

INTRODUCTION

- Colorectal cancer is the second leading cause of cancer-related death in developed countries.
- The etiology of colorectal cancer could arise from chronic inflammation or genetic mutation of polyps, (e.g. APC).
- Histone deacetylases (HDAC) regulate a number of genes involved in inflammation, cell proliferation and differentiation, cell cycle progression and cell survival. High expression of HDACs has been demonstrated in human colon cancer¹.
- There are a number of HDAC inhibitors, e.g. SAHA, MS275 and PXD101, emerging as a new class of anticancer agents which have shown good efficacy in clinical trials for treatment of hematological malignancies.
- None of these HDAC inhibitors have demonstrated good anti-tumor activity in solid tumors, which may be due to their poor pharmacokinetic properties and dose limiting toxicities.
- Here, we present the pharmacological profiling of SB939, a novel compound with improved metabolic, pharmacokinetic and pharmaceutical properties², in colon cancer models.

Figure 2: Inhibition of recombinant HDAC enzyme activities

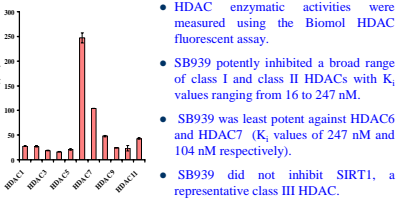
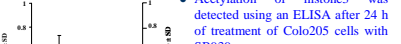


Figure 3: Cellular response to SB939 treatment

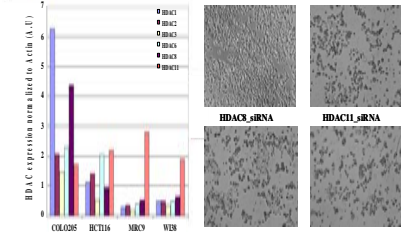
Colorectal cell lines	IC ₅₀ (µM); Mean±SD for inhibition of cell proliferation
Colo205	0.6 ± 0.1 (n = 41)
HCT116	0.5 ± 0.2 (n = 5)
SW620	0.5, 0.5 (n = 2)

- SB939 inhibited cell proliferation of 3 different colorectal cancer cell lines with similar potency.
- Acetylation of histone3 was detected using an ELISA after 24 h of treatment of Colo205 cells with SB939.
- Induction of acetylation of histone3, (EC₅₀ 0.6 ± 0.1 µM) occurred in the same concentration range as inhibition of cell proliferation (IC₅₀ 0.6 ± 0.1 µM). Data mean ± SD.



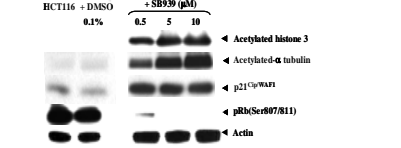
METHODS AND RESULTS

Figure 1: HDACs expression profile and gene knock down using siRNA



- Expression of different HDAC isoforms was quantified using quantitative real-time PCR.
- HDAC1, 2, 3, 6 and 8 were over-expressed in colon cancer cell lines.
- Knockdown of HDAC1, 8, and 11 in a colorectal cancer cell line (HCT116) resulted in changes in cell morphology and an increase in cell death.

Figure 4: Profiling of mechanistic biomarkers



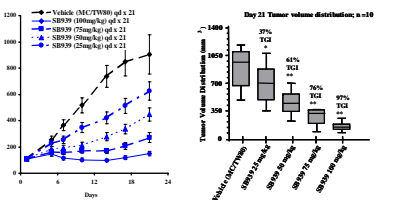
- The molecular effects of SB939 were evaluated in HCT116 cell line by western blot analysis.
- SB939 induced acetylation of histone3 and alpha tubulin, increased expression of the pro-apoptotic protein, p21^{Cip1/WAF1}, and reduced phosphorylation of retinoblastoma protein.

Figure 5: Induction of acetylation of histone3 in HCT116 tumor tissues

µM	Vehicle				SB939				SAHA			
	1	3	6	24	1	3	6	24	1	3	6	24
AcH3	[Western blot bands]											
Actin	[Western blot bands]											

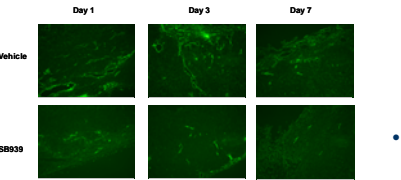
- Acetylation of histone3 in tumor samples was measured by western blot. Each blot represents tumors from 3 mice (10 µg protein each).
- After a single oral dose of SB939 (50 mg/kg) or SAHA (200 mg/kg), acetylation of histone3 was induced in tumor tissue within 1 h after dosing.
- The response persisted longer for SB939 than for SAHA.

Figure 6: Anti-tumor activity of SB939 in a model of advanced human colorectal cancer (HCT116 bearing nude mice)



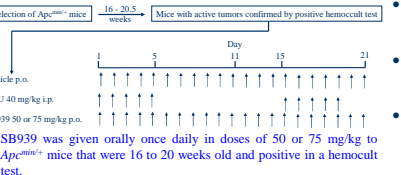
- After daily oral administration for 21 days at 25, 50, 75 and 100 mg/kg, SB939 induced significant and dose dependent tumor growth inhibition (TGI). Data mean ± SEM.
- All mice tolerated SB939 well. The mean group body weight loss on day 21 was 6%, 8%, 6% and 16% for the 25, 50, 75 and 100 mg/kg doses respectively.

Figure 7: Blood vessel formation in HCT116 tumor tissue

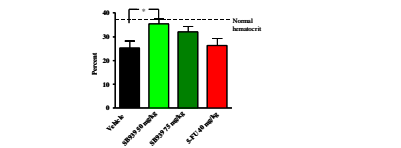


- SB939 was administered orally to HCT116 tumor bearing mice at a dose of 50 mg/kg for 7 days.
- Lectin is injected into the tail vein of HCT116 tumor bearing mice at different time points. The HCT116 tumor tissue was collected 30 min later.
- SB939 reduced the positive staining of lectin perfused blood vessels in HCT116 tumors.

Figure 8: Anti-tumor activity of SB939 in a mouse spontaneous colon cancer model, Apc^{min/+} Mice



- SB939 was administered at 40 mg/kg daily by intraperitoneal injection for 5 consecutive days followed by 9 days of rest for two cycles.
- 5-FU was administered at 40 mg/kg daily by intraperitoneal injection for 5 consecutive days followed by 9 days of rest for two cycles.
- SB939 significantly reduced tumor size and tumor number in both the small intestine and colon (46% and 61% at 50 mg/kg SB939; 53% and 67% at 75 mg/kg SB939 respectively) and was well tolerated.
- 5-FU significantly reduced tumor size and number in the small intestine by 39% but had no significant effect on lesions in the colon.



- SB939 but not 5-FU significantly increased hemocrit and reduced the number of immature erythrocytes (Ter-119⁺) in the spleen.

SUMMARY

- HDACs are over-expressed in colon cancer cell lines and knockdown of selective HDAC isoforms using siRNA leads to apoptotic cell death (Figure 1).
- SB939 potentially inhibits a broad range of class I and class II HDACs but is less potent against HDAC6 and HDAC7 (Figure 2).
- SB939 inhibits cellular HDAC enzyme activity, resulting in induction of acetylation of histone3 and inhibition of proliferation in colon cancer cell lines (Figure 3).
- SB939, in a dose dependent manner, arrests Colo205 at G₀/G₁ phase, HCT116 and SW620 at G₂/M phase, and increases apoptotic cell death at subG₀ phase (Figure 3).
- SB939 induces acetylation of histone3 and alpha tubulin, increases expression of the cyclin dependent kinase inhibitor and pro-apoptotic protein, p21^{Cip1/WAF1} in HCT116 colon cancer cells (Figure 4).
- SB939 induces H3 acetylation in HCT116 tumor tissues at 1 h after administration of an acute oral dose (50 mg/kg). The duration of this response persists for up to 24 h and is longer than that induced by SAHA at 200 mg/kg (Figure 5).
- SB939 induces a dose-dependent inhibition of tumor growth in a model of advanced human colorectal cancer (HCT116) (Figure 6).
- SB939 reduces the formation of blood vessels in HCT116 tumors (Figure 7).
- SB939 reduces tumor size and tumor count, and reduces progression of anemia in Apc^{min/+} mice, a model of early stage colorectal cancer (Figure 8).

CONCLUSION

SB939 is an effective anti-tumor drug in both *in vitro* and *in vivo* colon cancer models and has the potential to be an effective oral therapy for early and late stage colorectal cancer. SB939 is currently being evaluated in phase 1 clinical trials.

REFERENCES

¹B.H. Huang, M. Laban, C. Leung et al. (2005) Cell Death Differ 12; 395-404
²K. Sangthongpitag, H.S. Wang, P. Yeo, et al. (2006) EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics Prague Congress Centre, Prague, Czech Republic, Abstract number 166