

Pharmacokinetic-pharmacodynamic (PK-PD) relationship of novel HDAC inhibitors in HCT116 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
- Proof-of-Concept available with first HDAC inhibitor Zolinza (SAHA) approved for cutaneous T-cell lymphoma (CTCL), and several other HDAC inhibitors like FK228, PXD101, LBH589 and MGCD0103 in clinical trials
- Single agent pharmacodynamic activities reported in CTCL, peripheral T-cell lymphoma (PTCL), Lymphomas and myelodysplastic syndrome (MDS)
- PK/PD relationships have not been reported for HDAC inhibitors in either solid or hematological tumors. Linking the exposure of the drug to tumor growth dynamics could improve the optimization of lead compounds during drug discovery phase^{1,2}
- The pharmacokinetic-pharmacodynamic (PK-PD) predictors (Fig 1) such as ratio of overall exposure (AUC) to the minimum inhibition concentration (AUC/MIC), peak levels (C_{max}/MIC) and time drug remains above MIC (T > MIC) is a well-established paradigm in the antibacterial therapy³. However, such PK-PD relationships (Table 1) have not been thoroughly understood for HDAC inhibitors
- In this presentation, the relationship between the drug exposure (AUC and C_{max}) and *in vivo* anti-tumor activity (% tumor growth inhibition, %TGI) that best describes the efficacy of novel HDAC inhibitors in preclinical models will be discussed

Figure 1. PK-PD Predictors for Efficacy

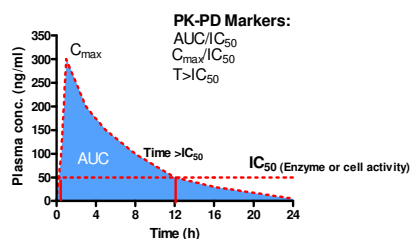


Table 1: PK-PD Markers

Area Under Curve (AUC)	The area under the plasma concentration versus time curve up to the last quantifiable time point, AUC was obtained by the linear and log-linear trapezoidal summation
Peak levels (C _{max})	Peak plasma concentration (C _{max}) is directly obtained from the PK data
Inhibitory Conc. (IC ₅₀)	Concentration of HDAC inhibitor that was required for 50% inhibition of its target (i.e. HDAC1 enzyme and HCT116 cell line)
AUC/IC ₅₀	The AUC/IC ₅₀ was defined as the ratio of area under the concentration-time curve (AUC) to the IC ₅₀
C _{max} /IC ₅₀	The concentration of C _{max} /IC ₅₀ was defined as the ratio of the maximum concentration of drug in plasma (C _{max}) to the IC ₅₀
T>IC ₅₀	The time over that concentration of HDAC inhibitor that was exceeded above IC ₅₀

MATERIALS AND METHODS

Table 2: *In vivo* Pharmacokinetics

Animals	Non-fasted female Balb/c nude mice 18-22 gm (8-10 weeks of age)
Dose & Formulation	Oral formulation for all drugs were prepared as a solution in 0.5% methyl cellulose and 0.1% Tween 80 in water
Protocol	At pre-defined time points (pre-dose, 5 or 10, 30min, 1, 2, 4, 8, and 24h), mice were sacrificed by overdose of CO ₂ and blood samples were collected by cardiac puncture. The blood samples were centrifuged for 10 min at 3000 rpm to separate plasma and the plasma was kept frozen at -80°C until analysis by LC/MS/MS ⁴
PK analysis	Pharmacokinetic parameters were calculated by a non-compartmental method using WinNonlin 5.2 software

Table 3: *In vivo* Efficacy Study

Species	Female athymic Balb/C nude mice, 10-12 weeks of age
Dose	SB939 (50 and 100 mg/kg), LBH589 (50 mg/kg), SAHA (200 mg/kg) and PXD101 (50 and 100 mg/kg), SB869 (50mg/kg) and SB207 (100 mg/kg)
Cell line	HCT-116 human colon carcinoma
Formulation	All the drugs were formulated in an appropriate vehicle (0.5% methylcellulose and 0.1% Tween 80 in water) for oral administration. Drugs were orally administered daily by a gavage for a period of 15 days
Tumor Growth Inhibition (%TGI)	%TGI = [(C _t -T _t)/(C ₀ -C ₁)*100] C _t = the median tumor size of the vehicle control group at time t, T _t = the median tumor size of the treatment group at the time t, C ₀ = the median tumor size of the vehicle control group first day of treatment

Table 4: *In vitro* Assays

Inhibition of HDAC enzymatic activity (HDAC1 IC ₅₀)	An <i>in vitro</i> fluorescence-based HDAC assay in 96-well format (BIOMOL Inc, Boston, USA) was used to screen and determine IC ₅₀ values for various HDAC isoforms. All drugs were tested against HDAC1 enzyme using different concentrations of the generic substrate <i>Fluor de Lys</i> (BIOMOL Inc, Boston, USA). Prism 3.0 software (GraphPad Software, San Diego, USA) was used to generate IC ₅₀ from triplicate data for each assay
<i>In vitro</i> cell proliferation assay (HCT116 IC ₅₀)	To determine the cellular activity of HDAC compounds, human cancer cell lines (HCT116) obtained from ATCC (Virginia, U.S.A.) were cultivated according to instructions provided by the supplier. HCT116 cells were seeded in 96-well plate at 3000 cells/well. The plates were incubated at 37°C, 5% CO ₂ , for 24 h. Cells were treated with compounds at various concentrations for 96 h. Cell growth was then monitored using the CyQuant™ cell proliferation assay (Invitrogen Pte Ltd). Dose response curves were plotted to determine IC ₅₀ values for the compounds using XL-fit (IDBS, New Jersey, U.S.A.)

RESULTS & DISCUSSION

- Comparative efficacy⁵ of SB939 and other HDAC inhibitors in HCT116 xenograft mouse model is illustrated in Fig 2
- After once daily oral dosing at the maximum tolerated dose for 15 days, the TGI for SB939 was 97% while the TGI for LBH589, SAHA, and PXD101 were 50, 40, and 35%, respectively
- PK-PD correlations using AUC/IC₅₀, C_{max}/IC₅₀ and T>IC₅₀ Vs % TGI as markers were examined for in-house lead compounds and other HDAC inhibitors like SAHA, PXD101, and LBH589 as presented in Table 5
- AUC/IC₅₀ or C_{max}/IC₅₀ (HCT116) Vs % TGI were the PK-PD parameters that best described the anti-tumor activity of HDAC inhibitors in mouse HCT116 xenograft model, with a correlation of 0.76 and 0.71 respectively (Fig 3)
- The plasma levels of a HDAC inhibitor above its IC₅₀ >1.5h yielded significant PD activity (Fig 4)
- AUC/IC₅₀ or C_{max}/IC₅₀ (HDAC1) Vs % TGI showed lesser degree of correlation indicating that other isoforms of HDAC could be important for the overall PD activity

Figure 2. Comparative efficacy of our clinical lead SB939 with other HDAC inhibitors in HCT116 xenograft model

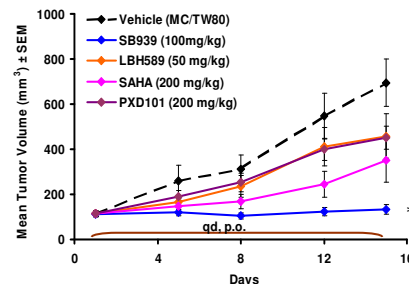


Table 5: Pharmacokinetic-Pharmacodynamic Parameters

PK-PD Attributes	SB939 (S*BIO)	SAHA (Merck)	PXD101 (Topotarget)	LBH589 (Novartis)	SB207 (S*BIO)	SB869 (S*BIO)
HDAC1 IC ₅₀ (ng/ml)	25	32	19	2.4	15	18
HCT116 IC ₅₀ (ng/ml)	190	875	185	22	1372	312
PD dose (mg/kg)	100 (PO)	200 (PO)	100 (PO)	50 (PO)	100 (PO)	50 (PO)
Mouse AUC (ng*hr/ml)	3682	2476	514	126	6118	1875
Mouse C _{max} (ng/ml)	5264	2004	978	116	6565	3980
Mouse AUC/IC ₅₀ (HCT116) (h)	19	3	3	6	5	6
Mouse C _{max} /IC ₅₀ (HCT116) (h)	28	2	5	5	5	13
Mouse AUC/IC ₅₀ (HDAC1) (h)	147	77	27	53	408	104
Mouse C _{max} /IC ₅₀ (HDAC1) (h)	211	80	39	5	263	159
Mouse T>IC ₅₀ (min)	240	60	30	60	60	90
HCT-116 mouse xenograft efficacy (% TGI)	97	40	35	50	57	68

Figure 3. PK-PD correlation using AUC/IC₅₀ (HCT116) and C_{max}/IC₅₀ (HCT116) for HDAC inhibitors

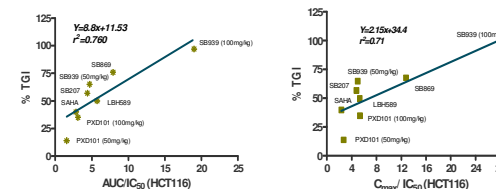
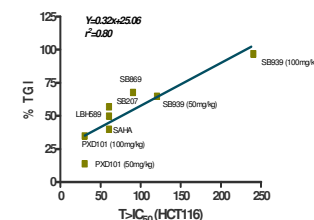


Figure 4. PK-PD correlation using T>IC₅₀ (HCT116) for HDAC inhibitors



SUMMARY

- HDAC inhibitors achieving AUC/IC₅₀ (HCT116) >5 and C_{max}/IC₅₀ (HCT116) >10, respectively, exhibited pronounced PD activity in mouse HCT116 tumor model
- T>IC₅₀ (HCT116) Vs % TGI also showed good correlation. This indicates that in addition to the concentration dependent PK/PD markers, the duration of the drug remaining above the cell proliferating concentration could be important for HDAC inhibitor's efficacy
- PK-PD optimization of compounds in the preclinical setting might help us identify best clinical candidates
- The PK-PD understanding also could speed up the efficacy evaluation of new oncology drugs during clinical development

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