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The histone deacetylase inhibitor SB939 acts synergistically with Sorafenib in an orthotopic model of hepatocellular carcinoma

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Introduction:

SB939 is an hydroxamic acid based class I, II and IV HDAC inhibitor, with very favourable physicochemical, pharmaceutical and pharmacokinetic properties, resulting in an excellent bioavailability and marked accumulation in tumor tissue. In clinical phase I studies with SB939 in solid tumors, drug exposure was demonstrated to be higher and side effects milder/fewer compared to first generation HDAC inhibitors such as SAHA. Only 6.7% Grade 3/4 fatigue (2/30 patients) or 6.9% (2/29 patients) were observed in two independent Phase I clinical studies making SB939 ideally suited for combination therapies. The aim of the present studies was to explore the potential of SB939 for the treatment of hepatocellular carcinoma (HCC).

Methods:

- Cell proliferation assays: YO-PRO-1 or CellTiter-Glo assay
- Mice: Female C.B-17-*Igh1b-Prkdc*^{scid}, 9-16 weeks
- Animal model: Hep3B, orthotopic liver xenograft (performed by VivoPharm)
- Vehicles: Sorafenib tablets were crushed and formulated in NNP-PEG300 (1:9); SB939 was dissolved in 0.5% MC, 0.1% Tween-80
- Doses: Sorafenib 10 or 45 mg/kg *p.o.* once daily (*q.d.*). SB939 125 mg/kg *p.o.* every second day (*q.o.d.*).
- Combination schedule: Sorafenib was dosed first, followed by SB939 6 h later
- PK and PD analysis was performed as described previously (*Novotny-Diermayr et al. Mol Cancer Ther; 9(3) 2010, p 642 ff*)
- The Ariol®Automated Image Analyzer was used to analyze immuno-histochemistry slides

Results:

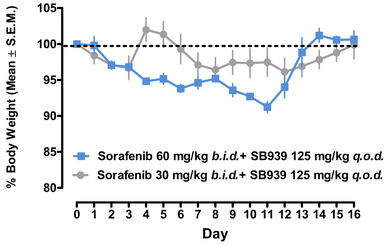
***In vitro* proliferation assay:**

- Hep3B cells do not have a B-Raf mutation and were relatively resistant to anti-proliferative effects of SB939 or Sorafenib (IC_{50} of 1.23 µM and 3.66 µM for SB939 and Sorafenib respectively)

MTD (Fig. 1):

- In non-tumor bearing mice the combination of Sorafenib (30 or 60 mg/kg *q.d.*) and SB939 (125 mg/kg *q.o.d.*) was well tolerated (max. BW loss of 3.6% and 8.7% respectively) (**Fig. 1**), but less well tolerated in tumor bearing mice. The dose of SB939 was reduced to 100 mg/kg *q.o.d* from days 14-20 in the efficacy study **(Figs 2 &3)**

Fig. 1: % Body weight during the MTD study in SCID mice



Anti-tumor Efficacy (Fig. 2 & 3) :

- In an orthotopic Hep3B xenograft model, the 10 mg/kg dose of Sorafenib was not efficacious on total liver weight (change in size + 0.7%). SB939 single agent treatment decreased diseased liver weight by 26 % and together with 10 mg/kg Sorafenib, a further decrease up to 37% was observed. Analysis using Clarke's Synergism calculation showed that there was a synergistic effect (CCI = -0.118).
- The high dose of Sorafenib had a slightly greater effect (40%) and this was further enhanced in combination with SB939 (54%) but the difference between the groups was not statistically significant.
- Similar effects were observed based on excised tumor wets weights but only the effects of the high dose Sorafenib alone and in combination with SB939 were statistically significant. H&E staining revealed that there was a lot of liver tissue excised together with the tumor.

Fig. 2: % Inhibition of diseased Liver- and Tumor weight

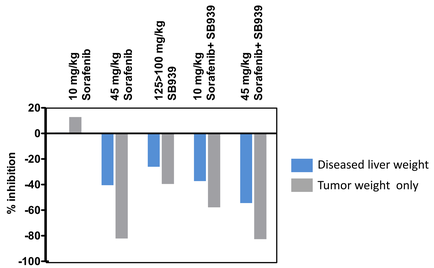
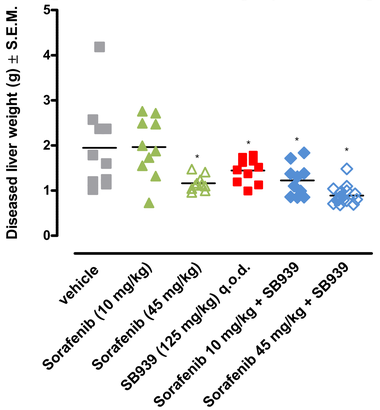


Fig. 3: Mean diseased Liver weights per treatment group



Pharmacokinetics (Fig. 4, Table 1):

- The plasma AUC of Sorafenib and SB939 on d21 were similar to published values for both compounds after chronic dosing. The AUC was higher on d1 which may be due to the diseased liver since both drugs are mainly metabolised by the liver (CYP3A4). The lower AUC on d21 compared to d1 was observed with both drugs and may have been due to an improvement in liver function after the large reduction in tumor burden induced by the therapy. The lower AUC of SB939 on d21 was also due to the dose reduction.
- The PK profiles of Sorafenib and SB939 were not affected by co-administration

Fig.4: PK profiles for SB939 and Sorafenib on d1 and d21 after co-administration

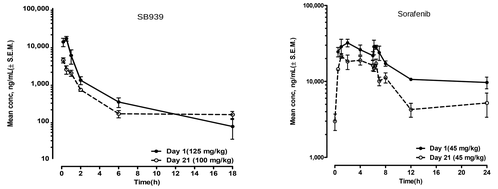


Table 1: PK parameters for SB939 and Sorafenib on d1 and d21 after co-administration

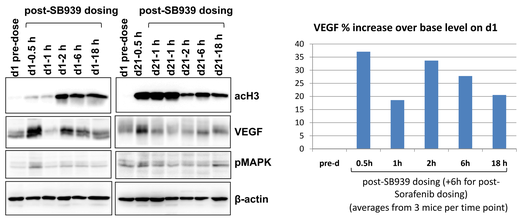
PK Parameter	SB939 Day 1	125 mg/kg Day 21*	Sorafenib Day 1	45 mg/kg Day 21
T_{max} (h)	0.5	0.17	2	1
C_{max} (µg/mL)	16	4	32	22
T_{last} (h)	18	18	24	24
C_{last} (ng/mL)	72	99	9697	5207
AUC_{last} (h*µg/mL)	18	7	380	217

*SB939 dose was reduced from 125 to 100 mg/kg *q.o.d.* from day 14 to 21

Biomarkers of Target Efficacy and Tumor Response: (Fig. 5)

- Acetylation of histone H3 (acH3) was induced in tumor tissue by SB939 treatment and remained upregulated for up to 18 h, indicating effective target inhibition by SB939.
- pMAPK was not inhibited in tumor tissue after treatment with the highest dose of Sorafenib, indicating no effect on the Raf signalling pathway.
- Tumor VEGF was increased compared to base levels 6.5 h after Sorafenib treatment, probably as a consequence of its anti-angiogenic effects (known effect of VEGF receptor tyrosine kinase inhibitors as a response to reduced tumor perfusion and increased hypoxia). The increase in VEGF was reduced to some extent by the addition of SB939.

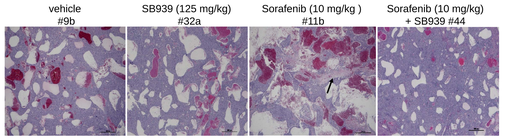
Fig. 5: Western blot analysis of tumor lysates treated with 45 mg/kg Sorafenib and 125 mg/kg SB939



Histological Analysis (Fig. 6 & 7):

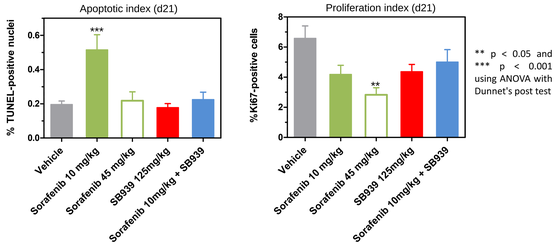
- Histological analysis of the tumor tissue at the end of the experiment revealed that both doses of Sorafenib, including the dose that did not reduce diseased liver weight, decreased the proliferation index (Ki67) but only 10 mg/kg Sorafenib increased apoptosis (TUNEL). The most striking effect seen with both doses of Sorafenib was an increase in necrosis and fibrosis.

Fig. 6: Representative pictures from H&E stained tumor sections on d21



- Interestingly, after treatment with SB939 alone or addition of SB939 to the low dose of Sorafenib (high dose Sorafenib + SB939 not analyzed) no necrosis or fibrosis could be detected, despite the significant reduction in diseased liver weight.
- SB939 alone or in combination slightly reduced the proliferation index at an effective anti-tumor dose (**Fig. 7**), but did not increase apoptosis, and in combination therapy, reduced the apoptotic effects of Sorafenib.
- The effects of SB939 on tumor morphology are still under investigation, but preliminary analysis suggests that SB939 leads to increased defects in mitotic spindle formation

Fig. 7: Automated analysis of apoptotic- and proliferation index in Hep3B tumors on d21



Conclusions:

- SB939 is effective as a single agent therapy, despite Hep3B cells being resistant to it *in vitro*
- At 10 mg/kg Sorafenib did not reduce tumor size but induced marked necrosis and fibrosis in the tumor tissue, which could be completely blocked in combination with SB939.
- The ineffective dose of 10 mg/kg Sorafenib showed synergistic anti-tumor activity in combination with SB939
- The mechanism of action for the combination of SB939 with Sorafenib warrants further investigation and may provide a rationale for a Phase II trial for a combination of SB939 with VEGF receptor tyrosine kinase inhibitors such as Sorafenib in HCC.