Evaluation of Human Pancreatic Cancer Cell Viability Following Administration of SBP-101 in the Presence and Absence of Gemcitabine and Nab-Paclitaxel

C. Baker, S. Arora, S. Gagnon, M. Cullen, A. Shah

Sun BioPharma, Inc., Gainesville, FL, USA

American Pancreatic Association 2015

ABSTRACT

Introduction: SBP-101 is an analogue of the native polyamine (PA) spermine. The PA uptake transport system is up-regulated in a number of neoplasms including pancreatic ductal adenocarcinoma (PDA) suggesting a promising therapeutic target. This study evaluated the anti-proliferative effect of SBP-101 in the presence and absence of gemcitabine (GEM) and nab-paclitaxel (NAB) in six human PDA cell lines.
Methods: AsPC-1, BxPC-3, Capan-1, HPAF-II, MIA PaCa-2 and PANC-1 cells were seeded in 96-well plates and allowed to adhere overnight. After 24 h fresh media was added containing 0.5-10 µM concentrations of SBP alone or + 0.5 µM GEM, + 5 nM NAB, or + both, or containing GEM, NAB or GEM + NAB alone. Cell proliferation was measured in triplicate 24, 48, 72 and 96 h post-treatment and IC_{50} values were calculated.
Results: SBP-101 produced an anti-proliferative effect in all cell lines; maximal inhibition most often occurred with 10 µM. At 96 h, maximum mean inhibition with 10 µM SBP-101 + GEM + NAB compared with GEM + NAB was 97.3% vs. 38.5% (BxPC-3), 90.1% vs. 47.1% (Capan-1), 89.7% vs. 38.3% (ASPC-1) and 39.4% vs. 8.0% (MIA PaCa-2) ($p<0.005$). SBP-101 alone produced greater inhibition than GEM + NAB in ASPC-1, BxPC-3 and Capan-1 cells ($p<0.005$). In most cell lines IC_{50} decreased with SBP-101 as treatment duration increased and combination treatments with SBP-101 resulted in a further decrease in IC_{50}, indicating an additive or synergistic effect with GEM and NAB on the decrease in cell viability.
Conclusion: SBP-101, both alone and in combinations with GEM and NAB, exhibited a marked anti-proliferative effect against PDA. SBP-101 alone and SBP-101 + GEM + NAB were more effective than GEM+NAB, the current standard of care. These results confirm the anti-neoplastic potential of SBP-101 and offer a rationale for its further investigation as a treatment for human pancreatic cancer.

INTRODUCTION

- In clinical practice patients with pancreatic ductal adenocarcinoma (PDA) treated with gemcitabine (GEM) develop resistance to this drug quickly, with disease progression occurring after only 3-4 months of treatment.[1,2] The addition of nab-paclitaxel (NAB) to GEM (the current standard of care) in patients with metastatic disease and good performance status has extended overall survival by only 7 weeks.[3,4] Thus there is a need to develop more effective treatments and regimens for PDA.
- SBP-101 is an analogue of the naturally occurring polyamine (PA), spermine.
- The polyamine transport uptake mechanism appears to be up-regulated in various tumor types, including PDA.
- Inducing polyamine depletion via the cellular uptake of synthetic polyamine analogues has been proposed as an antitumor strategy, making SBP-101 a promising therapy.[5-8]
- Prior studies examined the anti-neoplastic effects of subcutaneous administration of SBP-101 following orthotopic implantation of human L3.6pl pancreatic cancer cells into the pancreas of nude mice. SBP-101 25 mg/kg administered QD or 3x/wk inhibited the growth of human PDA and prolonged survival in mice. Co-administration of SBP-101 with GEM revealed an additive or synergistic effect.[9]

OBJECTIVES

- To determine the anti-proliferative effects of SBP-101 in the presence and absence of GEM and NAB in six different human pancreatic cancer cell lines.

METHODS

- Six human pancreatic cancer cell lines **(Table 1)** were plated in 96 well plates and allowed to adhere overnight.
- At 24 h, fresh medium containing various doses of SBP-101 (0.5 µM, 2.5 µM, 5 µM or 10 µM) either alone or in the presence of GEM (0.5 µM) and/or NAB (5 nM) was added **(Table 2).**
  - SBP-101 doses were selected to evaluate a dose-response relationship.
  - Doses for GEM and NAB were selected based on their *in vitro* activities in previous studies of human pancreatic cell lines.

ACKNOWLEDGEMENTS
This presentation is the intellectual property of Sun BioPharma, Inc.
Contact the author at (ashah@sunbiopharma.com) for permission to reprint and/or distribute.

METHODS

Table 1. Human Pancreatic Cell Lines

	Derived From
AsPC-1	primary ductal adenocarcinoma and metastatic ascites
BxPC-3	primary ductal adenocarcinoma and epithelial tissue
Capan-1	liver metastasis
HPAF-II	metastatic ascites
MIA PaCa-2	primary ductal adenocarcinoma and epithelial tissue
PANC-1	primary ductal adenocarcinoma and epithelial tissue

Table 2. Treatments

Single Agents	Combinations
Control alone	**SBP-101 + GEM**
SBP-101 alone (0.5 µM, 2.5 µM, 5 µM or 10 µM)	**SBP-101 + NAB**
GEM alone (0.5 µM)	**GEM + NAB**
NAB alone (5 nM)	**SBP-101 + GEM + NAB**

- Cell proliferation was measured 24, 48, 72, and 96 hours post-treatment by the Cell-Titer-Glo Luminescent Cell Viability Assay and an Optima Fluor Star Luminometer per the manufacturers' protocols.
- Anti-proliferative effects of treatments on each cell line were assessed by calculating the decrease or inhibition in cell viability as 100 x (Mean Control-Treatment) / Mean Control. The IC_{50} values were also calculated using an Emax inhibition model.
- Statistical differences between groups were determined using ANOVA with Tukey's post hoc multiple comparison test.

RESULTS

- SBP-101 alone produced an anti-proliferative effect in all cell lines with maximal inhibition generally occurring at 96 h and most frequently at the 10 µM dose level **(Table 3).**

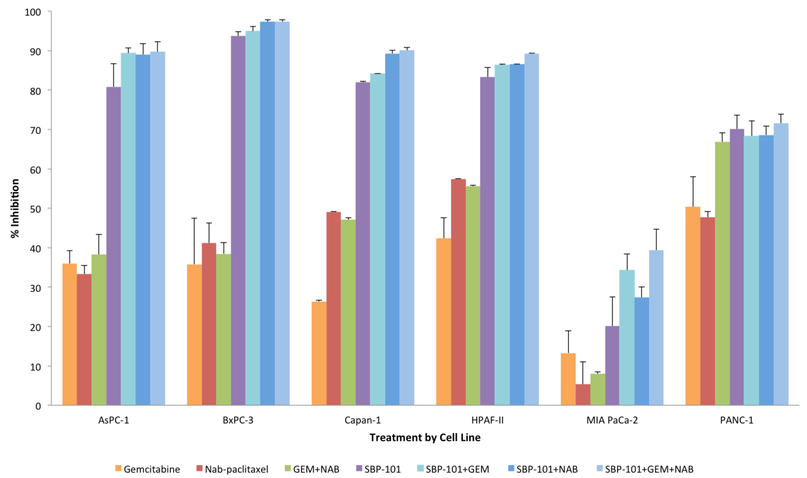
Table 3. Percent Inhibition in Cell Viability and IC_{50} Values for SBP-101 Alone at 96 Hours

Cell Line	SBP-101 0.5 µM	SBP-101 2.5 µM	SBP-101 5 µM	SBP-101 10 µM	IC_{50} (µM)
AsPC-1	54.0%	68.6%	63.7%	80.8%	1.3
BxPC-3	40.1%	85.7%	70.2%	93.7%	0.78
Capan-1	58.2%	72.1%	67.7%	81.9%	0.83
HPAF-II	71.6%	83.2%	44.3%	83.3%	--
MIA PaCa-2	15.3%	15.3%	25.2%	20.1%	30.0
PANC-1	73.5%	66.9%	64.6%	70.1%	0.60

- For ASPC-1, BxPC-3, Capan-1 and HPAF-II cells, IC_{50} values for SBP-101 decreased as the treatment duration increased and the combination treatments of SBP-101 with GEM and/or NAB resulted in a further decrease in IC_{50} indicating an additive or synergistic effect on decrease in the cell viability. For MIA PaCa-2 cells, the IC_{50} values for all treatments exceeded the highest concentration of 10 µM, suggesting a lack of consistent dose-related decrease in cell viability with treatments in this cell line. The IC_{50} values for PANC-1 decreased as treatment duration increased but combination treatments did not result in further decreases in the IC_{50} value.

RESULTS

Figure 1. Maximum Inhibition of Cell Viability (mean[SE]) at 96 h with SBP-101 (10 µM Dose) Alone and in Combination with GEM and/or NAB



- In AsPC-1, BxPC-3 and Capan-1 cell lines SBP-101, both alone and in all combinations, resulted in significantly greater inhibition of cell viability compared with GEM or NAB alone, and GEM + NAB ($p<0.05$).
- In the HPAF-II cell line, percent inhibition of cell viability was numerically greater with SBP-101 alone and in combinations compared with GEM, NAB, and GEM + NAB; however, the increases were not statistically significant.
- In the MIA PaCa-2 cell line, the triple combination of SBP-101 + GEM + NAB resulted in significantly higher inhibition compared with the GEM + NAB combination ($p<0.05$).
- In the resistant PANC-1 cell line, numerically greater inhibition was observed with SBP-101 treatments compared to GEM and NAB; however these differences were not statistically significant.

CONCLUSIONS

- SBP-101, both alone and in combinations with GEM and NAB, exhibited a marked anti-proliferative effect in the six human pancreatic cell lines studied.
- SBP-101 alone was more effective than GEM + NAB, the current standard of care, in most cell lines; however, the triple combination of SBP-101 + GEM + NAB exhibited the greatest effect.
- These results confirm the anti-neoplastic potential of SBP-101 and offer a compelling rationale for its clinical development as a potentially promising treatment for human pancreatic cancer.

REFERENCES
1.Shi X, et al. Acquired resistance of pancreatic cancer cells towards 5-Fluorouracil and gemcitabine is associated with altered expression of apoptosis-regulating genes. *Oncology* 2002; 4:354-362. 2. Thota R, Pauff JM, Berlin JD. Treatment of metastatic pancreatic adenocarcinoma: A Review. http://www.cancernetwork.com/oncology-journal/treatment-metastatic- pancreatic-adenocarcinoma-review. January 15, 2014. 3. Von Hoff DD, et al. Increased survival in pancreatic cancer with nab –paclitaxel plus gemcitabine. *N Engl J Med* 2013; 369:1691-1703. 4. Vogel A, et al. First-line nab-paclitaxel and gemcitabine in patients with metastatic pancreatic cancer from routine clinical practice. *In Vivo* 2014; 28:1135-1140. 5. Hector S, et al. Polyamine catabolism in colorectal cancer cells following treatment with oxaliplatin, 5-fluorouracil and N1, N11 diethylnorspermine. *Cancer Chemother Pharmacol* 2008; 62:517–527. 6. Jänne J, et al. Polyamines in rapid growth and cancer. *Biochem Biophys Acta* 1978;473:241–293. 7. Porter CW, et al. Enzyme regulation as an approach to interference with polyamine biosynthesis - an alternative to enzyme inhibition. *Adv Enz Reg* 1988; 27:57–79. 8. Casero RA Jr, et al. The role of polyamine catabolism in anti-tumour drug response. *Biochem Soc Trans* 2003;31:361–365. 9. Shah A, et al. Efficacy of diethyldihydroxyhomospermine against human pancreatic adenocarcinoma using orthotopic implantation of human pancreatic L3.6pl cells into the pancreas of nude mice. *Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5-9; San Diego, CA. Philadelphia (PA): AACR; 2014. Abstract nr 3128.*