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


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Introduction: SB939 is a hydrophilic acyl based class I and II IV HDAC inhibitor, with very favourable physicochemical, pharmacological and pharmacokinetic properties, resulting in an excellent bioavailability and moderated accumulation in tumor tissue. In clinical phase I studies with SB939 in solid tumors, drug exposure was demonstrated to be higher and safer effects (mild-to-moderate) compared to free generation (HD) inhibitors such as SAHA. Only 6.7% Grade 3/4 fatigue (13/19 patients) or 6.0% (2/20 patients) were observed in two independent Phase I clinical trials making SB939 study suited for combination therapies. The aim of the present study was to explore the potential of SB939 for the treatment of hepatocellular carcinoma (HCC).

Methods:
• Cell proliferation assays: 70-FLO-1 or CellTiter-Blue assay
• Ehrlich Tumoral CE-1 (Ct) subcutaneous tumor model.
• Animal model: Hepatitis induced liver cirrhosis performed by Gsh2Depr
• Vehicles: Sorafenib tablets were crushed and formulated in HEPES-PBS (1:10). SB939 was dissolved in 0.1% DMSO, 0.1% Tween 80
• Dose: Sorafenib 10 or 45 mg/kg. p.o. once daily (q.d.) or SB939 12.5 mg/kg. p.o. every second day (bid).
• Combination schedule: Sorafenib was dosed first, followed by SB939 3-4 h later.
• PK and PK analysis was performed as described previously (Novotny-Diermayr et al. Mol Cancer Ther; 13(10), 2014, 642-2).
• The Acrid/Aurometer Automated Analyzer was used to analyze immunochemistry slides

Results:
In vitro proliferation assays
• Hep3B cells do not have a Bcl-2 mutation and were relatively resistant to anti-proliferative effects of Sorafenib or SB939 (IC50 of 2.13 μM and 1.66 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 25 mg/kg bid) was well tolerated (mean B/W 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.d. from days 15-30 the efficacy study (Fig. 2 B&I).

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

Anti-tumor Efficacy (Fig. 2 & 3):
• In an orthotopic Hepatitis CE-1 model, the 20 mg/kg dose of Sorafenib was not effective on tumor liver weight (change in % vs. p.o.). SB939 single agent treatment decreased tumor liver weight by 28 % and together with 10 mg/kg Sorafenib, a further decrease up to 37% was observed. Analysis using Cochran’s Q-Summary calculation showed that there was a synergistic effect (χ2 = 0.116).
• The high dose of Sorafenib had a slightly greater effect (49%) and this was further enhanced in combination with SB939 (56%) but there were some differences between the groups that was statistically insignificant.
• Similar effects were observed based on excess tumor eaten 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 re-accumulated together with the tumor.

Fig. 2: Inhibition of diseased Liver and Tumor weight

Table 1: PK parameters for SB939 and Sorafenib on day 1 and day 22 after co-administration

Pharmacokinetics (Fig. 4, Table 1): The plasma AUC of SB939 and Sorafenib on day 1 were similar in published values for both compounds after chronic dosing. The AUC was higher on day 22 due to the disease liver increase and the mean accumulation factor from day 1 (MCAF). The mean AUC on day 21 compared to day 1 was observed with both drugs and may have been due to an improvement in liver function and increase in drug accumulation in liver and gut by the therapy. The lower AUC of SB939 on day 22 was also due to the dose reduction.

Fig. 4: PK profiles for SB939 and Sorafenib on day 1 and day 22 after co-administration

The proliferation index in Hep3B tumors on day 21 reduced to 60% of the control (Fig. 5).

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

Biomarkers of Target Efficacy and Tumor Response (Fig. 5):
• Accumulated beta tubulin III (tub III) was reduced in tumor tissue by SB939 treatment and remained upregulated for up to 18 h, indicating effective target inhibition by SB939.
• pJunA was not retained in tumor tissue after treatment with the highest dose of Sorafenib, indicating no effect on the Raf signaling pathway.
• Tumor VEGF was increased compared to base line 6.5 h after Sorafenib treatment, probably as a consequence of its anti-angiogenic effects (inhibition of VEGFR1 and VEGFR2 for further increasing the 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. 6: Representative picture from H&E stained tumor sections on day 21

• Interestingly, after treatment with 1803 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. 5), but did not increase apoptosis, and in combination therapy, reduced the apoptosis rate (Figs. 4 & 5).
• 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 day 21

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 this 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 successful combination.

Fig. 8: Representative picture from H&E stained tumor sections on day 21
• 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 (H&E) but not (10 mg/kg Sorafenib increased apoptosis (%)). The most striking effect seen with both doses of Sorafenib was an increase in necrosis and fibrosis.

Fig. 9: Western blot analysis of tumor lysates treated with 45 μg/ml SB939 and 125 μg/mg SB939