

# Pharmacokinetic/Pharmacodynamic (PK/PD) relationships of novel HDAC inhibitors in an HCT-116 mouse xenograft tumor model



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## INTRODUCTION

Inhibition of Histone deacetylase (HDAC) activity leads to cell cycle arrest and terminal differentiation of cancer cells, and represents a promising approach in the treatment of cancer<sup>1</sup>.

- Clinical proof-of-concept was shown with the first HDAC inhibitor Zolinza (SAHA), now approved for cutaneous T-cell lymphoma (CTCL). Other HDAC inhibitors like FK228, PXD101, LBH589 and MGD0103 are in clinical trials.
- Pre-clinical PK/PD relationships are not well characterized for inhibitors of HDACs. PK/PD relationships are critical for setting dose and regimen in pre-clinical and clinical stages of drug discovery<sup>2</sup>.
- The aim of this study was to explore relationships between PK and PD parameters, based on established paradigms used in anti-infective drug discovery
- PK parameters (AUC, C<sub>max</sub> and t<sub>1/2</sub>) were correlated with PD parameters (tumor cell proliferation inhibition [IC<sub>50,HCT116</sub>] and tumor growth inhibition [TGI]) in a human colorectal cancer (HCT-116) xenograft model in nude mice treated with a series of novel HDAC inhibitors and reference compounds.

## METHODOLOGY

**Compounds:** SB939, SB869, SB207, LBH589, SAHA, PXD101

**Inhibition of HDAC1 activity (IC<sub>50,HDAC1</sub>):** IC<sub>50</sub> values were determined against HDAC1 (in triplicates) using an *in vitro* fluorescence-based HDAC assay in 96-well format (BIOMOL Inc, Boston, USA) (Table 1)

**In vitro cell proliferation assay (IC<sub>50,HCT-116</sub>):** HCT-116 cells (ATCC, Virginia, U.S.A.) were seeded in 96-well plates at 3000 cells/well and then incubated at 37°C, 5% CO<sub>2</sub> for 24 h. Cells were treated with compounds at various concentrations for 96 h and cell proliferation was monitored using the CyQUANT™ assay (Invitrogen Pte Ltd). IC<sub>50</sub> values were estimated from dose response curves (XL-fit, IDBS, New Jersey, U.S.A) (Table 1)

**Pharmacokinetics: Animals:** Female Balb/c nude mice, 18-22 gm, 8-10 weeks. **Dose:** 50 mg/kg. **Route:** Oral (gavage). **Formulation:** 0.5% methyl cellulose and 0.1% Tween-80 in water.

**Time points:** Blood samples were taken at pre-dose, 5, 10, 30 min, 1 h, 2, 4, 8, and 24 h by cardiac puncture following CO<sub>2</sub> anesthesia. **Processing and bioanalysis:** Blood samples were centrifuged for 10 min at 3000 rpm and plasma harvested and analyzed by LC-MS/MS<sup>3</sup>. **PK Parameters:** Non-compartmental Analysis, WinNonlin (ver. 5.1, Pharsight) (Table 1). PK parameters for 100 mg/kg SB939 and PXD101, and 200 mg/kg SAHA were estimated by extrapolation of PK data from 50 mg/kg, assuming dose proportionality.

Table 1: Summary of PK/PD parameters

PK/PD Attributes	SB939	SAHA	PXD101	LBH589	SB207	SB869
IC <sub>50, HDAC 1</sub> (ng/ml)	25	32	19	2.4	15	18
IC <sub>50, HCT-116</sub> (ng/ml)	190	875	185	22	1372	312
Dose (mg/kg)	100	200	100	50	100	50
AUC (ng*h/ml)	3682	2476	514	126	6118	1875
C <sub>max</sub> (ng/ml)	5264	2004	987	116	6565	3980
AUC/IC <sub>50, HCT-116</sub>	19	3	3	6	5	6
C <sub>max</sub> /IC <sub>50, HCT-116</sub>	28	2	5	5	5	13
T>IC <sub>50, HCT-116</sub> (min)	240	60	30	60	60	90
TGI (%)	97	40	35	50	57	68

**In vivo Xenograft study: Animals:** Female Balb/c nude mice, 10-12 weeks. **Cell line:** Mice were implanted s.c. with 5 x 10<sup>6</sup> HCT-116 cells. Treatment began when tumor volumes were 75-144 mm<sup>3</sup>. **Doses:** SB939 (50 and 100 mg/kg), LBH589 (50 mg/kg), SAHA (200 mg/kg) and PXD101 (50 and 100 mg/kg), SB869 and SB207 at 50 mg/kg. **Regimen:** q.d. for 15 days. **Route:** Oral (gavage). **Formulation:** 0.5% methyl cellulose and 0.1% Tween-80 in water.

**Tumor Growth Inhibition:** (%TGI) = [(C<sub>t</sub>-T<sub>t</sub>)/(C<sub>t</sub>-C<sub>t1</sub>)\*100], where, C<sub>t</sub> = median tumor size of vehicle control group at time t, T<sub>t</sub> = median tumor size of treatment group at time t, C<sub>t1</sub> = median tumor size of vehicle control group first day of treatment (Table 1).

**PK/PD parameters:** The ratios AUC/IC<sub>50, HCT-116</sub>, C<sub>max</sub>/IC<sub>50, HCT-116</sub>, T>IC<sub>50, HCT-116</sub> were estimated (Table 1) and plotted individually against % TGI for each drug and the r<sup>2</sup> estimated (Figures 1, 2 and 3).

## SUMMARY OF KEY FINDINGS

- T>IC<sub>50,HCT-116</sub> (r<sup>2</sup>=0.80, Figure 1) and AUC/IC<sub>50,HCT-116</sub> (r<sup>2</sup>=0.76, Figure 2) showed high correlation with %TGI followed by C<sub>max</sub>/IC<sub>50,HCT-116</sub> (r<sup>2</sup>=0.71, Figure 3).
- Anti-tumor activity (%TGI~ 50) was observed when HDAC inhibitors achieved T>IC<sub>50,HCT-116</sub> > 1.5 h, AUC/IC<sub>50,HCT-116</sub> > 5 and, C<sub>max</sub>/IC<sub>50,HCT-116</sub> >10.
- SB939 at 100 mg/kg had the highest anti-tumor efficacy (%TGI=97) and the highest T>IC<sub>50,HCT-116</sub> (4 h), AUC/IC<sub>50,HCT-116</sub> (19), and C<sub>max</sub>/IC<sub>50,HCT-116</sub> (28) when compared to SAHA, PXD101, and LBH589 (Table 1).

## CONCLUSIONS

- Our data show that the superior preclinical profile and PK/PD relationships of SB939, compared to other HDAC inhibitors, has been translated in Phase 1 clinical trials<sup>4</sup>.
- Preclinical PK/PD studies, as we have described here, can help in rapid selection of the best candidates for advancing into clinical trials.

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