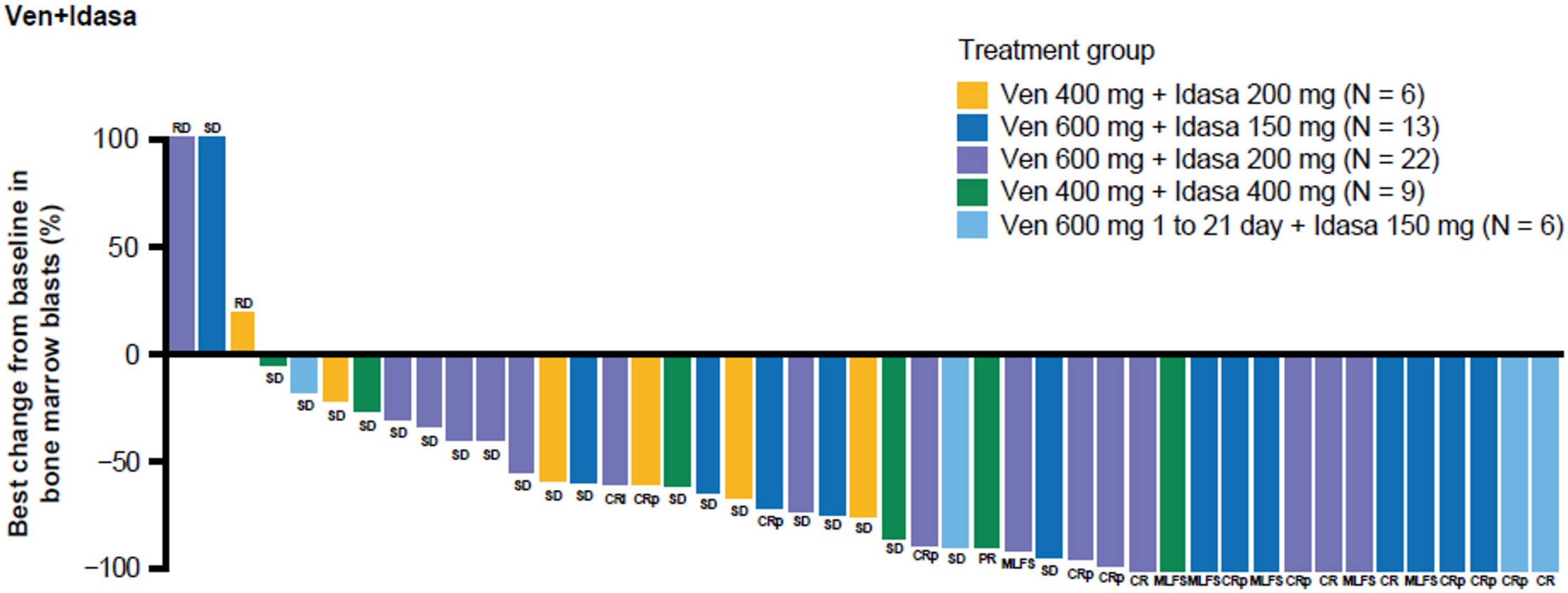
MDM2i + Ven is synergistic in *In Vivo* models of AML

- Superior anti-tumor activity of the MDM2 antagonist idasanutlin and the Bcl-2 inhibitor venetoclax in p53 wild-type acute myeloid leukemia models Tumor growth inhibition. in **a** MV4-11 subcutaneous model and TTE analysis of survival in **b** MV4-11 and **c** MOLM-13 orthotopic models (*n* = 10 mice per group). Combination treatment resulted in superior anti-tumor activity and enhanced survival.



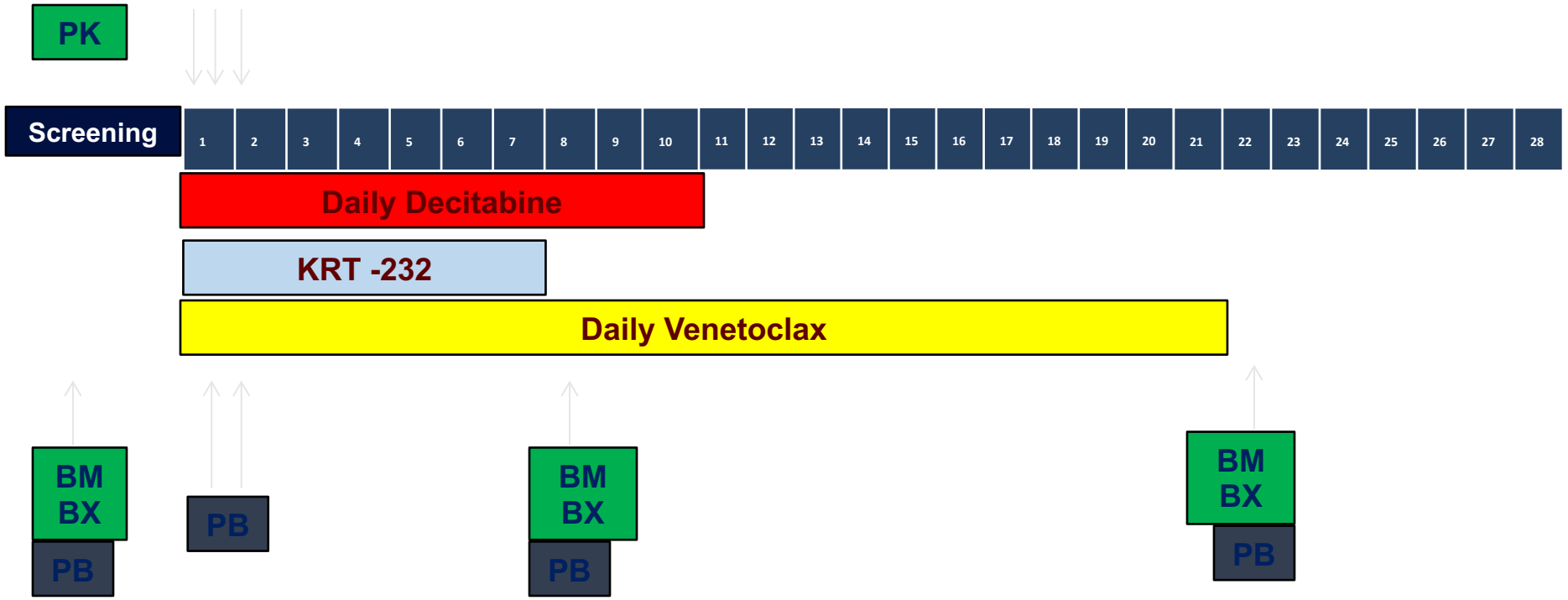
Christian Lehmann et al. Superior anti-tumor activity of the MDM2 antagonist idasanutlin and the Bcl-2 inhibitor venetoclax in p53 wild-type acute myeloid leukemia models. Journal of Hematology & Oncology

Venetoclax and MDM2i (Idasanutlin) is active in RR AMI



Daver et al. Venetoclax and idasanutlin or cobimetinib in relapsed/refractory AML patients ineligible for cytotoxic chemotherapy. Under Review

Amendment: Addition of Venetoclax



Decitabine: 20 mg/m^2/day IV for 10 days per cycle on Days 1 to 10
KRT-232: **PO** Days 1 to7
Venetoclax: 100 mg Day 1, 200 mg Day 2, 400 mg Day 3 to 21 or until BM Blast <5% if not in at least MLFS on Day 21.

BM Bx = Bone Marrow Biopsy; PB = Peripheral Blood AML Blasts;
PK= Pharmacokinetic Sample

A phase 1b study with expansion cohort of escalating doses of KRT-232 (AMG 232) administered in combination with standard induction chemotherapy (cytarabine and idarubicin) in newly diagnosed acute myelogenous leukemia (AML)

Study Objectives

- **Primary Objectives**

- To evaluate the toxicities of KRT-232 (AMG 232), cytarabine and idarubicin, and to determine the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of KRT-232 (AMG 232), cytarabine and idarubicin.

- **Secondary Objectives**

- To observe and record anti-tumor activity. Although the clinical benefit of these drugs has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.
- To evaluate the pharmacokinetic (PK) profiles of KRT-232 (AMG 232), cytarabine and idarubicin when used in combination.
- To evaluate p53 signaling induced by KRT-232 (AMG 232), cytarabine and idarubicin.
- To correlate KRT-232 (AMG 232), cytarabine and idarubicin exposure with pharmacodynamics endpoints (efficacy, toxicity, changes in p53 signaling).

- **Exploratory Objectives**

- To evaluate the Response Rate (RR) and Progression Free Survival (PFS) of KRT-232 (AMG 232), cytarabine and idarubicin in acute myeloid leukemia (AML).
- To evaluate potential predictive biomarkers, including MTF2 and H3K27me3, of response to KRT-232 (AMG 232), cytarabine and idarubicin in AML.
- To evaluate the pharmacodynamic (PD) effects of KRT -232 and induction chemotherapy in AML Blasts.

Study Design

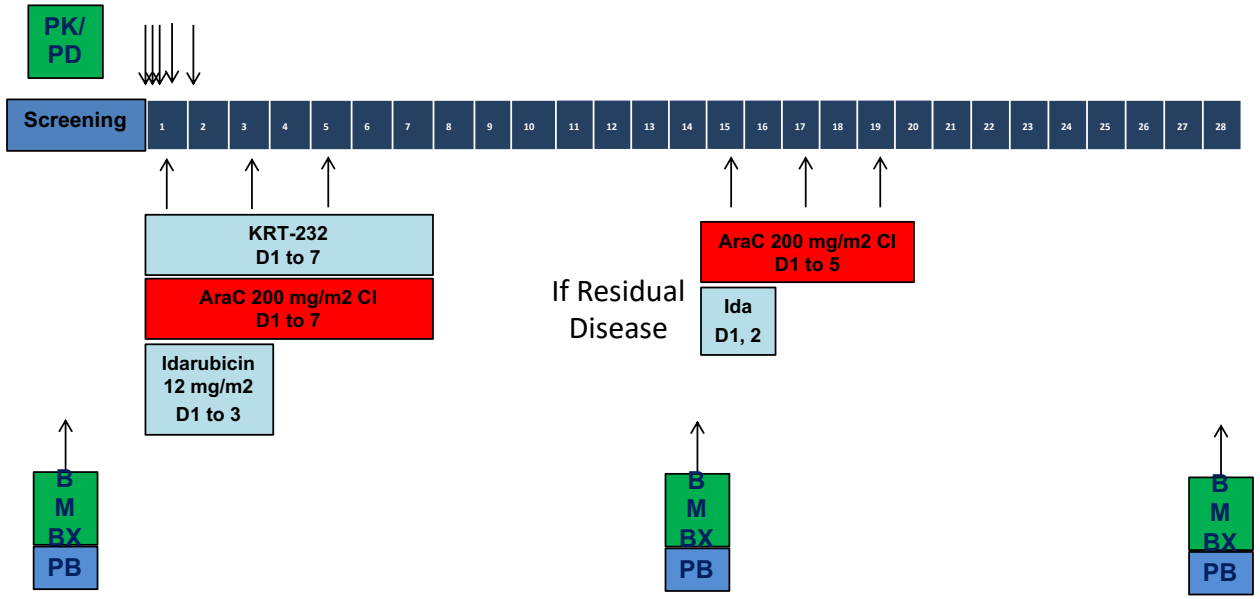
Dose Escalation Schedule			
Dose Level	Dose		
	KRT-232 (AMG 232) (mg; PO)	Cytarabine* (mg/m^2; IV)	Idarubicin* (mg/m^2; IV)
Level -2	60	200	12
Level -1	90	200	12
Level 1**	120	200	12
Level 2	180	200	12
Level 3	240	200	12
Level 4	300	200	12
Level 5	360	200	12
* Dose rounding per institutional guidelines is allowed. **Starting Dose Level			

Dose Expansion Cohort: Once the RP2D is reached, up to an additional 12 will be treated at this dose (for 18 total patients at this dose).

Study Eligibility

- Newly diagnosed and previously untreated AML (except APL) (≥20% blasts in bone marrow or extramedullary leukemia) according to the World Health Organization (WHO), 2016 criteria. Note that patients who have received treatment with hypomethylating agents alone or in combination with venetoclax, ivosidenib or enasidenib for myelodysplastic syndrome (MDS) and have now transformed to AML are eligible.
- Eligible patients must show evidence of wild-type (WT) p53 as assessed by DNA sequencing before initiation of KRT-232 (AMG 232).
- Patients must be considered candidates for intensive chemotherapy treatment with standard doses of cytarabine and idarubicin (“7+3 regimen”).
- Left ventricular ejection fraction (LVEF) ≥50% as assessed by echocardiogram or radionuclide angiography.

Treatment Plan/Schema



Results

CCCP ID	Date of Initial Tx	Dose Level	Age at Enroll	#Cyc	Off Tx Reason	End of Last Course/Eval	Best Response	PD	Any DLT's	Highest Grade Tox. (Attr. | Any)
MD015-0003	2/19/2021	1	41.3	5	COMPLETED	9/20/2022	CR	No	No	4 | 4
MD015-0005	3/25/2021	1	38.4	4	COMPLETED	1/9/2023	CR	No	No	3 | 3
MD015-0007	4/27/2021	1	52.1	4	COMPLETED	9/12/2022	CR	No	Yes	3 | 3
MD015-0008	4/29/2022	1	66.6	3	COMPLETED	11/23/2022	CR	11/17/2022	No	3 | 4
MD015-0009	5/20/2022	1	49.9	4	Still On	12/29/2022	CR	N	No	3 | 4
MD015-0011	7/27/2022	1	67.7	3	Still On	10/27/2022	CR	N	No	2 | 3
MD015-0013	10/25/2022	2	46.0	1	Still On	11/21/2022	CR	N	No	3 | 4

MD015-0001, CA011-0002, OK003-0004, CA011-0006 and MD015-0010 were screen fails. Patient 7 DLT was prior to risk mitigation amendment.

Arm Descriptions:

DL 1: KRT-232 120mg (D1-7) + 200mg/m2 Cytarabine (D1-7) + 12mg/m2 Idarubicin (D1-3)

DL 2: KRT-232 180mg (D1-7) + 200mg/m2 Cytarabine (D1-7) + 12mg/m2 Idarubicin (D1-3)

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