Polo-like kinase-1 as a novel target in neoplastic mast cells: demonstration of growth-inhibitory effects of small interfering RNA and the Polo-like kinase-1 targeting drug BI 2536

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Online Supplemental Table S1. Patients' characteristics, expression of pPlk-1 in MC, and response to BI 2536.

N.	Male/female	Age (years)	Tryptase (ng/L)	Diagnosis	*KIT* D816V	pPlk-1 IHC	% clonal cells in sample*	BI 2536 IC_{50}
#01	f	59	49.6	ISM	-	+	<10%	ND
#02	f	60	188.0	ISM	+	+	50%	ND
#03	m	61	650.0	ISM	ND	+	50%	ND
#04	m	41	14.5	ISM	-	+	10%	ND
#05	m	44	69.4	ISM	+	+	10%	ND
#06	m	54	60.1	ISM	+	+	10%	ND
#07	m	40	15.8	ISM	+	+	10%	ND
#08	f	50	53.7	ISM	+	+	50%	ND
#09	m	22	72.0	ASM	-	+	>80%	ND
#09	m	26	1910.0	MCL	-	+	>90%	ND
#10	f	47	104.0	SSM	+	+	>90%	100-1000
#11	m	60	212.0	ASM	+	+	>80%	ND
#11	m	60	393.0	ASM→MCL	+	+	>100%	ND
#12	m	33	36.0	ISM	+	+	10%	ND
#13	m	42	100.0	ISM	+	+	10%	ND
#14	m	43	20.0	ISM	-	+	10%	ND
#15	f	46	790.0	ISM	+	+	50%	10-25
#16	m	42	630.0	SSM	+	+	50%	n.d.
#17	f	39	11.5	ISM	+	ND	10%	>1000
#18	f	46	14.4	ISM	+	ND	10%	100-1000
#19	f	44	14.0	ISM	+	ND	10%	10-50
#20	m	82	30.7	ISM	+	+	10%	5-10
#21	m	51	53.2	ISM	+	+	10%	1
#22	m	66	63.4	ISM	+	+	10%	100-1000
#23	f	69	16.8	ISM	+	+	10%	100-1000
#24	m	31	122.0	ISM	+	+	10%	100-1000
#25	m	52	290.0	ISM	+	+	10%	5-10
#26	m	74	17.6	ISM	-	+	10%	5-10
#27	m	74	1140.0	ASM	+	ND	>80%	10-50
#28	m	39	22.7	ISM	+	ND	10%	5-10
#29	f	47	64.7	ISM	+	+	10%	10-50
#30	f	53	14.1	ISM	+	+	10%	5-10
#31	f	36	489	MCL	-	ND	>80%	ND
#32	f	2	ND	ICM	ND	+	ND	ND

*f: female; m: male; ISM: indolent systemic mastocytosis; SSM, smoldering systemic mastocytosis; ASM: aggressive systemic mastocytosis; MCL: mast cell leukemia; ICM: isolated cutaneous mastocytoma; IHC: immunohistochemistry; ND: not determined; pPlk-1: phosphorylated Polo-like kinase 1. *The percentage of clonal cells was estimated from morphological examination, analysis of* KIT *D816V (detection limit: 10% clonal cells), and the diagnosis (variant of SM).*

Online Supplementary Table S2. Expression of Plk-1 and pPlk-1 in hematopoietic cells in SM and normal bone marrow.

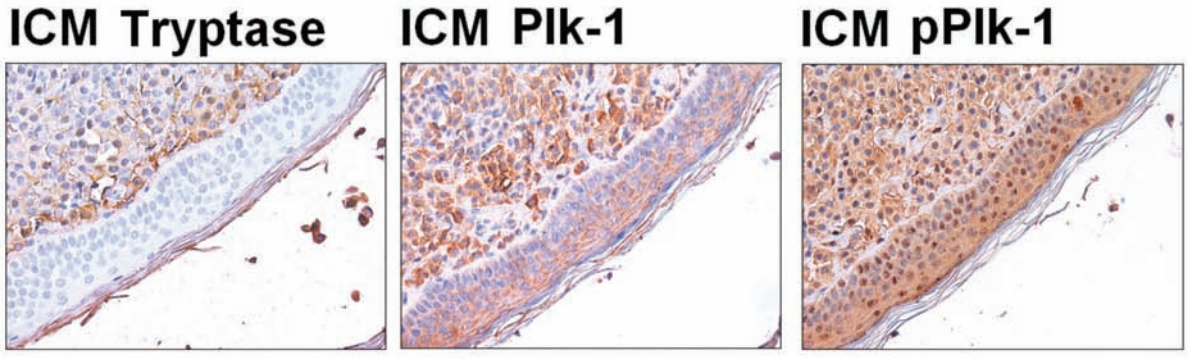
	Normal bone marrow		Systemic mastocytosis	
Cell type	Plk-1	pPlk-1	Plk-1	pPlk-1
Erythroid cells	+/	-	+/-	-
Myeloid progenitors	+	+/-	+	+
Neutrophils	+	+/-	+	+
Eosinophils	n.d.	n.d.	+	-
Megakaryocytes	+	+	+	+
Lymphocytes	n.d.	n.d.	n.d.	+/-
Mast cells	n.d.	n.d.	+	+
Endothelial cells	+	+	+	+
Endosteal cells	n.d.	+	n.d	+
Fat cells	+	+/-	+	+/-

Expression of Plk-1 and pPlk-1 in bone marrow cells was determined by indirect immunohistochemistry. Plk-1, Polo-like kinase-1; pPlk-1, phosphorylated Plk-1; n.d. not determined.

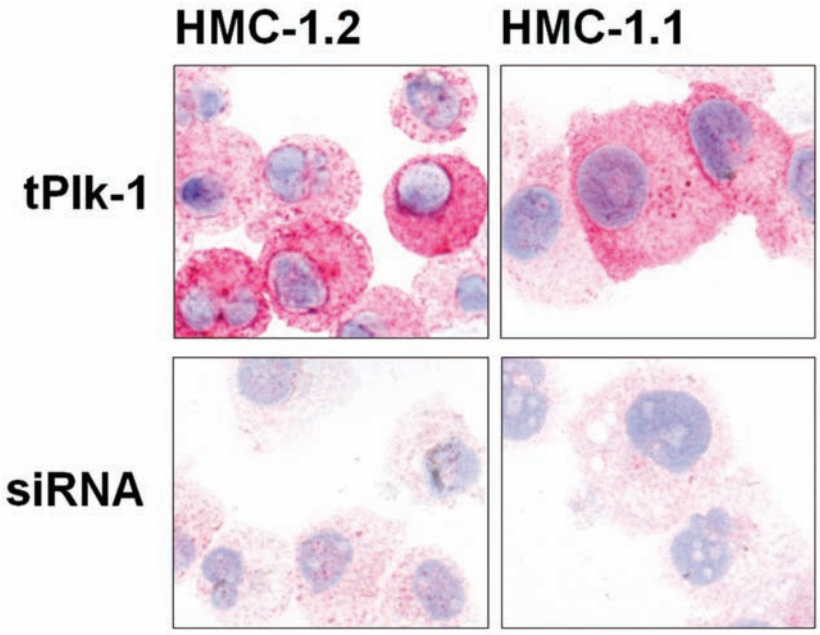
Online Supplementary Table S3. Effect of BI 2536 on various leukemic cell lines.

		Known key molecular response to BI 2536	
Cell line	Origin	Defect/mutation	IC_{50} values (nM)
MV4-11	AML	*FLT3* ITD	1-5
MOLM-13	AML	*FLT3* ITD	1-5
KG-1	AML	-	5-10
U937	AML	-	5-10
HL60	AML	-	5-10
K562	CML	*BCR/ABL*	5-15
KU812	CML	*BCR/ABL*	10-50
HMC-1.1	MCL	*KIT* V560G	5-10
HMC-1.2	MCL	*KIT* V560G & *KIT* D816V	5-10

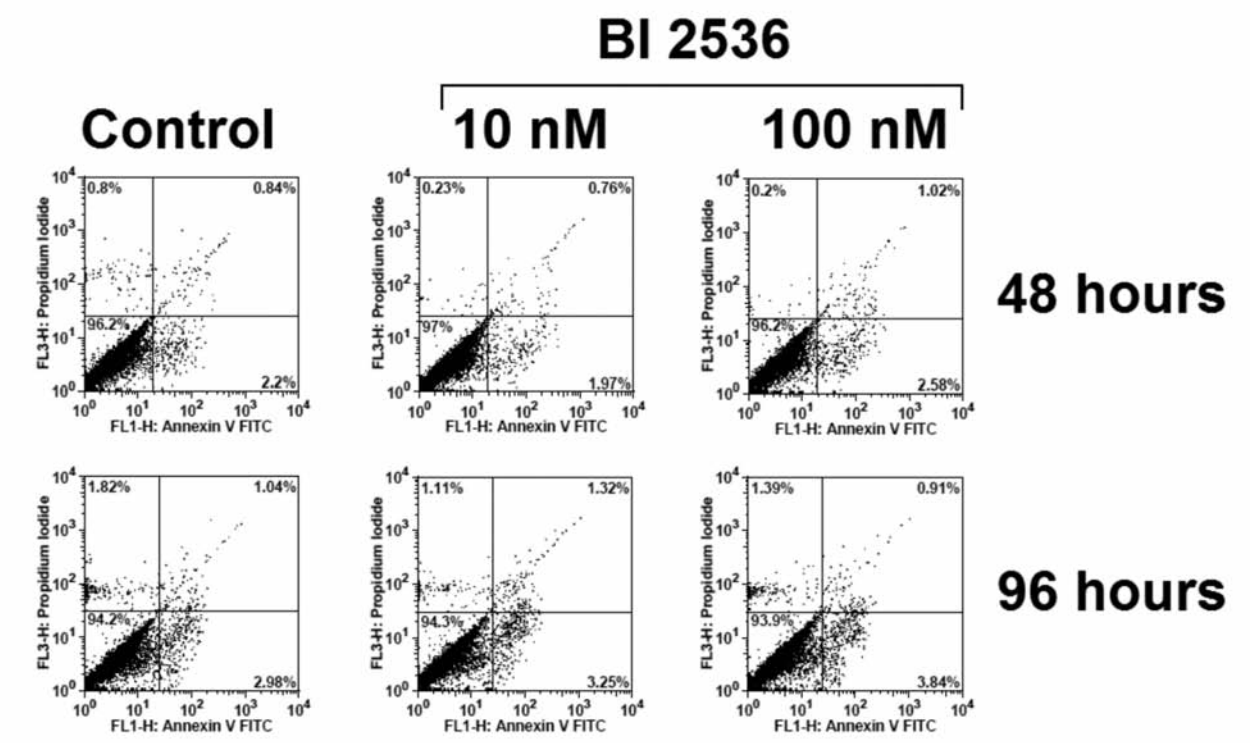
AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MCL: mast cell leukemia.



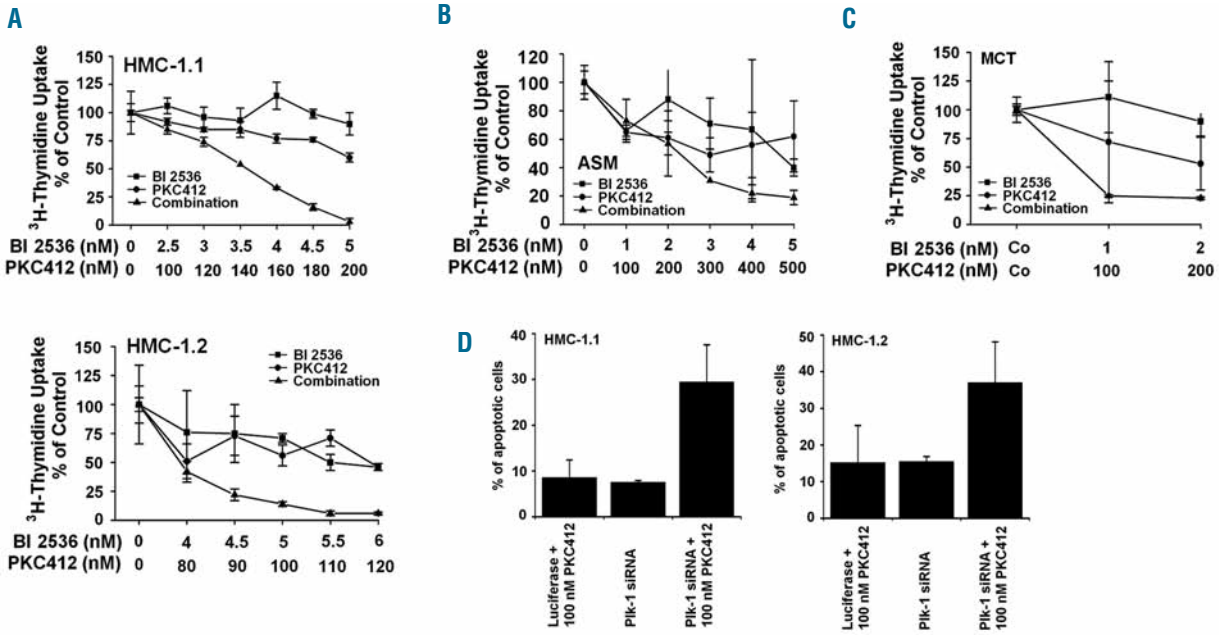
Online Supplementary Figure S1. Detection of tryptase, Plk-1 and phosphorylated Plk-1 (pPlk-1) in serial sections obtained from a patient with isolated cutaneous mastocytoma (ICM; patient #32 in *Online Supplementary Table S1*). Skin sections were stained with antibodies against tryptase, total Plk-1, and pPlk-1 as described in the text. Magnification x 40. Images were viewed under an Olympus BX50F4 microscope (Olympus, Hamburg, Germany) and prepared using an Olympus DP11 camera and Adobe Photoshop CS2 software version 11.0 (Adobe Systems, San Jose, CA, USA) to adapt for brightness and contrast.



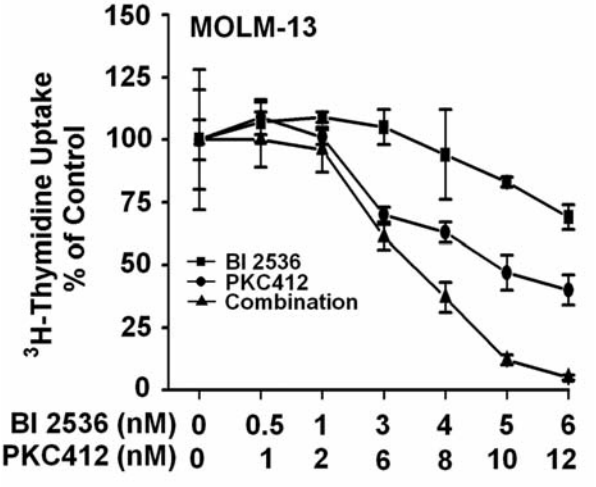
Online Supplementary Figure S2. Immunocytochemical detection of Plk-1 in HMC-1.1 cells and HMC-1.2 cells. Staining was performed on untransfected cells (upper panels) and cells transfected with a Plk-1 specific siRNA (lower panels). Immunocytochemistry was performed as described in the text using antibodies against Plk-1. Magnification x 100. Images were viewed under an Olympus BX50F4 microscope (Olympus, Hamburg, Germany) and prepared using an Olympus DP11 camera and Adobe Photoshop CS2 software version 11.0 (Adobe Systems, San Jose, CA, USA).



Online Supplementary Figure S3. Normal cultured cord blood-derived MC were incubated with control medium or 10-100 nM BI 2536 at 37°C for 48 or 96 h. Thereafter, the percentages of apoptotic cells were analyzed by combined annexin V/propidium iodide staining as described in the text.



Online Supplementary Figure S4. BI 2536 synergizes with midostaurin/PKC412 in producing growth arrest in neoplastic mast cells (MC). (A) HMC-1.1 cells and HMC-1.2 cells and C2 cells were incubated with various concentrations of BI 2536 (▪-▪), PKC412 (●-●), or a combination of both drugs (▲-▲) at a ratio of 1:40 (HMC-1.1) or 1:20 (HMC-1.2, C2 cells). (B) Primary neoplastic MC (aggressive SM) were incubated with various concentrations of BI 2536 (■-■), PKC412 (●-●), or a combination of both drugs (▲-▲) at a ratio of 1:100. After incubation with drugs (48 h, 37°C), ^{3}H-thymidine uptake was measured. (C) Primary canine neoplastic MC were incubated in various concentrations of BI 2536 (■-■), PKC412 (●-●), or a combination of both drugs (▲-▲) at a ratio of 1:100. After incubation with drugs (48 h, 37°C), ^{3}H-thymidine uptake was measured. Results represent the mean±standard deviation from triplicate experiments. (D) HMC-1.1 cells and HMC-1.2 cells were transfected with Plk-1 siRNA or luciferase siRNA and then exposed to PKC412 as described in the text. Results represent the mean±standard deviation from three independent experiments.



Online Supplementary Figure S5. Cells were incubated with control medium or various concentrations of BI 2536 (▪-▪), PKC412 (●-●) or a combination of both drugs (▲-▲) at a ratio of 1:2. After incubation with drugs (48 h, 37°C), ^{3}H-thymidine uptake was measured. Results represent the mean±standard deviation from triplicate experiments.