



A robust and quantitative biomarker assay for SB939, a potent, orally-active HDAC inhibitor

Veronica Novotny-Diermayr, Vasantha M. Nayagam, Nina Sausgruber, Ai Leng Liang, Loh Yung Kiang, Hannes Hentze, Ramesh Jayaraman, Pauline Yeo, Kantharaj Ethirajulu and Jeanette Wood

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S*Bio Pte Ltd, 1 Science Park Rd, #05-09 The Capricorn, Science Park II, SINGAPORE 117528, Tel: +65/6827 5000, www.sbio.com

Background:

- Histone deacetylase inhibitors (HDACi) are therapeutic agents, which induce tumor cell cytosolysis, differentiation and apoptosis.
- SB939 is a pan-HDACi with superior pharmaceutical and pharmacokinetic properties that is currently in Phase I trials. For a synopsis see poster #136 (H.S. Wang).
- We have developed a sensitive and quantitative Western blot assay for acetylated histone 3 (acH3) as a pharmacodynamic (PD) readout for the target efficacy of SB939.
- This assay was used to demonstrate target inhibition in animal cancer models and in PBMCs of solid tumor patients in a Phase I clinical trial.

Materials and Methods:

- To validate the biomarker assay RAMOS or HCT-116 cells were purchased from ATCC and cultivated according to the supplier's instructions.
- PBMCs to establish the assays were isolated from healthy donors using CPT tubes. After washing with PBS they were cultivated in RPMI with 10% FBS. (Treatment after 2h of resting)
- Cells were lysed in modified RIPA, containing a HAT-inhibitor (Garcinol; final conc. 10 μ M) and protease inhibitors.
- 25 μ g lysate per sample were separated on 15% SDS-PAGE gels, followed by transfer to a PVDF membrane.
- Membranes were cut in two halves, the top part probed for actin (Sigma), the bottom part probed for acH3 (Cell Signaling K9/K14 acH3), both at 1:1000 for 1.5h at RT, followed by 1h anti-Rb-HRP secondary Ab (CST) at 1:1000 and developing using ECL (GE-Lifescience).

Biomarker Validation:

Fig. 1A: SB939 can be detected at 0.125 μ M in PBMCs treated ex-vivo

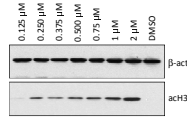


Fig. 1B: A minimum of 1.6 μ g of total protein is required to detect acH3 in RAMOS cell lysate

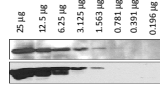
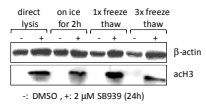


Fig. 1C: This assay is very robust (here shown using HCT-116 cell lysate)



Preclinical Validation:

Fig. 2A: Experimental protocol of animal studies in nude mice

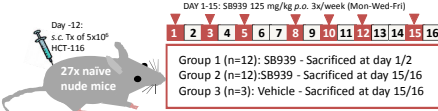
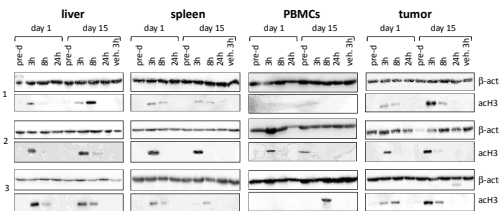


Fig. 2B: Western blot analysis of different tissues in 3 sets of tumor bearing nude mice



- Images were digitally captured with a LAS-3000 (Fujifilm) for 100s (actin) or 2000s (acH3).
- Densitometric analysis was performed using MultiGauge software (Fujifilm). AcH3 values were normalized by actin values and equalized using the acH3/actin ratio of the positive control. The positive control used is 25 μ g cell lysate from RAMOS cells, treated for 24h with 2 μ M SB939.
- Animal studies: 10 – 12w old female, athymic nude mice were inoculated subcutaneously with 5x10⁶ HCT-116 colon cancer cells. Tumors grew for 12d to an average size of 200 mm³. Nude mice and naive Balb/c mice were fed p.o. 125 mg/kg 3x weekly (following the clinical schedule).
- Tumor size was determined by caliper measurements and tumor growth inhibition (TGI) was calculated according to %TGI = 100*(C_T)/(C_C - C_T), using the mean tumor volumes. All statistical analyses were performed using Prism Graphpad.
- Plasma levels of SB939 in the clinical samples were estimated in a CR0 (MPI Research, USA). A synopsis of the Phase I dosing regimen and the clinical protocol can be found in poster #413 (W.P. Yong).

Fig. 2C: Densitometric analysis of acH3 levels in different tissues of HCT-116 xenografted nude mice

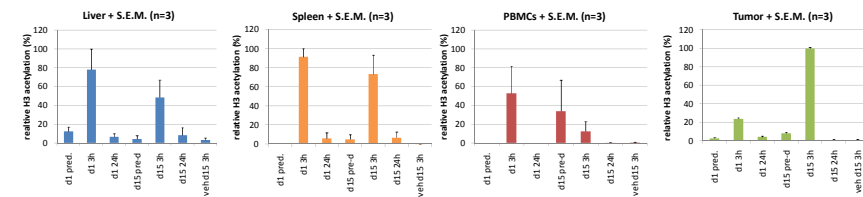


Fig. 3: Dose response curve for SB939 in different tissues of naive (Balb/c) mice, 3h after dosing on d1

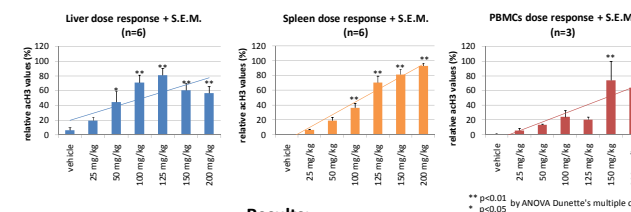
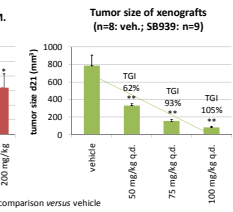


Fig. 4: Efficacy of SB939 in HCT-116 xenografts



Results:

Biomarker Validation:

- AcH3 could be detected after ex-vivo treatment of tumor cell lines or PBMCs with SB939 for 24h. The lowest concentration of SB939 yielding a detectable signal was 60 nM for RAMOS cells and 125 nM (44 ng/ml) for PBMCs (Fig. 1A).
- The minimum amount of protein needed to detect acH3 in cells was 1.56 μ g (Fig. 1B).
- The assay gives reproducible signals, even after freeze-thawing the lysate, and is very robust (Fig. 1C).

Preclinical Validation:

- In the animal study orally dosed SB939 led to a strong induction of acH3 (peak at 3h, d1 and d15) in all tissues with no basal level detected on d1 pre-dose.
- In general the acH3 was less on d15 than on d1, except for tumor tissue, indicating selectivity of SB939 for tumor tissue after repeated dosing (Fig. 2). The plasma levels of SB939 observed correlated well with the acH3 peaks (data not shown).
- The acH3 signal increased dose dependently in all tissues (Fig. 3). This also correlated with the dose dependency and the anti-tumor efficacy in HCT-116 xenografts (Fig. 4).

Clinical Biomarker Analysis:

- In the solid tumor patients of the Phase I trial, the relative acH3 values measured in PBMCs increased dose dependently from 10 mg to 60 mg (Figs. 5 & 7).
- At the recommended dose (60 mg) the highest acH3 response was at the 3h measurement point on d1 and d15 (Figs. 5 & 6).
- The dose-dependent increases in acH3 followed the increased plasma levels of SB939 (Fig. 7).

CONCLUSION:

SB939 shows superior dose-dependent exposure and target inhibition in animal tumor models that translates to solid tumor patients in a Phase I clinical trial.

Clinical Biomarker Analysis:

Fig. 5: PD analysis for acH3 levels (densitometric analysis) in PBMCs from solid tumor patients

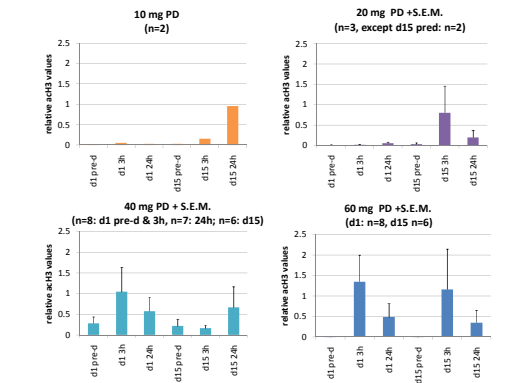
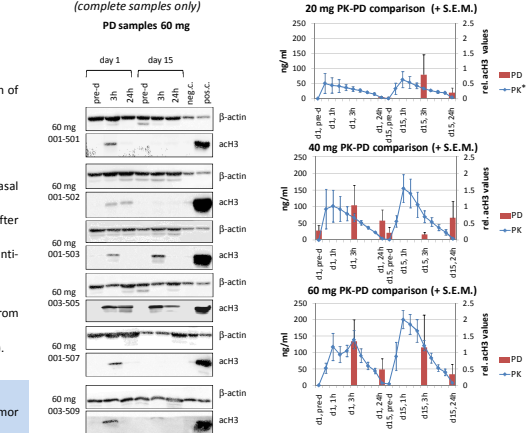


Fig. 6: Western blots from 60 mg cohort



* Time points for PK were: pre-d, 0.5h, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h and 24h on d1 and d15