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Preclinical characterization of SEL24-B489, a dual PIM/FLT3 inhibitor for the treatment of hematological malignancies

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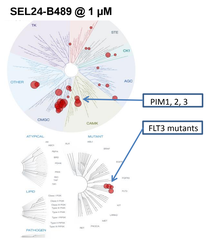
Introduction

PIM kinases represent an emerging therapeutic target in multiple hematological malignancies, as exemplified by the ongoing phase I clinical trials by AstraZeneca (AZD1208) and Novartis (LGH447) in acute myeloid leukemia and multiple myeloma. Selvita has developed a potent and selective dual PIM/FLT3 mutant kinase inhibitor - SEL24-B489 showing high inhibitory activity on all three PIM kinase isoforms and FLT3 kinase mutants. We have previously reported that PIM kinases are important downstream effectors of FLT3 signaling and play a crucial role in cell survival and inhibition of apoptosis upon expression. Due to heterogeneous nature of AML, dual inhibition of FLT3 mutant kinase and PIM kinases led to improved efficacy of our compound in comparison to selective inhibitors of either PIM of FLT3 kinases.

Herewith, we would like to report further progress of characterizing SEL24-B489 inhibitor beyond AML. We assessed PIM kinase expression levels in a panel of lymphoid malignancies and found that PIM1 and PIM2 exhibit high expression levels in a fraction of mantle cell lymphoma (MCL), diffuse large-B-cell lymphoma (DLBCL), follicular lymphoma (FL), Hodgkin's lymphoma (HL), chronic lymphocytic leukemia (CLL) and mucosa associated lymphoid tissue-type (MALT) lymphoma cell lines and primary tumors. High levels of PIM kinases were associated with certain established adverse prognostic factors and clinical outcome of the patients and correlated with aggressiveness of the disease in some of these tumors. Inhibition of PIM kinases with inhibitors was shown to influence cellular proliferation and translational inhibition. Comparison of SEL24-B489 to competitive PIM inhibitors revealed higher cellular activity and biomarker response, as shown by inhibition of phospho-S6 phosphorylation in sub-micromolar concentrations. The presented data validates further SEL24-B489 as a successful example of rational drug design and showcases a promising therapeutic approach in multiple hematological malignancies, both standalone and in combination with standard of care and targeted therapies currently in clinical development.

SEL24-B489 shows cytotoxic activity in AML cancer cell lines and high selectivity on a panel of 451 kinases

A)



B)

Kd determination @Dx [nM]	SEL24-B489	AZD 1208 *	AC 220*
PIM1	2	0.2	-
PIM2	2	0.8	-
PIM3	3	0.9	-
CDK2	2000	-	-
FLT3wt	160	-	1
FLT3(ITD)	16	-	9

C)

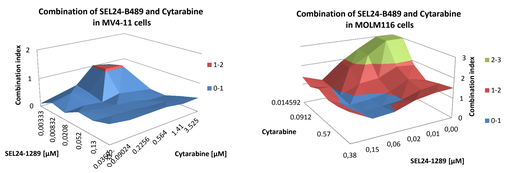
AML cell lines, FLT3-ITD positive

	MV4-11 IC50 (μM)	MOLM-13 IC50 (μM)	MOLM-16 IC50 (μM)	KG-1 IC50 (μM)	CMK IC50 (μM)	HL-60 IC50 (μM)
SEL24-B489A	0.15	0.29	0.1	0.48	0.11	~7.75
AZD1208 *	2.24	>10	0.07	0.8	0.54	4.19
AC220 *	0.003	0.001	>10	2.48	>10	>0.1
Cytarabine	0.57	0.15	0.66	0.44	0.06	0.44

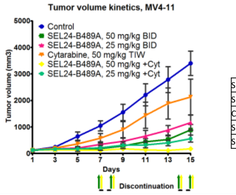
Figure 1. Overview of *in vitro* activity for SEL24-B489, AZD1208, AC220 and cytarabine – reference PIM kinase inhibitors reported in the literature and patent applications (AZD1208 – phase I PIM inhibitor by Astra Zeneca; AC220 – phase III FLT3 inhibitor from Ambit); A) SEL24-B489 shows very high selectivity when tested on a panel of 451 kinases. B) In addition to high PIM kinase inhibition it shows strong binding to FLT3 mutant kinases. C) SEL24-B489 shows strong cytotoxicity in AML cell lines independently from the status of FLT3 mutation.

SEL24-B489 shows strong synergistic effects in combination with cytarabine on AML cell lines *in vitro* and *in vivo*

A)



B)



	TGI (%)
SEL24-B489A, 50 mg/kg	82
SEL24-B489A, 25 mg/kg	77
Cytarabine, 50 mg/kg	60
SEL24-B489A, 50 mg/kg + Cytarabine, 50 mg/kg	99
SEL24-B489A, 25 mg/kg + Cytarabine, 50 mg/kg	89

Figure 2. Combination study of B489 with cytarabine shows strong synergistic effects both *in vitro* and *in vivo* in AML cell lines. A) In vitro combination experiment. Compound B489 shows synergistic effects with cytarabine in MOLM16 and MV-4-11 cells. Combination Index values calculated using CompuSyn Software. The following guidelines were implemented: CI value < 1 indicates synergism, CI value = 1 indicates additive effect and CI value > 1 indicates antagonism.

B) *In vivo* combination of B489 and cytarabine in MV-4-11 subcutaneous xenografts implanted in immunodeficient mice (SCID/beige). B489 compound was administered *per os* at a schedule twice a day (BID) in two doses 50 and 25 mg/kg alone or in combination with cytarabine dosed intraperitoneally in 50 mg/kg dose three times a week (TIW). Tumor growth inhibition (TGI) revealed strong synergistic antitumor effect dependent on dose.

Comparison of selective PIM inhibitor AZD1208, selective FLT3 inhibitor AC220 and dual PIM/FLT3 inhibitor B489 in AML cells

A)

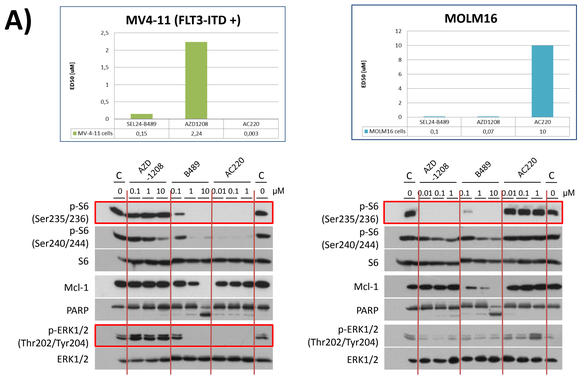


Figure 3. A) Dual PIM/FLT3 inhibitor B489 shows superior activity in AML cell lines irrespective of the mutation background. B) MV-4-11 and MOLM16 (both AML) expressing high levels of PIM kinases, were treated with SEL24-B489 compound for 4 hours in a dose-dependent manner *in vitro* and analyzed for phosphorylation of FLT3/PIM kinase downstream targets using Western blot. C – negative control (untreated cells). AZD1208 – selective PIM inhibitor, AC220 - selective FLT3 inhibitor and SEL24-B489 dual PIM and FLT3 inhibitor.

Primary AML cell survival after inhibitor treatment

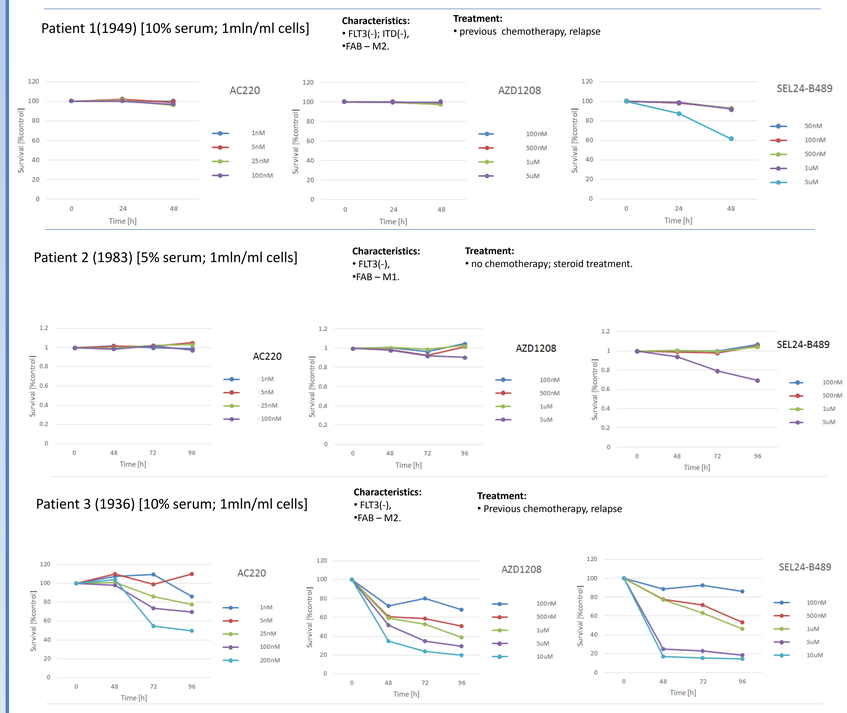
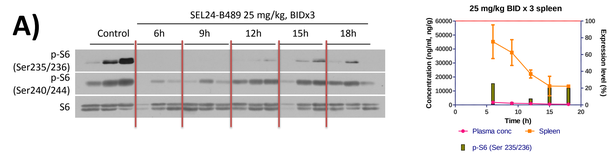


Figure 4. Survival of primary AML blasts after treatment with SEL24-B489 compared with AZD1208 and AC220 shows superior activity of the dual inhibitor SEL24-B489 over AZD1208.

Pharmacokinetic-pharmacodynamic relationship and high efficacy *in vivo* in mouse xenograft AML models

A)



B)

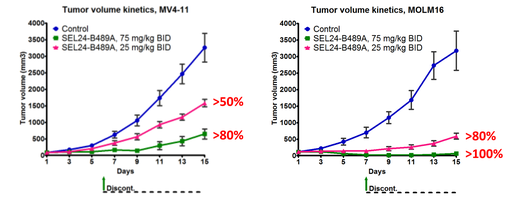


Figure 5. MV-4-11 and MOLM16 AML xenografts in immunodeficient mice (SCID/beige). Cells were implanted subcutaneously and tumors were allowed to reach >150 mm^3 (for A) or 100 to 150 mm^3 (for B). A) PK/PD relationship between SEL24-B489 concentration and biomarker expression in spleen and tumor (not shown) was tested in a MOLM16 xenograft in immunodeficient mice (SCID/beige). B489 compound was administered *per os* for three consecutive days at a schedule twice a day (BID) in dose 25 mg/kg. After the last administration the animals were sacrificed at indicated time points and the plasma and spleen samples were harvested for compound concentration analysis (PK) and biomarker response analysis (PD). B) Efficacy of SEL24-B489 was investigated B489 compound was administered *per os* at a schedule twice a day (BID) in doses 75 or 25 mg/kg. Tumor size was monitored every other day and revealed strong antitumor effect dependent on dose in MV-4-11 and MOLM16 xenografts.

Assessment of the safe dose in repeated 5-days and 10-days toxicology studies of SEL24-B489 in rats

A)

	Lethality	BW gain Day 0-5 (g)	Day of experim.	
Control, water	0/3	14	D6	
SEL24-B489A, 100 mg/kg QD, males	0/3	-20	D6	safe
SEL24-B489A, 25 mg/kg QD, males	0/3	7	D6	
Control, water	0/3	6	D6	
SEL24-B489A, 100 mg/kg QD, females	0/3	-22	D6	safe
SEL24-B489A, 25 mg/kg QD, females	0/3	0,2	D6	

B)

	Lethality	BW gain Day 0-9 (g)	Day of experim.	
Control, water	0/5	23	D10	
SEL24-B489A, 25 mg/kg BID, males	0/5	-10	D10	safe
SEL24-B489A, 10 mg/kg BID, males	0/5	20	D10	
Control, water	0/5	7	D10	
SEL24-B489A, 25 mg/kg BID, females	0/5	-17	D10	
SEL24-B489A, 10 mg/kg BID, females	0/5	3	D10	safe

C)

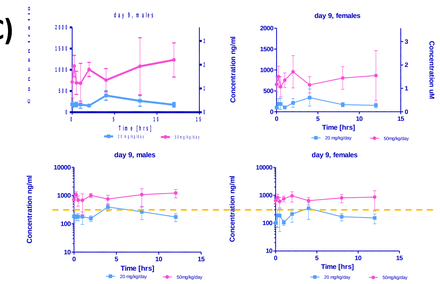


Figure 6. Repeated A) 5-days and B) 10-days toxicology studies of SEL24-B489 in Sprague-Dawley rats. The doses were administered *per os* once a day (QD) for 5 days or twice a day (BID) for 10 days. The doses 100 mg/kg QDx5 and 25 mg/kg BIDx10 are considered as safe, based on the obtained data from body weight gain (left panel), clinical chemistry, hematology, necropsy and histology of liver, kidney, spleen and stomach (data not shown). C) SEL24-B489 toxokinetics after 10 days of BID administration in rat. Dose of 20 mg/kg/day reaches therapeutic plasma levels of biomarker inhibition (~300ng/ml, dashed line), while dose of 25 mg/kg/day leads to stable compound concentration that is over 3 times higher than predicted therapeutic levels.

Summary of SEL24-B489 properties

Target(s) activity	PIM1 Kd = 2nM, IC50 = 5 nM	Physicochemical properties	MW:~440	PK profile rat PO 25mg/kg	T1/2 10h
	PIM2 Kd = 2nM, IC50 = 12 nM		cLOGP=3.96		Tmax 6.7h
	PIM3 Kd = 3nM, IC50 = 17 nM		cLOGD=1.45		Cmax 913 ng/ml
	FLT3-ITD Kd = 16 nM		PSA=75.7		AUC 19630 [ng*h/ml]
	FLT3wt Kd = 160 nM				F: 52%
Cellular activity (MTS viability assay)	MOLM-16 (AML) 0.1 μM	ADME	Drug interactions	PK profile dog PO 5mg/kg	T1/2 17.6h
	MV4-11 (AML) 0.2 μM		CYP3A4 IC50 15.7 μM		Tmax 1.75h
	KG-1 (AML) 0.2 μM		CYP1A2 IC50 >20 μM		Cmax 155ng/ml
	CMK (AML) 1.3 μM		CYP2B6 IC50 19μM		AUC 3078 [ng*h/ml]
	U-2932 (DLBCL) 0.5 μM		CYP2C9 IC50 14.2 μM		F: 45%
	OCI-LY-7 (DLBCL) 0.1 μM		CYP2D6 IC50 2.5 μM (>10 μM @ RBC)	Safety	Safe dose in mice >150mg/kg QD (14 days continuous administration) or 50 mg/kg BID
	SU-DHL-6 (DLBCL) 4.6μM		CYP2C19 IC50 16.6 μM		
	OCI-LY-1 (DLBCL) 5.8 μM		Metabolic stability		
	Maver-1(MCL) 0.5μ M		Stable in 59 and microsomes – human, rat, dog, mouse, cynomolgus		Safe dose in rats >100 mg/kg QD (5 days) and >25 mg/kg BID (10 days)
	Z138(MCL) 0.6 μM				
	Jeko-1 (MCL) 0.3 μM		hERG IC50 = 3.6 μM		
	Mino (MCL) 0.5 μM			In vivo efficacy	>80% TGI in MV4-11 xenograft 75mg/kg BID, PO
	REC1(MCL) 0.1 μM				>100 % TGI in MOLM16 xenograft 75mg/kg BID, PO
	HepG2 0.6 μM				dose dependent efficacy

Figure 7. Summary of SEL24-B489 properties – clinical candidate with first in class dual PIM/FLT3 profile. Summary of target(s) activity, cellular activity, physicochemical properties, ADME properties, PK profile in rat and dog, safety dose and in vivo efficacy are shown.