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First-in-class dual PIM/FLT3 inhibitor SEL24-B489 for the treatment of hematological malignancies

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Introduction

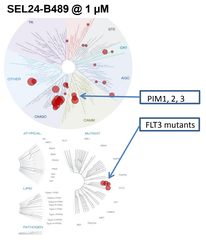
We have previously reported that PIM kinases are important downstream effectors of FLT3 signaling – a therapeutic target in multiple hematological malignancies, including acute myeloid leukemia and multiple myeloma. As inhibition of PIM kinases was shown to influence cellular proliferation and translational inhibition. PIM1 and PIM2 exhibit high expression levels in a fraction of lymphoma cell lines and in primary tumors. High levels of PIM kinases were associated with certain established adverse prognostic factors, clinical outcome of the patients, and aggressiveness of the disease in some of these tumors.

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 on FLT3 kinase mutants. As predicted from a 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 or FLT3 kinases. SEL24-B489 revealed higher cellular activity and biomarker response than competitive PIM inhibitors, as shown by inhibition of pS-S6 and pS-STAT5 phosphorylation at sub-micromolar concentrations. Further B489 characterization *in vitro* showed its superior potency over other PIM and FLT3 inhibitors in AML patient samples.

As reported previously, B489 efficiently inhibited tumor growth *in vivo* as monotherapy and in combination with standard of care and targeted therapies in clinical development. Repeated 14-days toxicology in rats and 10-days toxicology in dogs studies revealed that safe doses reached therapeutic plasma levels of biomarker inhibition in mice. B489 affected lymphoid tissues and released bone marrow marginal neutrophils, which should be regarded as an added value of the B489 inhibitor developed to cure leukemia patients.

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

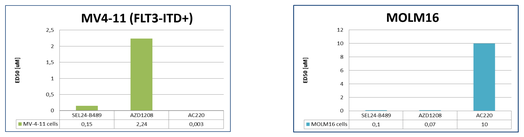
C)

	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-B489	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 – a 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 a AML cell lines independently from the status of FLT3 mutation.

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

A)



B)

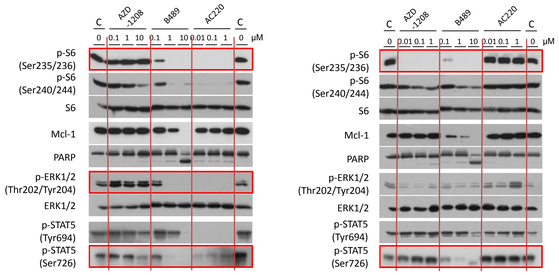


Figure 2. A) Dual PIM/FLT3 inhibitor B489 shows superior activity in AML cell lines irrespective of the mutation background. B) MV-4-11 and MOLM16 (both AML) expressing high levels of PIM kinases, were treated with SEL24-B489 compound for 4 hours in a dose-dependent manner *in vitro* and analyzed for 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 3. Head to head comparison of three inhibitors, selective PIM inhibitor AZD1208 (Astra Zeneca), selective FLT3 inhibitor AC220 (Ambit) and dual PIM/FLT3 inhibitor (Selvita) in AML patient samples.

SEL24-B489 shows *in vivo efficacy* in FLT3-ITD positive and wild type FLT3 AML models

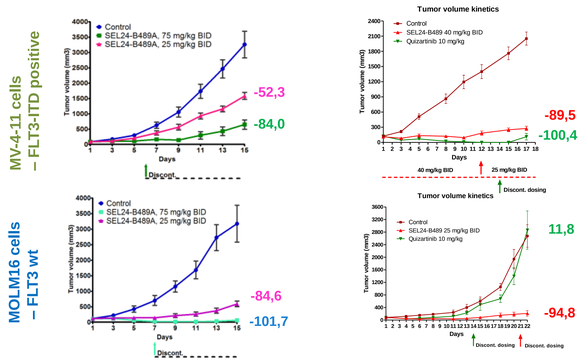


Figure 4. SEL24-B489 is active *in vivo* bothin FLT3-ITD positive and FLT3 wt, PIM kinase expressing cell lines.

Summary of SEL24-B489 properties

Target(s) activity	PIM1 Kd = 2 nM, IC50 = 5 nM	Physicochemical properties	MW:~440	PK profile - rat PO 25 mg/kg	T1/2 10 h
	PIM2 Kd = 2 nM, IC50 = 12 nM		cLOGP=3.96		Tmax 6.7 h
	PIM3 Kd = 3 nM, IC50 = 17 nM		cLOGD=1.45		Cmax 913 ng/ml
	FLT3-ITD Kd = 16 nM		PSA=75.7		AUC 19630 [ng*h/ml]
	FLT3 wt Kd = 160 nM				F: 52%
Cellular activity (MTS viability assay)	MOLM-16 (AML) 0.1 µM	ADME	Drug interactions	PK profile - dog PO 5 mg/kg	T1/2 17.6 h
	MV4-11 (AML) 0.2 µM		CYP3A4 IC50 15.7 µM		Tmax 1.75 h
	IG-1 (AML) 0.2 µM		CYP1A2 IC50 >20 µM		Cmax 155 ng/ml
	CMK (AML) 1.3 µM		CYP2B6 IC50 19 µM		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 >100 mg/kg QD (5 days) or 50 mg/kg BID (>14 days)
	SU-DHL-6 (DLBCL) 4.6 µM		CYP2C19 IC50 16.6 µM		
	OCI-LY-1 (DLBCL) 5.8 µM		Metabolic stability		Safe dose in rats >100 mg/kg QD (5 days), >25 mg/kg BID (10 days), 20 mg/kg QD (14 days)
	Maver-1 (MCL) 0.5µM		Stable in S9 and microsomes – human, rat, dog, mouse, cynomolgus		
	Z138 (MCL) 0.6 µM				Safe dose in dogs 10 mg/kg QD (10 days)
	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 75 mg/kg BID, PO
	REC1 (MCL) 0.1 µM				>100 % TGI in MOLM16 xenograft 75 mg/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.

Repeated 14-days toxicology study of SEL24-B489 in rats: assessment of the safe dose

A)

	Lethality	ΔBW (%)	Food consumption (%)
Control, water	0/5	140	100
SEL24-B489, 40 mg/kg QD, males	0/5	123	75
SEL24-B489, 20 mg/kg QD, males	0/5	135	96
Control, water	0/5	115	100
SEL24-B489, 40 mg/kg QD, females	0/5	110	67
SEL24-B489, 20 mg/kg QD, females	0/5	115	89

	Lesions		
	Bone marrow	Spleen	Thymus
Control, water	0/5	0/5	0/5
SEL24-B489, 40 mg/kg QD, males	4/5	2/5	5/5
SEL24-B489, 20 mg/kg QD, males	5/5	0/5	5/5
Control, water	0/5	0/5	0/5
SEL24-B489, 40 mg/kg QD, females	3/5	0/5	5/5
SEL24-B489, 20 mg/kg QD, females	0/5	0/5	5/5

B)

MALE	RBC 10^6/L	Reticuloc % RBC	WBC 10^3/L	Neut % WBC	Lymp % WBC	Mono % WBC	Platelets 10^3/L	PT s	ALT U/L	AST U/L	ALP U/L	GGT U/L	Creat umol/L	Cholest mmol/L	Proteins g/L	Na mmol/L	K mmol/L
Control	7.1	0.33	4.1	0.4	3.5	0.1	1286	17.4	20	171	205	5.2	39	1.8	59.2	143	4.8
SEL24-B489, 40 mg/kg QD	6.9	0.29	3.4	0.8	2.5	0.1	994	19.7	34	105	300	5.8	40	2.1	55.0	143	4.6
SEL24-B489, 20 mg/kg QD	7.0	0.29	4.4	0.8	3.5	0.1	1234	18.5	19	95	243	4.8	35	1.7	54.6	142	4.7

FEMALE	RBC 10^6/L	Reticuloc % RBC	WBC 10^3/L	Neut % WBC	Lymp % WBC	Mono % WBC	Platelets 10^3/L	PT s	ALT U/L	AST U/L	ALP U/L	GGT U/L	Creat umol/L	Cholest mmol/L	Proteins g/L	Na mmol/L	K mmol/L
Control	7.6	0.25	4.5	0.5	3.8	0.1	1193	17.6	15	121	112	4.4	43	1.9	62.4	141	5.3
SEL24-B489, 40 mg/kg QD	7.4	0.30	2.3	0.4	1.8	0.04	1159	18.3	22	80	174	5.2	42	2.0	60.3	141	4.8
SEL24-B489, 20 mg/kg QD	7.0	0.28	2.6	0.5	2.0	0.05	1182	17.1	15	80	137	4.4	47	2.1	64.4	140	4.5

C)

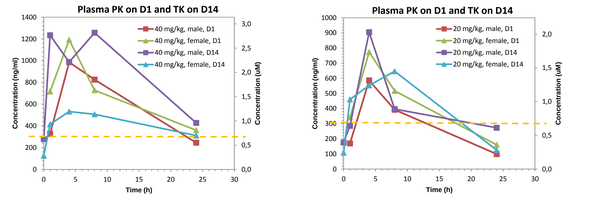


Figure 5. Sprague-Dawley rats were administered *per os* once a day (QD) for 14 days. The dose of 20 mg/kg QDx14 is considered as safe, based on the obtained data from body weight changes (A, left table), clinical chemistry, hematology (B), necropsy and histopathology (not shown). At both dose levels, lesions observed in the primary and/or secondary lymphoid tissues (A, right table) are regarded as beneficial in the context of targeted disorders. SEL24-B489 pharmacokinetic (day 1) and toxicokinetic (day 14) prove that the safe dose of 20 mg/kg/day reaches therapeutic plasma levels of biomarker inhibition (~300 ng/ml; C, dashed line).

Repeated 10-days toxicology study of SEL24-B489 in dogs: assessment of the safe dose

A)

	Lethality	ΔBW %	Food consumption (%)
Control, water	0/1	100	100
SEL24-B489, 30 mg/kg QD, male	0/1	89	70
SEL24-B489, 10 mg/kg QD, male	0/1	102	107
Control, water	0/1	100	100
SEL24-B489, 30 mg/kg QD, female	0/1	84	36
SEL24-B489, 10 mg/kg QD, female	0/1	94	73

	Atrophy	
	Peyer's patches	Thymus
Control, water	-	-
SEL24-B489, 30 mg/kg QD, males	slight	marked
SEL24-B489, 10 mg/kg QD, males	-	minimal
Control, water	-	-
SEL24-B489, 30 mg/kg QD, females	slight	moderate
SEL24-B489, 10 mg/kg QD, females	-	minimal

B)

MALE	RBC 10^6/L	Reticuloc % RBC	WBC 10^3/L	Neut % WBC	Lymp % WBC	Mono % WBC	Plateiets 10^3/L	PT s	ALT U/L	AST U/L	ALP U/L	GGT U/L	Creat umol/L	Cholest mmol/L	Proteins g/L	Na mmol/L	K mmol/L
Control	6.58	0.9	7.0	46.8	44.9	6.1	390	8.4	43.1	32.3	89	3.0	72.7	3.72	57.7	147.6	4.01
SEL24-B489, 30 mg/kg QD	7.56	0.1	26.5	76.3	18.2	4.3	142	8.2	47.2	25.6	75	5.1	73.3	5.26	49.0	142.3	4.51
SEL24-B489, 10 mg/kg QD	5.92	0.5	17.2	72.6	21.6	4.1	259	8.4	30.7	31.4	216	2.6	64.5	6.49	54.6	146.5	4.05

FEMALE	RBC 10^6/L	Reticuloc % RBC	WBC 10^3/L	Neut % WBC	Lymp % WBC	Mono % WBC	Plateiets 10^3/L	PT s	ALT U/L	AST U/L	ALP U/L	GGT U/L	Creat umol/L	Cholest mmol/L	Proteins g/L	Na mmol/L	K mmol/L
Control	7.32	0.8	8.9	52.8	41.7	3.8	274	8.7	21.3	31.5	147	2.2	60.7	3.93	56.1	148.3	3.95
SEL24-B489, 30 mg/kg QD	9.32	0.1	28.4	79.1	15.3	4.3	252	8.1	64.8	35.5	120	2.1	70.8	4.56	54.4	140.7	4.46
SEL24-B489, 10 mg/kg QD	5.85	0.1	20.0	72.1	21.0	5.5	423	7.8	37.8	28.7	204	2.9	68.9	4.67	53.9	146.0	4.41

C)

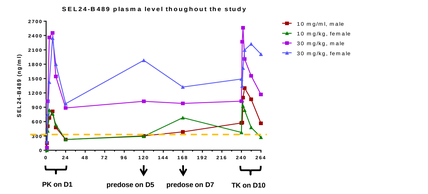


Figure 6. Beagle dogs were administered *per os* once a day (QD) for 10 days. The dose of 10 mg/kg QDx10 is considered as safe, based on the obtained data from body weight changes (A, left table), clinical chemistry, hematology (B), necropsy and histopathology (not shown). At both dose levels, secondary lymphoid tissues atrophy (A, right table) and neutrophilia (B) are regarded as beneficial in the context of targeted disorders. SEL24-B489 plasma level measured throughout the study course prove that the safe dose of 10 mg/kg/day reaches therapeutic plasma levels of biomarker inhibition (~300 ng/ml; C, dashed line).