The potential of spermine analogue SBP-101 (diethyl dihydroxyhomospermine) as a polyamine metabolism modulator in ovarian cancer

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The naturally occurring polyamines, putrescine, spermidine and spermine, are polycationic alkylamines that are essential for cellular growth and proliferation. As such, many cancers are reliant on elevated polyamine levels that are maintained through dysregulated polyamine metabolism. Polyamine metabolism is thus a promising target for cancer therapeutics, and modulation of polyamine metabolism has been attempted with numerous enzyme inhibitors and polyamine analogues. SBP-101 (diethyl dihydroxyhomospermine) is a novel spermine analogue that has shown efficacy in slowing pancreatic tumor progression both *in vitro* and *in vivo*.

Here we determined the effect of SBP-101 treatment on polyamine metabolism in a variety of cancer cell types *in vitro* including lung, ovarian, prostate, pancreatic and breast. We evaluated the activity of four enzymes involved in the polyamine pathway following treatment with either SBP-101 or the well-characterized spermine analogue, BENSpm (N^1,N^{11}-bisethylnorspermine). Additionally, we determined by high performance liquid chromatography the effect of SBP-101 on intracellular polyamine pools and the accumulation of the analogue itself. The activity of the biosynthetic enzymes ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMdc) and the catabolic enzymes spermidine/spermine-N-(1)-acetyltransferase (SSAT) and spermine oxidase (SMOX) were determined with and without treatment with the polyamine analogues. SBP-101 treatment resulted in a varying increase in the activity of polyamine catabolic enzymes in a subset of tested cell lines, while it downregulated the activity of the biosynthetic enzyme ODC across all cell types studied. These results indicate that SBP-101 likely exerts its effects predominately through decreased polyamine biosynthesis with minor upregulation of catabolism in contrast to the structurally similar BENSpm where the increase in polyamine catabolism is the predominant response.

A sustained elevation of polyamine levels plays a role in the immunosuppressive environment of some cold tumors, and the pharmacologic and genetic modulation of polyamine metabolism have demonstrated success in reducing immunosuppressive phenotypes. Therefore, to extend our *in vitro* results, we evaluated the efficacy of SBP-101 in the immunosuppressive VDID8$^+$ murine ovarian cancer model. SBP-101 caused a marked increase in median survival comparable to that of some promising combination therapies. Future studies will determine the synergistic effects, if any, of SBP-101 in combination with other polyamine metabolism modulators as well as with immune modulators.

Results

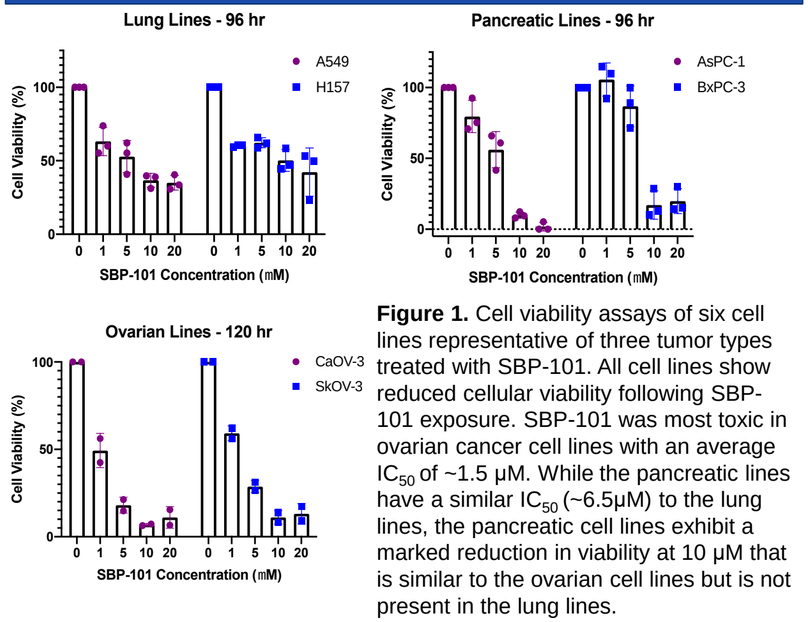

Figure 1. Cell viability assays of six cell lines representative of three tumor types treated with SBP-101. All cell lines show reduced cellular viability following SBP-101 exposure. SBP-101 was most toxic in ovarian cancer cell lines with an average IC_{50} of ~1.5 μM. While the pancreatic lines have a similar IC_{50} (~6.5μM) to the lung lines, the pancreatic cell lines exhibit a marked reduction in viability at 10 μM that is similar to the ovarian cell lines but is not present in the lung lines.

Results

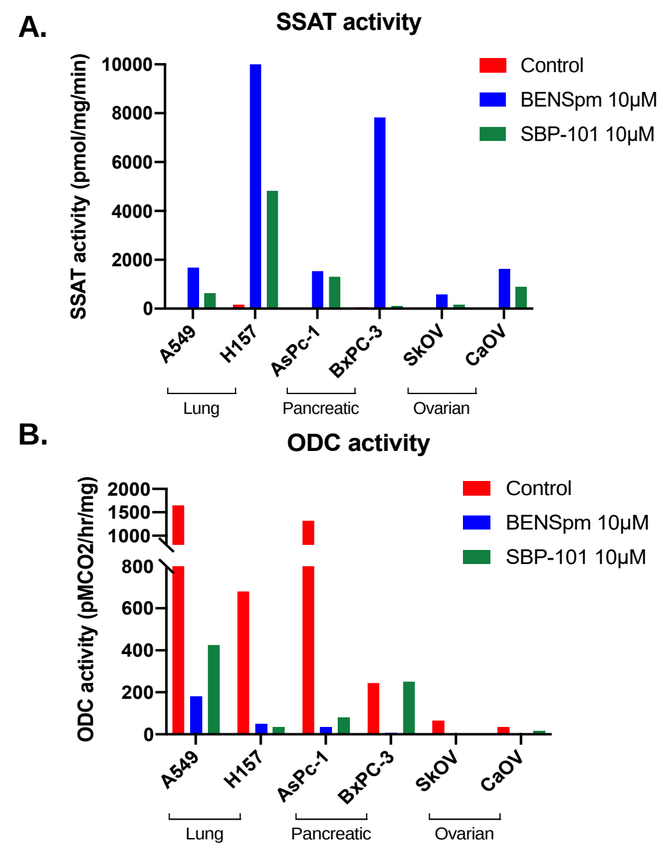

Figure 2. Polyamine enzymatic activity following treatment with either BENSpm or SBP-101. **(A)** SBP-101 treatment upregulates the catabolic enzyme SSAT similarly, but not as extensively as BENSpm. **(B)** SBP-101 treatment downregulates ODC, a rate-limiting biosynthetic enzyme, in all tested cell lines apart from BxPC-3. The downregulation is comparable to BENSpm.

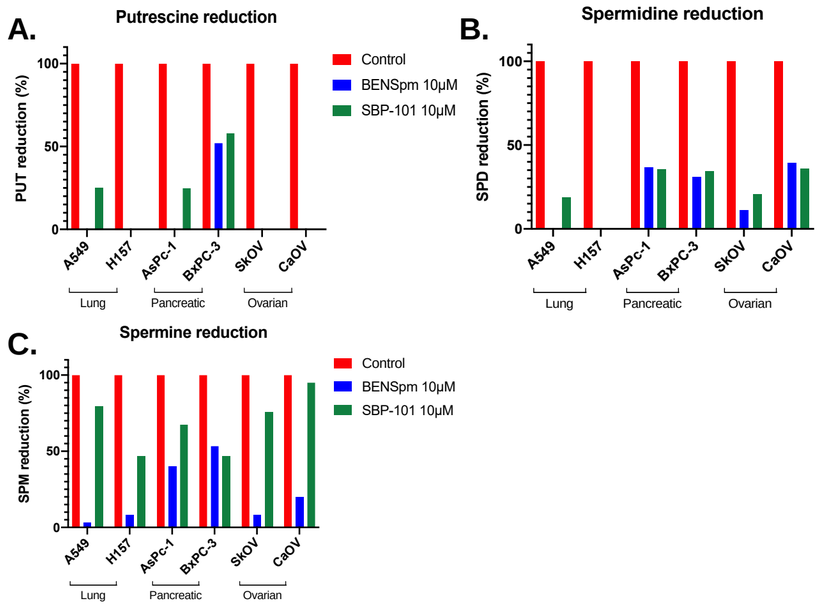

Figure 3. HPLC analysis of intracellular polyamine content following treatment with a polyamine analogue. **(A,B)** Treatment with SBP-101 reduced intracellular putrescine and spermidine content comparably to BENSpm whereas SBP-101 had a less marked effect on intracellular spermine concentrations **(C)**.

Results

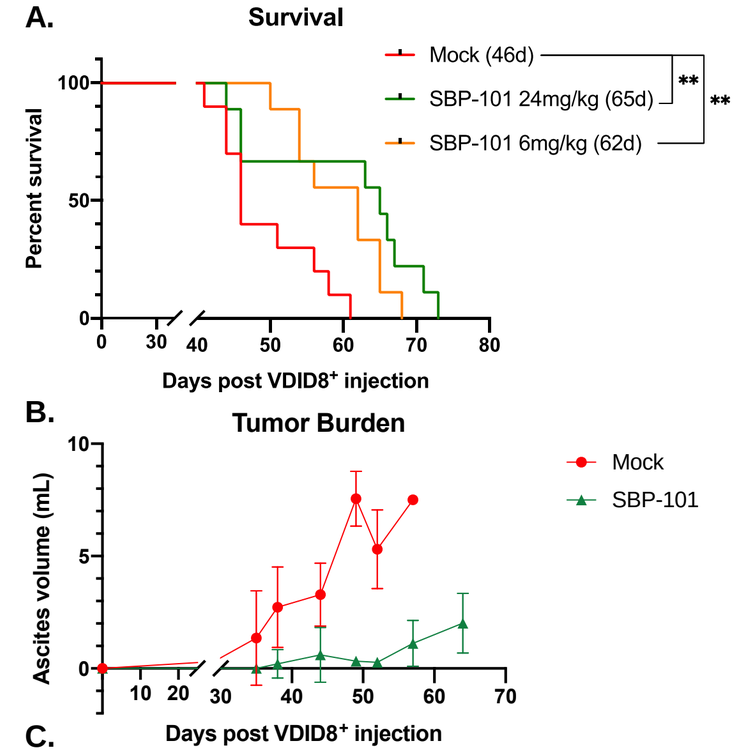

Figure 4. Efficacy of SBP-101 in ovarian murine model. Utilizing the VDID8$^+$ murine ovarian cancer model (ID8$^+$ C57Bl/6 ovarian cells overexpressing both VEGF and Defensin) we treated mice with SBP-101 at either 24 mg/kg or 6 mg/kg alternating MWF. **(A)** Both doses of SBP-101 produced a statistically significant prolongation of survival (24mg/kg p=.0049, 6 mg/kg p=.0042). There was no significant difference in response between the two SBP-101 doses. **(B)** The prolonged survival was correlated with a delay in the production of ascites, the indication of tumor burden in this model. Additionally, when SBP-101 treated mice succumbed to the disease, their overall tumor burden was lower when compared to control mice. **(C).** HPLC analysis of organs following euthanasia showed an accumulation of SBP-101 predominately in the kidneys and the liver.

Conclusions

The polyamine analogue, SBP-101, modulates polyamine metabolism across a variety of cancer cell types *in vitro* and significantly prolongs the survival of an immunosuppressive ovarian murine model *in vivo.* SBP-101 produces a modest upregulation of polyamine catabolism, however it appears that the majority of SBP-101's effects are through downregulation of polyamine biosynthesis. Treatment of C57Bl/6 mice injected with VDID8$^+$ ovarian cancer with SBP-101 significantly prolongs survival and decreases overall tumor burden. Future studies will evaluate the effects of SBP-101 in combination with other polyamine metabolism modulators as well as with immune modulators.