Evaluating the efficacy of spermine analogue ivospemin (SBP-101) in combination with chemotherapy in ovarian cancer

Cassandra E. Holbert[1], Jackson R. Foley[1], Michael J. Walker[2], Elizabeth M. Bruckheimer[2], Jennifer K. Simpson[2], Tracy Murray Stewart[1], and Robert A. Casero, Jr.[1]

[1]Johns Hopkins Medicine, Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland

[2]Panbela Therapeutics, Inc., Waconia, Minnesota

Abstract #4944

Polyamines are small cationic alkylamines that play critical roles in essential cellular processes governing growth and proliferation. As such, cancers are fully reliant on increased polyamine pools maintained through dysregulation of polyamine metabolism. Pharmaceutical modulation of polyamine metabolism is a promising avenue in cancer therapeutics and has been attempted with enzyme inhibitors, including DFMO (difluoromethylornithine), and polyamine analogues. Ivospemin (SBP-101) is a spermine analogue that has shown efficacy in slowing pancreatic and ovarian tumor progression both *in vitro* and *in vivo* and demonstrated encouraging results in pancreatic cancer clinical trials.

We have shown that ivospemin decreases polyamine content through depression of the activity of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) in a variety of cancer cell lines [1]. Treatment of the VDID8$^+$ murine ovarian cancer model with ivospemin resulted in a marked increase in survival [1]. Here we examine the potential of combining ivospemin and chemotherapeutic agents that are used to treat platinum-resistant ovarian cancer. Treatment with gemcitabine, topotecan, and doxorubicin increased the *in vitro* toxicity of ivospemin, while paclitaxel and docetaxel did not have any added benefit over ivospemin alone. Using the VDID8$^+$ model, we further evaluated the efficacy of ivospemin in combination with gemcitabine, topotecan, and doxorubicin *in vivo*. Ascites fluid was used as a marker of tumor burden and evaluated for polyamine content. Addition of ivospemin improved the survival of mice treated with any of the three chemotherapeutics. The ivospemin and doxorubicin combination mice had the greatest median survival time; this combination is being further evaluated in mechanistic studies and additional murine studies. This combination was further evaluated with a reduced dose of doxorubicin and maintained benefit over doxorubicin or ivospemin alone.

Ovarian cancers have extremely immunosuppressive tumor microenvironments (TME) and metabolic reprogramming of the TME to reduce immunosuppressive phenotypes is a promising approach for treatment. Sustained elevation of polyamine levels supports an immunosuppressive TME, and evidence suggests that pharmacologic depletion of polyamines may reduce immunosuppressive phenotypes. DFMO treatment in the immunosuppressive VDID8$^+$ model influences the immune cells of the TME, and we therefore are investigating the combination of ivospemin and DFMO in ovarian cancer. In addition to the cooperativity of ivospemin and chemotherapeutic agents, we have observed a cooperative antiproliferative response in ovarian cancer cells following DFMO and ivospemin cotreatment. Together, these studies suggest the potential of polyamine modulation by ivospemin and DFMO in combination with standard of care chemotherapy. Future studies will determine influences on the immune microenvironment and will evaluate cooperativity between ivospemin, DFMO, and chemotherapy.

[1] Holbert CE, *et al.* Int J Mol Sci. 2022. 23(**12**): 6798.

Polyamine Metabolism

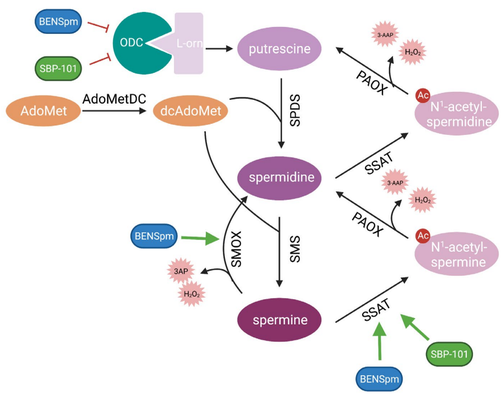

Figure 1. Polyamine metabolic pathways. The polyamine precursor L-ornithine is converted to putrescine (PUT) by the biosynthetic enzyme ornithine decarboxylase (ODC). PUT is converted to spermidine (SPD) and then spermine (SPM) by spermidine synthase (SPDS) and spermine synthase (SMS) respectively. This utilizes decarboxylated S-adenosyl-L-methionine (dcAdoMet) produced by S-adenosylmethionine decarboxylase (AdoMetDC). SPM can be directly catabolized to SPD by spermine oxidase (SMOX) with the production of 3-aminopropanal (3-AP) and hydrogen peroxide as byproducts. Both SPM and SPD can be back-converted to their predecessors by SSAT utilizing an acetylated intermediate with hydrogen peroxide and 3-acetylaminopropnaal (3-AAP) as byproducts.

Results

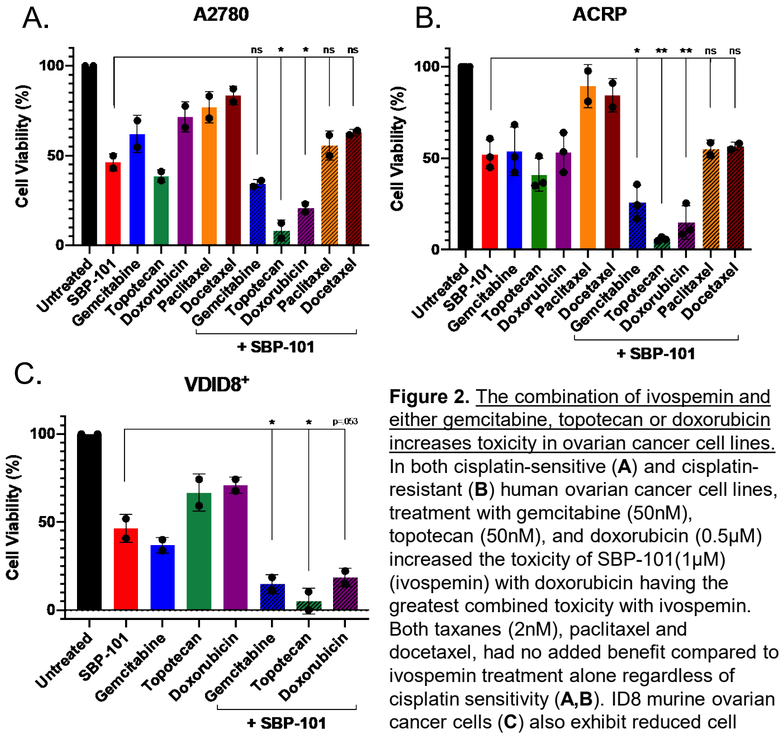

Figure 2. The combination of ivospemin and either gemcitabine, topotecan or doxorubicin increases toxicity in ovarian cancer cell lines. In both cisplatin-sensitive (**A**) and cisplatin-resistant (**B**) human ovarian cancer cell lines, treatment with gemcitabine (50nM), topotecan (50nM), and doxorubicin (0.5μM) increased the toxicity of SBP-101(1μM) (ivospemin) with doxorubicin having the greatest combined toxicity with ivospemin. Both taxanes (2nM), paclitaxel and docetaxel, had no added benefit compared to ivospemin treatment alone regardless of cisplatin sensitivity (**A,B**). ID8 murine ovarian cancer cells (**C**) also exhibit reduced cell viability when cotreated with SBP-101 and either gemcitabine, topotecan or doxorubicin.

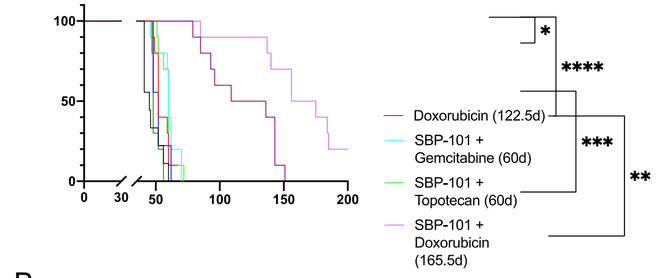

Figure 3. Ivospemin (SBP-101) increases survival and decreases tumor burden in doxorubicin-treated mice in the VDID8$^+$ C57Bl6 model of ovarian cancer. Mice were injected with 350,000 VEGF+, Defensin+ ID8 ovarian cancer cells on day 0. Treatment with all four drugs began on day 3 at the following doses: ivospemin (24 mg/kg 2qw, alternating weeks); gemcitabine (30 mg/kg qwx4); topotecan (1 mg/kg 3qwx4); doxorubicin (1 mg/kg 3qwx4) **(A)**. All doses are clinically relevant. SBP-101 and doxorubicin were the only single agents with a statistically significant survival advantage over control mice. While doxorubicin treatment more than doubled the median lifespan of mock mice, the addition of SBP-101 still resulted in a 35% increase in median lifespan over doxorubicin treatment alone (p-value: 0.0026). Addition of SBP-101 to the chemotherapies also decreased overall tumor burden **(B)** as shown by lower ascites volume at time of second drain. This decrease is statistically significant when compared to the chemotherapy alone for all three treatments with p-values = <0.01

Results

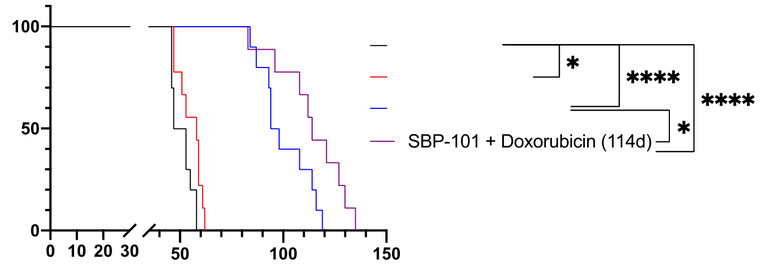

Figure 4. Subclinical dosing of doxorubicin in combination with ivospemin is sufficient to increase survival of VDID8$^+$-injected C57Bl6 female mice. SBP-101 treatment was completed 2qw on alternating weeks at a dose of 24 mg/kg for a total of 8 treatments (4 treatment weeks). Doxorubicin treatment was completed 2qw every week for four weeks (8 total treatments) at a dose of 0.5 mg/kg. The doxorubicin dosing in this experiment represents a 66% reduction in total doxorubicin exposure compared to Figure 3. Even with the sub-clinical dose of doxorubicin (66% reduction in total doxorubicin exposure), the addition of SBP-101 resulted in a 20% increase in median lifespan.

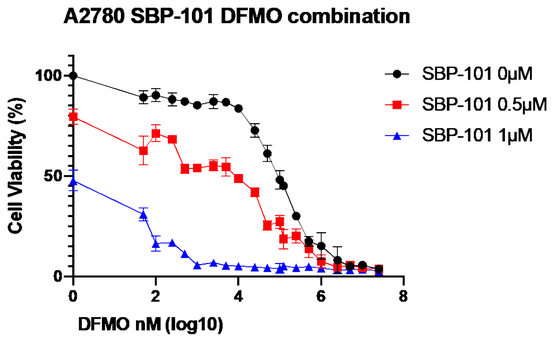

Figure 5. The addition of the ODC inhibitor, difluoromethylornithine (DFMO), to SBP-101 results in a cooperative antiproliferative response in A2780 ovarian cancer cells. Cells were treated with DFMO for 48 hours prior to SBP-101 addition. The 95% CI for IC_{50}s values for treatment are [7 – 11 μM] for DFMO alone, [0.15 – 13 μM] for DFMO + 0.5μM SBP-101, and [11 – 14 nM] for DFMO + 1μM SBP-101.

Conclusions

Ivospemin (SBP-101), an analogue of the naturally occurring polyamine spermine, suppresses the growth of ovarian cancer both *in vitro* and *in vivo* through modulation of polyamine metabolism. The addition of ivospemin to chemotherapeutics gemcitabine, topotecan, and doxorubicin increases their toxicity *in vitro* regardless of the tumor cell's cisplatin sensitivity. The combination of ivospemin and doxorubicin significantly increases overall survival in an immunosuppressive murine ovarian cancer model even at sub-clinical doxorubicin dosing levels. There is a significant unmet need in the treatment of women with platinum-resistant ovarian cancer, and the results presented here suggest that the combination of doxorubicin and ivospemin may be useful in the treatment of these patients. Future studies will evaluate other molecular pathways influenced by the combination treatment to help determine other potential combinatorial strategies. Additionally, combining DFMO with ivospemin *in vitro* results in a cooperative antiproliferative response. DFMO is notably well-tolerated and can influence immune cells to promote a more immune-friendly tumor microenvironment. Future experiments will evaluate the effect of adding DFMO to ivospemin treatment as well as the influence on immune cells within the tumor microenvironment.

Work in the Casero and Stewart laboratory is supported by grants from the US National Institutes of Health (ROICA235963 and T32CA009110), and through a sponsored research agreement with Panbela Therapeutics.