Co-targeting PIM and PI3K/mTOR pathways with a single molecule: Novel orally available combined PIM/PI3K and PIM/PI3K/mTOR inhibitors.

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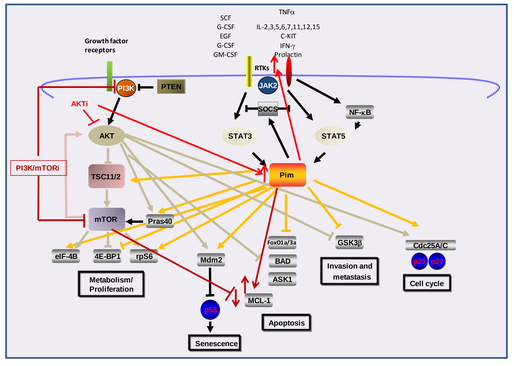
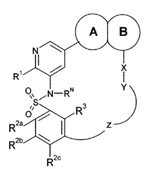
Fig 1. Crosstalk between PI3K/AKT, mTOR and PIM pathways



- The PI3K/AKT pathway is commonly activated in human cancer.
- The efficacy of PI3K/mTOR or AKT inhibitors is compromised by the stimulation of compensatory signaling pathways.
- PIM kinases, produce parallel oncogenic signals to AKT and mTOR and share several downstream molecular targets.
- PIM mediates resistance to rapamycin, AKT and PI3K/mTOR inhibition (Schatz et.al. 2011, Cen et al. 2013, Zang et al. 2013).
- Combination of PI3K inhibitor GDC-0941 with a PIM selective inhibitor, ETP-45299, is strongly synergistic in antiproliferation experiments in MV4:11 AML cells (Blanco-Aparicio et al. 2011).

Identification of PIM/PI3K and PIM/PI3K/mTOR inhibitors

Compounds from our PI3K program were screened to evaluate their PIM activity identifying in such a way hits with weak dual PI3K-PIM activities. These hits came from an internally generated collection of macrocycles, which were explored to fine tuning dual PI3K/mTOR activities. Crystal structure of the hits in PIM1 protein helped us to understand the key interactions of these compounds required for PIM activity. Taking this into account, a chemical exploration was done around them trying to balance the dual (PIM, PI3K) or triple (PIM, PI3K, mTOR) activities and to optimize the drug like properties of the compounds. As a result of this exploration compounds ETP-539/IBL-202 and ETP-339/IBL-301 were identified.



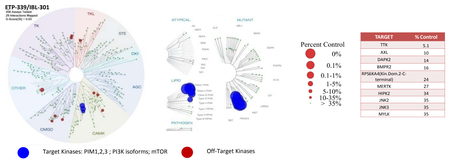
Biochemical and ADME profile of ETP-539/IBL-202 and ETP-339/IBL-301

A

CHEMISTRY	PIM1 IC_{50} (nM)	PIM2 IC_{50} (nM)	PIM3 IC_{50} (nM)	PI3K IC_{50} (nM)	MTOR IC_{50} (nM)
ETP-539/IBL-202	30.4	21.5	15.0	10.1	5960.0
ETP-339/IBL-301	25.7	21.4	3.7	3.0	90.8

CHEMISTRY	MLM (1h', 1µM)	MMM (1h', 1µM)	RAM (1h', 1µM)	P450 1A2 IC_{50} (µM)	P450 2C9 IC_{50} (µM)	P450 2C9 IC_{50} (µM)	P450 2D6 IC_{50} (µM)	P450 3A4 IC_{50} (µM)	hERG IC_{50} (µM)
ETP-539/IBL-202	77.8	83.9	85.8	>50	31.2	4.5	>50	>50	>30
ETP-339/IBL-301	87.5	89.8	87.3	>50	>50	4.6	>50	>50	>30

B



TARGET	% Control
TTK	5.1
AXL	10
DAPK2	14
BMPR2	16
RPS6KA4(Kin.Dom.2-C-terminal)	24
MERTK	27
RIPK2	34
JNK2	35
JNK3	35
MYLK	35

Percent Control: 0% · 0.1% · 0.1-1% · 1-5% · 5-10% · 10-35% · > 35%

Similar results were obtained for ETP-539/IBL-202

ETP-339/IBL-301 and ETP-539/IBL-202 are panPI3K inhibitors

Figure 2. A) Biochemical potency towards PIM isoforms, PI3Kα and mTOR. *In vitro* ADME properties: microsome stability, cytochromes and hERG inhibition. B) KINOMEscan™ in vitro binding assays was used to evaluate ETP-339/IBL-301 and ETP-539/IBL-202 against a panel of 456 kinases.

Downregulation of PIM and PI3K /mTOR by dual and triple action inhibitors

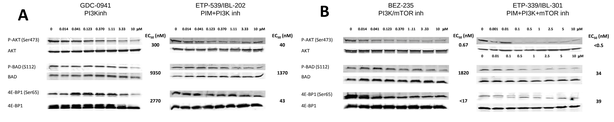


Figure 3. Dose dependent inhibition of PIM, PI3K and mTOR biomarkers in MV4:11 acute leukemia cell line treated 1h with A) GDC-0941 and ETP-539/IBL-202 and B) BEZ-235 and ETP-339/IBL-301.

Cell cycle and apoptotic profile of Dual PIM/PI3K inhibitors

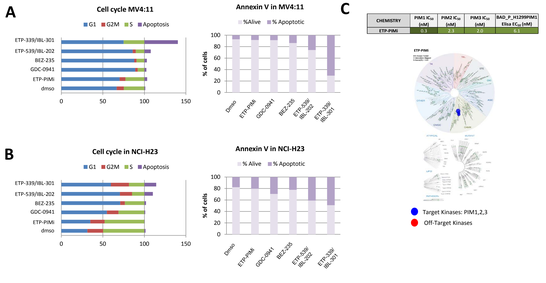
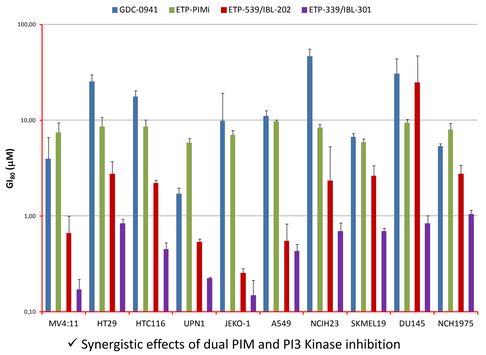


Figure 4. Cell cycle analysis and annexin V of ETP-PIMi, GDC-0941, ETP-539/IBL-202, BEZ-235 and ETP-339/IBL-301 at 1µM. A) MV4:11 acute myeloid leukemia cell line treated 24h. B) NCI-H23 non small cell lung cancer cell line treated 48h. C) ETP-PIMi: biochemical potency towards PIM isoforms and selectivity by KINOMEscan™.

Antiproliferative activity of dual and triple action inhibitors



✓ Synergistic effects of dual PIM and PI3 Kinase inhibition

Figure 5. Antiproliferative activity in different cell lines (72h) of ETP-PIMi, GDC-0941, ETP-539/IBL-202 and ETP-339/IBL-301

Pharmacokinetic profile of ETP-539/IBL-202 and ETP-339/IBL-301 in mouse and rat exposure

A

	ETP-539/IBL-202		
	Mouse PK		Rat exposure
PARAMETER	I.V.	ORAL	ORAL
F(%)	-	87.05	
Cmax(ng/ml)	-	2243.83	2347
Tmax (h)	-	8.00	7.3
AUC inf (h*ng/ml)	21329.05	29709.35	47058
T ½ (h)	2.27	-	
Cl((L/h/kg)	0.04	-	
Vd (L/kg)	0.13	-	
MRT (h)	2.95		

B

	ETP-339/IBL-301		
	Mouse PK		Rat Exposure
PARAMETER	I.V.	ORAL	ORAL
F(%)	-	34.51	
Cmax(ng/ml)	-	2929.00	1817
Tmax (h)	-	0.25	6
AUC inf (h*ng/ml)	16596.24	57280.69	28000
T ½ (h)	3.15	-	-
Cl(L/h/kg)	0.04	-	-
Vd (L/kg)	0.49	-	-
MRT (h)	10.89		

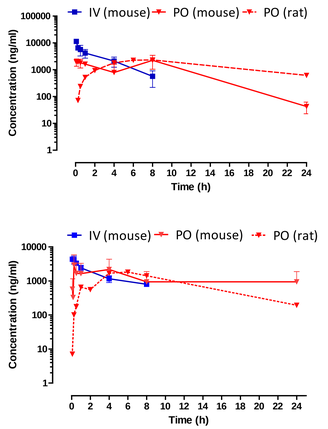


Figure 6. A) Plasma comparative levels of ETP-539/IBL-202 in mouse (1mg/kg i.v. and 1.6mg/kg p.o) and rat (10 mg/kg p.o). B) Plasma levels of ETP-339/IBL-301 (1 mg/kg i.v and 10mg/kg p.o.) in mouse and rat (Rat PK data was generated by Pharmidex).

Preliminary low dose study: in vivo biomarkers downregulation and efficacy in K-Ras^{G12V}-Induced NSCLC

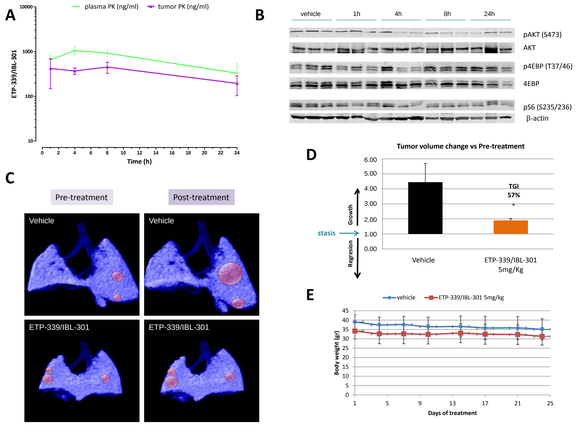


Figure 7. A) ETP-339/IBL-301 exposure in plasma and tumor at different time points after compound p.o administration 5mg/kg in K-Ras+/LSLG12Vgeo ; RERT ert/ert conditional mice (Guerra, Cancer Cell 2003). The K-Ras mutation was induced by tamoxifen and six and a half months later when lung tumors developed, mice were treated. B) Western blot analysis of the effect ETP-339/IBL-301 on PI3K, mTOR and PIM kinases downstream protein targets in KRas^{V12} lung tumors. C) Computed tomography (CT) scans of K-Ras+/LSLG12Vgeo ; RERT ert/ert conditional mice before and after treatment. Mice were treated orally with ETP-339/IBL-301 5mg/kg, 5 days a week for 3 weeks. D) Tumor volumes of five mice in each treatment group after three weeks of daily treatment are shown as percentage of relative change to pre-treatment tumor volumes. Values are means ± s.e.m. E) Body weight variation during the efficacy study.

Conclusions

-Compounds with dual PIM/PI3K or PIM/PI3K/mTOR inhibition activities have been developed. ETP-539/IBL-202(PIM/PI3Ki) and ETP-339/IBL-301(PIM/PI3K/mTORi) have been identified as selective orally bioavailable compounds.

- Both ETP-539/IBL-202 (PIM/PI3Ki) and ETP-339/IBL-301 (PIM/PI3K/mTORi) showed more potent antiproliferative activity than PIM and PI3K selective inhibitors alone. This correlated with higher induction of apoptosis and strong donwregulation of PIM, PI3K, mTOR pathways.

- Dual and triple compounds show synergistic effect in cancer cell lines over single agent compounds.

- ETP-339/IBL-301 (PIM/PI3K/mTORi) has been characterized *in vivo*, showing downregulation of PIM, PI3K, mTOR pathway biomarkers in PK/PD mechanistic studies, together with efficacy in GEMM of NSCLC at a low dose of 5mg/kg.