SUPPLEMENTARY APPENDIX

MDM2- and FLT3-inhibitors in the treatment of *FLT3*-ITD acute myeloid leukemia, specificity and efficacy of NVP-HDM201 and midostaurin

Katja Seipel,[1,2] Miguel A.T. Marques,[1] Corinne Sidler,[1] Beatrice U. Mueller[2] and Thomas Pabst[2]

[1]Department for Biomedical Research, University of Bern and [2]Department of Medical Oncology, Inselspital, Bern University Hospital, Switzerland

 doi:10.3324/haematol.2018.191650

Received: February 20, 2018.

Accepted: June 29, 2018.

Pre-published: July 5, 2018.

Correspondence: thomas.pabst@insel.ch

MDM2- and FLT3-inhibitors in the treatment of FLT3-ITD acute myeloid leukemia, specificity and efficacy of NVP-HDM201 and midostaurin

Research Article

Authors: [1,2]Katja Seipel, [1]Miguel A.T. Marques, [1]Corinne Sidler, [1]Beatrice U. Mueller, [2]Thomas Pabst.

Affiliations: [1] Department of Biomedical Research University of Bern, 3008 Bern, Switzerland; and [2] Department of Medical Oncology; University Hospital Bern; 3010 Bern; Switzerland.

Running title: Targeting FLT3-ITD in AML

Key words: acute myeloid leukemia (AML); FMS like tyrosine kinase 3 (FLT3); internal tandem duplication (ITD); tumor suppressor p53 (TP53); mouse double minute 2 homolog (MDM2); midostaurin (PKC412); HDM201.

Funding: This work was supported by a grant from the Swiss National Science Foundation (SNF) #310030_127509 to TP.

Correspondence: Thomas Pabst, Department of Medical Oncology, University Hospital, 3010 Bern, Switzerland. Phone +41 31 632 8430; Fax +41 31 632 3410; thomas.pabst@insel.ch.

Supplemental Figure S1

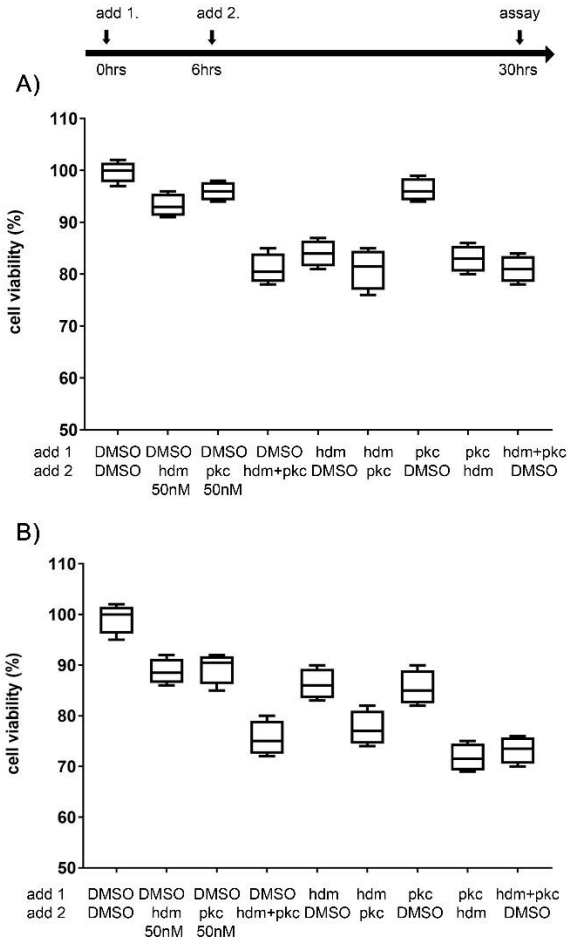

Fig. S1 Synergistic effect on cell viability in AML cells treated with midsotaurin and NVP-HDM201 independent of sequence of drug application.

Cell viability measurements in AML cells pretretead for 6 hours and treated for 24 hrs with midostaurin (PKC412) and NVP-HDM201 in all combinations. NVP-HDM201 pretreatment followed by midostaurin treatment had the same effect on cell viability as midostaurin pretreatment followed by NVP-HDM201 treatment. Moreover both sequential treatments had nearly the same effect on cell viability as 30hrs of direct combination treatment in OCI-AML2 cells (A) and MOLM-13 cells (B).

Supplemental Figure S2

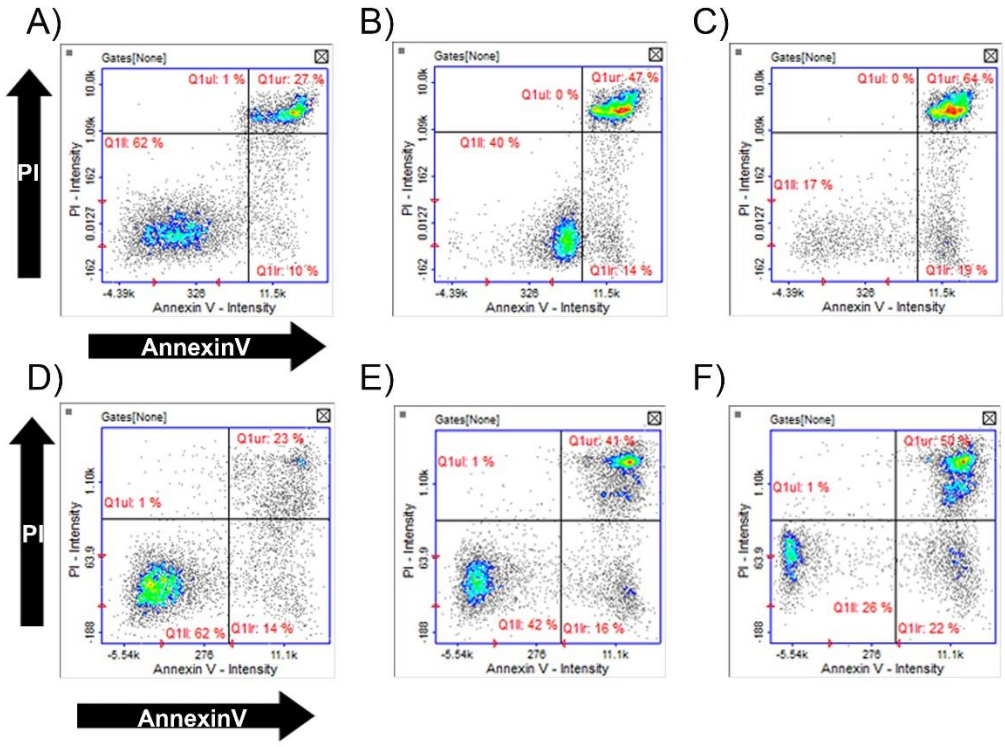

Fig. S2 Dose-dependent induction of apoptosis in FLT3-ITD AML cells.

Cytometric assays were performed using single compounds and combination treatments with midsoaturin and NVP-HDM201 in *FLT3*-ITD AML cells to measure induction of apoptosis using AnnexinV/PI staining. MV4-11 cells were treated for 24hrs with DMSO (A), 50nM NVP-HDM201 and 20nM midostaurin (B), 200nM NVP-HDM201 and 100nM midostaurin (C). MOLM-13 cells were treated for 48hrs with DMSO (D), 50nM NVP-HDM201 and 20nM midostaurin (E), 100nM NVP-HDM201 and 50nM midostaurin (F). Samples were analyzed on the NC-3000 imager.

Supplemental Table I: AML cell lines and patient samples characteristics.								
subgroup	ID	status	blast (%)	cyto[1] genetic	molecular genetics [2]			
					FLT3[3]	TP53	NPM1	mutation
FLT3wt TP53wt NPM1wt	OCI-AML2	AML cell line	100		0	wt	wt	DNMT3A
	1	primary AML PB[4]	85	normal	0	wt	wt	
	2	primary AML BM[4]	80	normal	0	wt	wt	
	3	primary AML BM	90	inv(Y)	0	wt	wt	
	4	primary AML BM	70	normal	0	wt	wt	
	5	primary AML BM	80	normal	0	wt	wt	
	6	primary AML PB	85	del(7)	0	wt	wt	
	7	primary AML BM	**30**	t(6;9)	<0.05	wt	wt	KRAS, NRAS
FLT3wt TP53wt NPM1mut	OCI-AML3	AML cell line	100		0	wt	mut	DNMT3A, NRAS
	8	primary AML PB	85	normal	0	wt	mut	
	9	primary AML BM	90	inv(9)	0	wt	mut	
	10	primary AML PB	70	normal	0	wt	mut	
	11	primary AML BM	90	normal	0	wt	mut	
FLT3ITD TP53wt NPM1wt	MOLM-13	AML cell line	100	t(9;11)	1,77	wt	wt	mTOR
	MV4-11	AML cell line	100	t(4;11)	>99	wt	wt	
	12	primary AML PB	90	normal	0,78	wt	wt	
	13	primary AML PB	84	normal	2,12	wt	wt	
	14	relapsed AML PB	99	inv(4)	0,77	wt	wt	
	15	primary AML PB	80	normal	0,49	wt	wt	
	16	primary AML BM	85	normal	0,71	wt	wt	DNMT3A, WT1
FLT3ITD TP53wt NPM1mut	17	primary AML PB	74	normal	0,92	wt	mut	
	18	primary AML PB	80	normal	0,84	wt	mut	
	19	primary AML PB	**38**	normal	0,55	wt	mut	
	20	primary AML PB	91	del(18)	0,83	wt	mut	
	21	primary AML PB	95	normal	0,74	wt	mut	
	22	primary AML PB	90	normal	0,86	wt	mut	
TP53mut	MOLM-16	AML cell line	100		0	mut	wt	
	PL-21	AML cell line	100		0	wt/P36fs	wt	KRAS
	23	primary AML PB	95	normal	19,1	mut	wt	
	24	primary AML BM	**25**	normal	0,75	mut	wt	RUNX1
	25	primary AML PB	70	normal	0,64	mut	wt	
	26	primary AML PB	72	normal	0,44	mut	mut	
TP53del	HL-60	AML cell line	100		0	del	wt	NRAS

1) inversion, translocation and deletion are abbreviated inv, t and del

2) wild type, mutated and deleted are abbreviated wt, mut and del

3) FLT3 allelic ratio (ITD/wt)

4) peripheral blood (PB); bone marrow (BM)