Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Apogee Biotechnology Corporation

2. **Reporting Period (start and end date of grant award period):** June 1, 2012–May 31, 2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Charles D. Smith, PhD

4. **Grant Contact Person's Telephone Number: (**717) 531-4758

5. **Grant SAP Number:** 4100059191

6. **Project Number and Title of Research Project:** 1 - Development of ABC294640 for Combination Chemotherapy of Pancreatic Cancer

7. **Start and End Date of Research Project:** 06/01/2012 -05/31/2014

8. **Name of Principal Investigator for the Research Project:** Lynn W. Maines, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $834,062.34

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Maines, Lynn	Principal Investigator	8.2	27,449.96
Smith, Charles	Co-Investigator	32.4	132,353.25
Zhuang, Yan	Co-Investigator	22.9	56,324.07
Green, Cecelia	Technician	20.3	23,117.09
Smith, Lisa	Office Administrator	3.8	4,550.00
Schrecengost, Randy	Senior Research Scientist	15.0	11,728.57
Keller, Staci	Technician	34.4	26,666.70
Smith, Ryan	Technician	49.9	40,450.01

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X___ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Clinical trial of ABC294640 in pancreatic cancer patients	NIH ☐ Other federal (specify:_____) ☒ Nonfederal source (specify: __State of PA__)	7/2012	$500,000	$500,000
Evaluation of ABC294640 as a medical counter measure for the prevention of gastrointestinal acute radiation syndrome	NIH ☒ Other federal (specify:_DOD__) ☐ Nonfederal source (specify:_)	10/2012	$688,000	$688,000
Mitigation of radiation-induced pulmonary fibrosis by a sphingosine kinase inhibitor	☒NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:_)	11/2012	$600,000	$593,448
Protection against chemotherapy-induced gastrointestinal mucositis by a sphingosine kinase inhibitor	☒NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:_)	4/2013	$224,116	$224,116
Clinical trial of ABC294640 in liver cancer patients	NIH ☐ Other federal (specify:____) ☒ Nonfederal source (specify: __State of PA__)	7/2013	$500,000	$500,000
Targeting prostate cancer with a sphingosine kinase inhibitor	☒NIH ☐ Other federal (specify:_____)	8/2013	$225,000	$not funded

	☐ Nonfederal source (specify:_)			
Modulation of cancer radiotherapy by ABC294640	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	12/2013	$200,000	$not funded (in negotiation stage)
An early-phase clinical trial evaluating ABC294640 in patients with refractory/relapsed DLBCL	☒NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:_)	1/2014	$1,500,00	$not funded (in review)
Radiosensitizing prostate cancer by downregulation of androgen receptors and c-Myc	☒NIH ☐ Other federal (specify:___) ☐ Nonfederal source (specify:_)	4/2014	$225,000	$not funded (in review)
Optimization of dual-targeted compounds for the treatment of leukemia	☒NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:_)	7/2014	$225,000	$not funded (in review)
Mitigation of pulmonary fibrosis following exposure to ionizing radiation by the sphingosine kinase 2 inhibitor ABC294640	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	7/2014	$5,663,047	$not funded (in review)

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

Apogee will be submitting a phase 2 SBIR grant to support a clinical trial of ABC294640 in pancreatic cancer patients. This will be submitted for the December 5, 2014 deadline with a proposed budget of approximately $1,500,000.

12. Future of Research Project. What are the future plans for this research project?

This project was important for several reasons and so is of high priority at Apogee for continuation. The most important outcome is that it demonstrated the ability of ABC294640, as a single-agent, to inhibit pancreatic tumor growth in the most clinically-relevant animal

model available, i.e. an orthotopic pancreatic cancer model in mice. This allows us to greatly simplify plans for the clinical testing of the drug, in that we will move forward with single-agent trials, rather than moving into combination chemotherapy trials. This avoids the issue of potential drug-drug interactions in the patients, and also removes any potential issue of needing to define the optimal scheduling of multiple drugs to avoid mechanism-based reductions in activity, eg. cell-cycle arrest resulting in reduced sensitivity to Abraxane. The demonstration of specific critical signaling pathways blocked by ABC294640 may allow us to select patients for treatment based on the overexpression of those pathways in their tumors. Finally, demonstration that circulating levels of S1P can be used to monitor the in vivo activity of ABC294640 may allow real-time monitoring of individual patient response to the drug.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

    Yes___X___ No_____

    If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female				1
Unknown				
Total			**1**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	2
Unknown				
Total			**1**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	2
Black				
Asian				
Other				
Unknown				
Total			**1**	**2**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This funding was essential for completing the preclinical development of ABC294640 for future clinical trials in pancreatic cancer patients. The information generated in this project is also being used to refine preclinical and clinical development of this drug for other types of cancer. It also provided critical information that was used to secure other grants from federal sources, i.e. SBIR grants.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes__X_____ No_____

If yes, please describe the collaborations:

Apogee collaborates closely with the Penn State College of Medicine / Hershey Medical Center (and is a spin-out company from the Department of Pharmacology). We have maintained strong research collaborations with Penn State faculty, e.g. we have just submitted an STTR grant application with a Dept of Pharmacology faculty member (Dr. Jong Yun) as the Principal Investigator. When funded, this will provide additional research funding to both Apogee and Penn State.

16(B) Did the research project result in commercial development of any research products?

Yes___X____ No_____

If yes, please describe commercial development activities that resulted from the research project:

We are actively discussing opportunities for commercialization of the Apogee research portfolio, including out-licensing ABC294640 for late-stage clinical testing and the subsequent commercialization activities, e.g. manufacturing and sales. This funding

allowed the project on pancreatic cancer to progress closer to the pivotal clinical testing that will be essential for further development of the drug.

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Overview

The research objectives of this project were to complete a preclinical efficacy and safety data package and preclinical regulatory activities to support the approval of an IND application for a Phase I/IIa clinical trial evaluating ABC294640 in combination with Gemzar and/or Abraxane in pancreatic cancer patients.

The Specific Aims were:

1. To evaluate the effects of ABC294640, alone and in combination with gemcitabine or paclitaxel, on pancreatic cancer cells in vitro. Using a panel of human pancreatic cancer cell lines, we will determine the effects of ABC294640 on proliferation, apoptosis, migration (invasion) and cytokine production. The ability of ABC294640 to synergize with gemcitabine or paclitaxel in the proliferation and apoptosis assays will also be determined. Mechanism-based pharmacodynamic (PD) endpoints will be assessed in the treated cells to provide biomarkers for drug action.

2. To evaluate the effects of ABC294640, alone and in combination with gemcitabine or Abraxane, on pancreatic tumors in vivo. The antitumor activities of ABC294640, alone and in combination with gemcitabine or Abraxane, will be determined in an orthotopic pancreatic tumor model in SCID mice using bioluminescent detection. Mechanism-based PD endpoints will be assessed in the tumors, and plasma sphingosine 1-phosphate levels will be determined as a potential clinical biomarker of drug action.

3. To complete preclinical tasks necessary to initiate clinical testing of ABC294640 in combination with Gemzar and/or Abraxane, including limited additional toxicology studies; development of the clinical trial protocol; and submission of an IND application for the drug combination trial. It is important to note that these regulatory tasks will be highly leveraged by previous work completed by Apogee and utilized in the acquisition of the IND approval for testing ABC294640 as a single-agent, a clinical trial that is currently ongoing.

Summary of Research Completed

<u>Specific Aim 1. To evaluate the effects of ABC294640, alone and in combination with gemcitabine or paclitaxel, on pancreatic cancer cells in vitro</u>.
Inhibitors of sphingosine kinase 1 and 2 (SphK1, SphK2) diminish the production of sphingosine-1-phosphate, a bioactive lipid involved in tumor cell survival, motility, invasion and angiogenesis. ABC294640 (Figure 1) is a selective inhibitor of SphK2 and has demonstrated pre-clinical utility as an anti-tumor and anti-inflammatory agent.

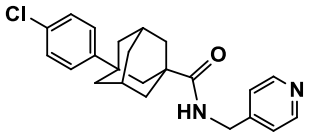


Figure 1. ABC294640

A. Expression of SphKs in human tumors.
Studies were done to assess the level of expression of SphK1 and SphK2 in normal pancreatic tissue and pancreatic tumors. We have previously shown that RNA for SphK1 is frequently overexpressed in various human tumor types, i.e. up to 4-fold increases were found in

approximately 80% of tumor tissues compared with matched normal tissue from the same patient. Data for the expression of SphK1 and SphK2 in pancreatic tumors have not been published to date; however, we have conducted a study using human tumor cDNA arrays available from Origene. As shown in Figure 2, SphK2 expression is markedly elevated in pancreatic tumors compared with normal pancreatic tissue (5.9 ± 0.4 fold; control n=3, tumor n=16); while only a minor increase in the expression level of SphK1 (1.4 ± 0.2 fold) was observed. Overexpression of SphK2 was also observed in samples from breast, colon and prostate tumors; whereas, SphK1 overexpression was more pronounced in liver, lung, ovary and thyroid tumors. Therefore, targeting SphK2 may be particularly pertinent to the treatment of pancreatic cancer.

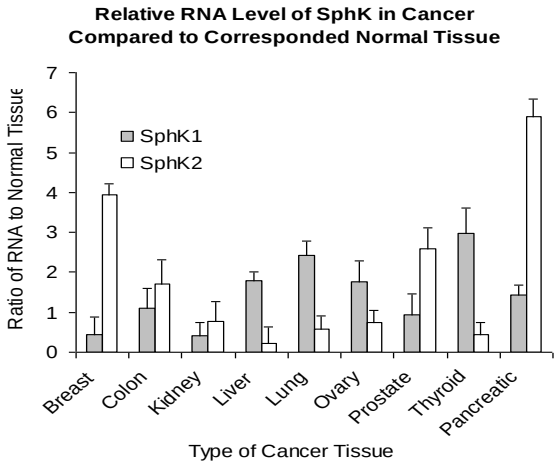


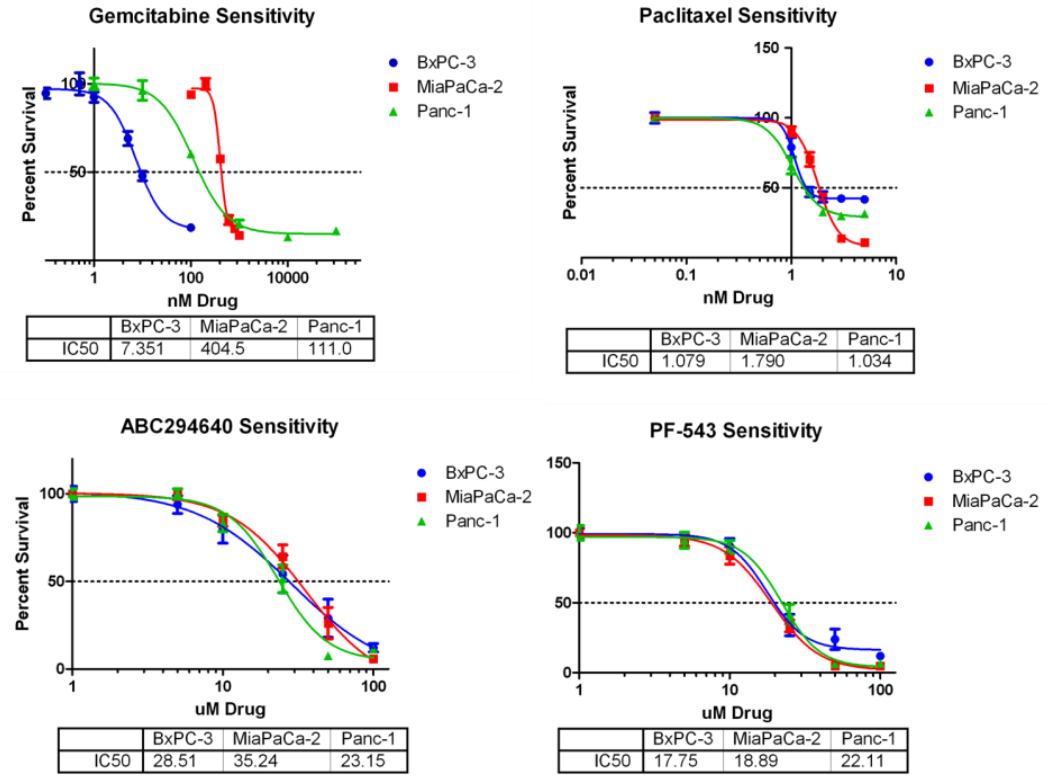
Figure 2. Expression of SphK1 and SphK2 message in human tumors. A PCR array from Origene containing cDNA from areas of normal tissue or cancer was analyzed by real-time quantitative PCR. Expression levels of SphK1 and SphK2 were normalized to β-actin mRNA in each sample. Values represent the mean ± sd fold expression of SphK1 (filled bars) and SphK2 (open bars) in the various tumors compared with expression levels in normal samples.

B. Evaluation of the effects of ABC294640 alone and in combination with gemcitabine or paclitaxel on pancreatic cancer cell proliferation.

At the time this project was initiated, the antimetabolite gemcitabine was the standard drug given to pancreatic cancer patients, and while it is typically able to provide some improvement in the quality of life of the patients, it does not cause tumor regression and only marginally increases overall survival. More recently, the drug Abraxane, which is nanoparticle-bound paclitaxel (PTX), has been found to provide modest increased survival when given to pancreatic cancer patients in combination with gemcitabine. Because we believed that ABC294640 will be most effective when added to the existing drugs, we completed a detailed series of studies assessing the combined effects of ABC294640 and either gemcitabine or paclitaxel on pancreatic cancer cell proliferation.

Figure 3 demonstrates the cytotoxicity of these 3 drugs as well as PF-543 which is a selective SphK1 inhibitor at low concentrations (nM) and a dual SphK1 + SphK2 inhibitor at higher

concentrations (μM) in a panel of 3 human pancreatic cancer cell lines (BxPC-3, MiaPcCa-2 and Panc-1).



	BxPC-3	MiaPaCa-2	Panc-1
IC50	7.351	404.5	111.0

	BxPC-3	MiaPaCa-2	Panc-1
IC50	1.079	1.790	1.034

	BxPC-3	MiaPaCa-2	Panc-1
IC50	28.51	35.24	23.15

	BxPC-3	MiaPaCa-2	Panc-1
IC50	17.75	18.89	22.11

Figure 3. Cytotoxicities of study drugs toward pancreatic cancer cell lines.

Whereas the pancreatic cancer cell lines show a 50-fold range in sensitivity to gemcitabine, they are all essentially equally sensitive to paclitaxel, ABC294640 and PF-543. It is notable that PF-543 inhibits cell proliferation only at high doses, supporting our hypothesis (based on RNA-interference studies) that SphK2 is more important for regulation cancer cell proliferation than is SphK1. The consistency in the IC50s for ABC294640 in the pancreatic cancer cell line panel suggests that it is blocking a pathway essential for all tumor cell growth, and therefore is unlikely to be circumvented by common mechanisms for resistance to other anticancer drugs. The effects of combined treatment with ABC294640 + gemcitabine and ABC294640 + paclitaxel are summarized in Figures 4 and 5. The Combination Index is a statistical measure of drug-drug interaction, with a CI = 1 indicating that the two drugs are additive, while CI< 1 indicates synergy and CI > 1 indicates antagonism. The data demonstrate that combination of ABC294640 with gemcitabine (Figure 4) or paclitaxel (Figure 5) results in synergistic cytotoxicity at essentially all concentrations of the two drugs. Therefore, there is excellent potential for improving the antitumor activity of the standard drugs for pancreatic cancer (Gemcitabine and paclitaxel (as Abraxane)) by the addition of ABC294640 to the chemotherapy protocol.

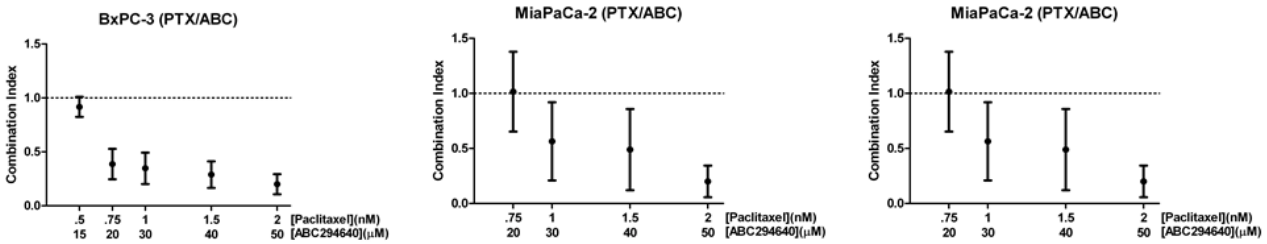


Figure 4. Interactions between ABC294640 and Paclitaxel in pancreatic cancer cell lines.

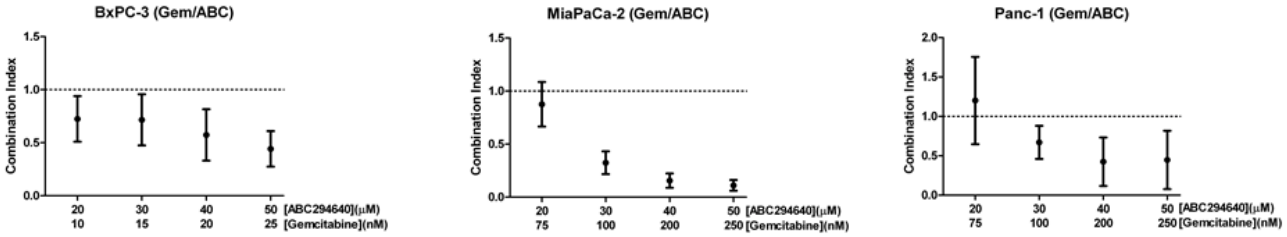


Figure 5. Interactions between ABC294640 and gemcitabine in pancreatic cancer cell lines.

C. Pro-apoptotic and pro-autophagic sphingolipids are elevated in ABC/PTX treated cells. Tumor cells contain sphingolipids having a variety of fatty acid chains, and these different molecular species may have differing cell activities. Alterations in the sphingolipid profiles were determined in PANC-1 cells at 72 h post-treatment with ABC294640 and/or PTX. Treatment of cells with ABC294640 resulted in increased ceramides with concurrent depletion of S1P (Table 1), consistent with inhibition of SphK2. Treatment with PTX had marginal effects on ceramide levels; however, dihydrosphingosine and S1P levels were surprisingly decreased. Compared with either single-agent, more pronounced elevations in ceramides occurred following ABC294640/PTX combination treatment. In particular, the pro-apoptotic species, C18-ceramide as well as C18:1-ceramide were elevated in ABC294640/PTX treated cells at both drug doses. However, only C18:1-ceramide reached statistical significance in cells treated with the 25 μM ABC294640/100 nM PTX combination (Table 1). C20:1 ceramide was elevated, albeit not statistically significantly, following the combined treatment. Surprisingly, 12.5 μM ABC294640 caused a consistent 20% decrease in both of these lipids (** $P < 0.01$); whereas 25 μM ABC294640 elevated these lipid species. Dihydro-C16-ceramide is associated with autophagy and cell cycle arrest, and was markedly elevated in ABC294640-treated and ABC/PTX-treated cells, but not PTX alone, consistent with induction of an autophagic response by ABC294640. Conversely, dihydrosphingosine (dhSph) was significantly decreased by the 12.5 μM ABC294640/50 nM PTX combination treatment, but increased following ABC294640 alone. The proliferation-associated lipid, S1P, was markedly reduced following all doses of PTX and the higher dose of ABC294640 (Table 1).

Table 1. Effects of ABC294640 and PTX on sphingolipid profiles in PANC-1 cells. PANC-1 cells were treated with the indicated concentrations of ABC294640 and/or PTX for 72 h. Sphingolipids were then quantified by LC-MS and normalized to vehicle-treated control cells. Values represent the mean ± standard error of 3 independent experiments. $P <$ *0.05, **0.01, ***0.001 versus vehicle control samples.

	C18-Cer	C18:1-Cer	C20:1-Cer	dhC16-Cer	dhSph	Sph-1P
ABC 12.5 μM	80 ± 3**	78 ± 2***	61 ± 14	202 ± 20**	176 ± 20*	91 ± 31
PTX 50 nM	123 ± 8*	110 ± 32	101 ± 8	111 ± 28	44 ±11*	21 ± 6
ABC/PTX	146 ± 18	123 ± 35	159 ± 23	146 ± 22	62 ± 12*	36 ± 10
ABC 25 μM	137 ± 35	131 ± 27	178 ± 70	210 ± 22**	133 ± 32**	24 ± 2
PTX 100 nM	121 ± 9	119 ± 36	123 ± 18	110 ± 27	48 ± 4***	25 ± 2
ABC/PTX	203 ± 48	187 ± 32*	364 ± 91	223 ± 19**	80 ± 22	37 ± 15

D. G2/M cell cycle arrest and apoptosis occurs following treatment with the ABC294640/PTX combination. Cell cycle analyses were conducted after 72 h of drug treatment in PANC-1 cells (Figures 6A and 6B). Treatment with ABC294640 alone increased the percentage of cells in G1 while suppressing the G1/S transition in a dose-dependent fashion. Cells treated with ABC294640 alone display no increase in the pre-G1 apoptotic region, consistent with a previous demonstration of autophagic cell death and cell cycle arrest elicited by ABC294640 treatment. As expected, PTX alone caused a marked G2/M arrest, consistent with its ability to stabilize microtubules, resulting in apoptosis and autophagy in a number of cell lines. Changes in cell cycle for the ABC294640/PTX combination were no different than effects of PTX alone, suggesting that the majority of the impact of ABC/PTX on the cell cycle was PTX-induced. Using a lower dose of PTX (5 nM, 25% survival for MIA PaCa-2, 50% survival for PANC-1) and 25 μM ABC294640, we examined caspase 3/7 activation at 24 and 48 h post-treatment in PANC-1 (PTX-resistant) and MIA PaCa-2 (PTX-sensitive) cells (Figures 6C and 6D, respectively). By 48 h post-treatment, ABC294640 alone caused a significant decrease in caspase 3/7 activity in both cell lines, suggesting that ABC294640-induced autophagy may antagonize caspase-mediated apoptosis. Both cell lines showed an increase in caspase activation after treatment with either PTX or ABC294640/PTX. Caspase activation was significantly lower in ABC294640/PTX-treated MIAPaCa-2 cells than in cells treated with PTX alone, suggesting antagonism of the apoptotic pathway.

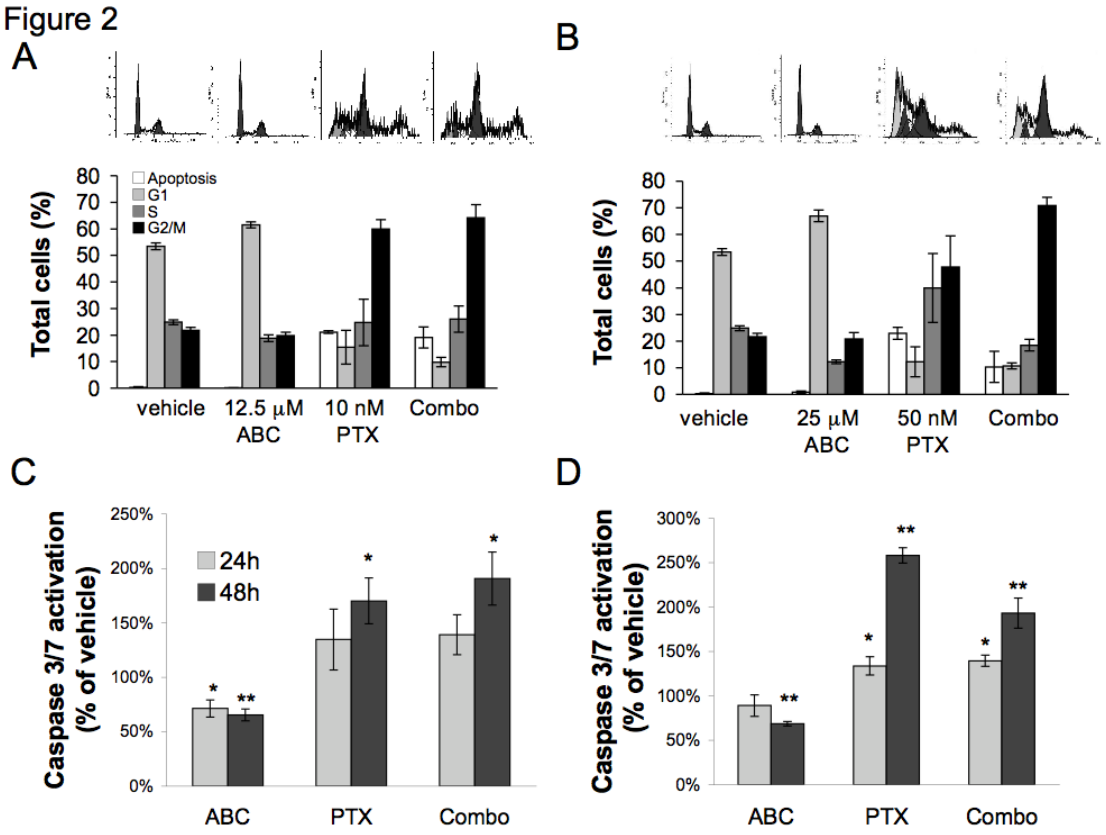


Figure 6. Cell cycle and apoptotic effects of ABC294640 and PTX. PANC-1 cells were treated with 12.5 μM ABC294640 and/or 10 nM PTX (Panel A) or 25 μM ABC294640 and/or 50 nM PTX (Panel B) for 72 h and analyzed by flow cytometry. Histogram values are the mean ± standard error of the percentage of cells in apoptosis (sub-G_1, white bars), G_1 (light gray bars), S (gray bars) or G_2/M (black bars) phases of the cell cycle from 3 independent experiments. Representative histograms (DNA content versus cell count) are provided. Caspase 3/7 activity was examined 24 (gray bars) or 48 (black bars) h after treatment of PANC-1 (Panel C) or MIA PaCa-2 (Panel D) cells treated with 25 μM ABC and/or 5 nM PTX. * $P < 0.05$ and ** $P < 0.005$ compared with the vehicle controls.

E. Markers of autophagy are altered following ABC294640/PTX treatment. We previously demonstrated that toxic doses of ABC294640 activate autophagy in tumor cells, which can be measured as the cleavage of LC3 protein on autophagosomal membranes, as well as increased expression of beclin-1. Western analyses were conducted to determine the effects of the ABC294640/PTX combination on induction of these autophagic markers. LC3 cleavage was elevated in cells treated with the low-dose ABC294640/PTX combination for 72 h (12.5 μM ABC294640 and 10 nM PTX, Figure 7A), as well as the high-dose combination for 24 h (50 μM ABC294640 and 100 nM PTX; Figure 7C) compared to vehicle or ABC294640-treated cells. Interestingly, beclin-1 expression was significantly decreased for the same ABC294640/PTX combination dose at 72 h (Figure 7B). This marker of autophagy was not significantly altered from the vehicle control in the high dose 24 h samples (Figure 7D). This suggests that beclin-1 up-regulation may be uncoupled from the LC3 cleavage response in PANC-1 cells and/or is inhibited by the concurrent apoptotic pathway.

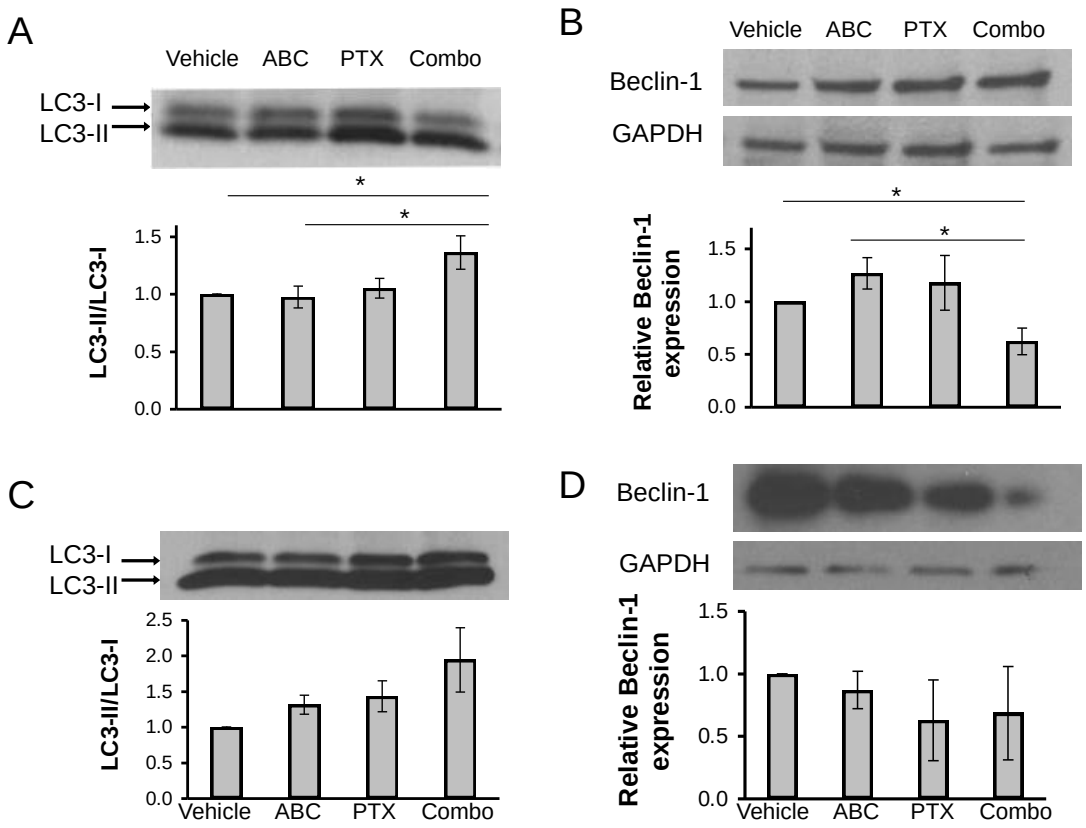


Figure 7. Effects of ABC294640 and PTX on autophagy markers in PANC-1 cells. PANC-1 cells were treated with 12.5 μM ABC294640 and/or 10 nM PTX (Panels A and B) or 50 μM ABC294640 and/or 100 nM PTX for 24 h (Panels C and D) for 72 h. Total cell protein was then analyzed by immunoblotting for LC3 (Panels A and C) or Beclin-1 (Panels B and D). For LC3, the histogram data represents the ratio of LC3-II : total LC3 (LC3-I + LC3-II), a value that increases during autophagosome formation. Beclin-1 levels are normalized to that of GAPDH. Values represent the mean ± standard error for 3 independent experiments. *$P < 0.05$ for the indicated comparisons.

F. ABC/PTX causes cell rounding and cytoskeletal changes. Because PTX binds to and stabilizes microtubules, we examined the impact of ABC294640 (25 μM) and PTX (50 nM), alone or in combination on microtubule structure in PANC-1 cells by β-tubulin immunocytochemistry. While networks of microtubules were still intact in vehicle- and ABC294640-treated cells (Figures 8A and 8B, respectively), PTX and to a greater extent, ABC294640/PTX, treatment caused significant cell rounding and a reduced number of visibly intact microtubules (Figures 8C and 8D, respectively). These results are consistent with apoptotic cell death following arrest of tubulin dynamics by paclitaxel. Focal adhesion kinase (FAK) activation, an indicator of cellular detachment and reattachment to a substratum via focal adhesions, was elevated in PTX and ABC/PTX treated cells (Figures 8E and 8F, respectively).

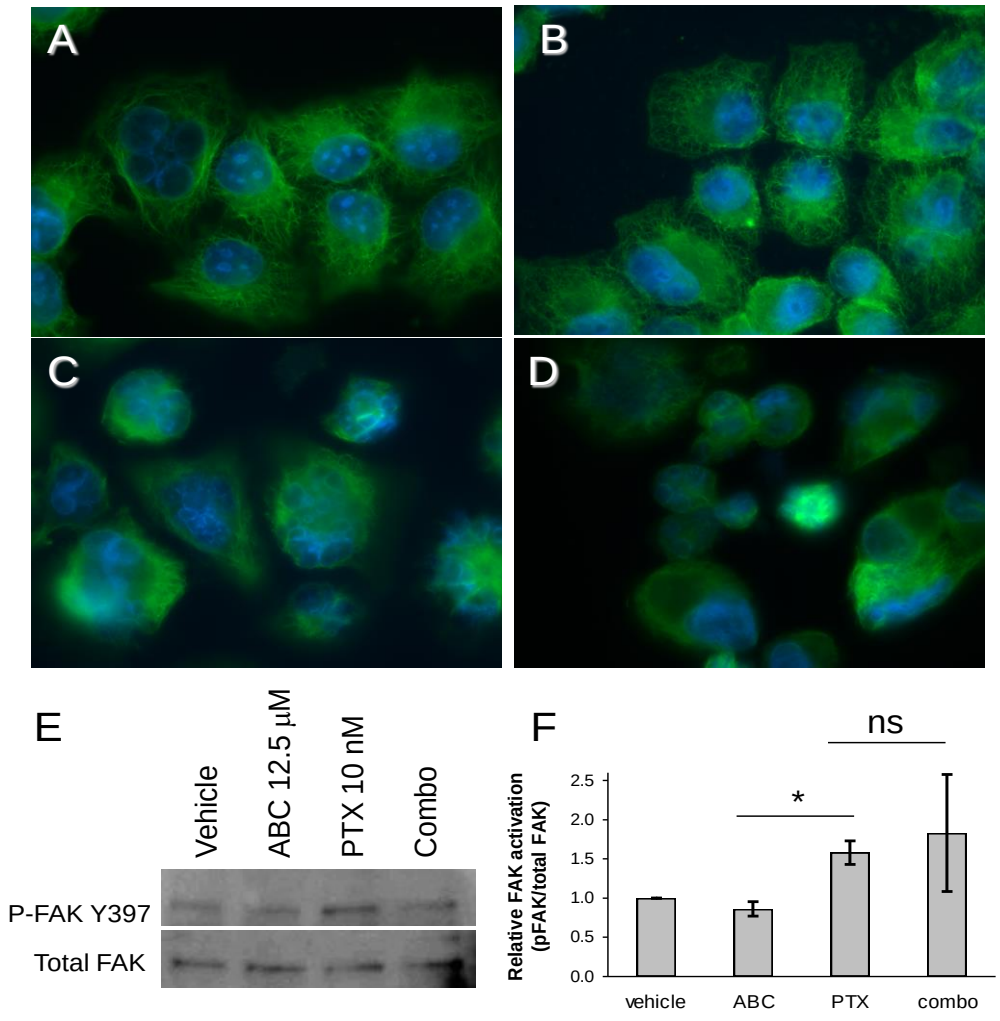


Figure 8. Effects of ABC294640 and PTX on PANC-1 cell cytoskeleton. PANC-1 cells were treated with vehicle (Panel A), 12.5 μM ABC294640 (Panel B), 50 nM PTX (Panel C), or ABC294640 and PTX in combination (Panel D) for 48 h and microtubule structure was analyzed by β-tubulin immunocytochemistry (green) with nuclei being counterstained with DAPI (blue). Samples from the cell treatments were also assessed for FAK activation by immunoblotting (Panels E and F). Values represent the mean ± standard error for 3 experiments. ns = not significant, $P > 0.05$; *$P < 0.01$ for the indicated comparisons.

G. Effects of ABC294640 on signaling in pancreatic cancer cells.

We sought to better understand the mechanism for the antitumor activities of ABC294640 in pancreatic cancer cell lines and so measured its effects on critical signaling pathways involved in tumor growth. These studies involved measurement of either protein levels by western blotting or measurement of mRNA levels by quantitative PCR. A large number of studies are summarized in the following Figures:

We demonstrated that ABC294640 treatment suppresses cellular markers of proliferation and survival, specifically phosphorylated ERK1/2 and AKT (S473). Here, we analyzed PANC-1 cells that had undergone low-dose, single-agent ABC294640 or PTX or combination treatment for 72 h. A sub-toxic dose of ABC294640 alone (12.5 μM) slightly elevated the relative AKT activation (phospho-AKT: total AKT); whereas PTX alone (10 nM) had a larger effect on AKT activation; and this was substantially abrogated by combining PTX with ABC294640 (Figure 9A). Relative ERK activation was also slightly increased by ABC294640 alone and markedly increased by PTX alone (Figure 9B). The addition of ABC294640 reduced the effect of PTX on pERK2, but not pERK1, which is surprising given that the target of ABC294640, SphK2, is phosphorylated and activated by ERK1. Of note, the expression of total ERK protein decreased slightly with single agent treatment, but resulted in almost undetectable protein expression in cells treated with the ABC294640/PTX combination. Higher doses (50 μM ABC294640, 100 nM PTX) were used for a shorter time period (24h; Figures 9C and D) to examine their effects on cell survival signaling. In other cell lines, a 24 h treatment with 100 nM PTX caused a significant decrease in AKT activation; however, PANC-1 cells in this study did not show any change. Rather, ABC294640 and the combination of ABC294640/PTX caused an increase in AKT activation at these doses. In contrast to the increase in ERK activation seen with the lower doses of ABC294640/PTX (Figure 9B), a slight decrease in ERK phosphorylation was observed with higher doses of the combination (Figure 9D) with no change in total ERK expression. These effects could be temporally dependent since ERK expression may decrease after 24 h. However, at the higher doses, most cells died by 72 h and levels of ERK and AKT were below detection (not shown).

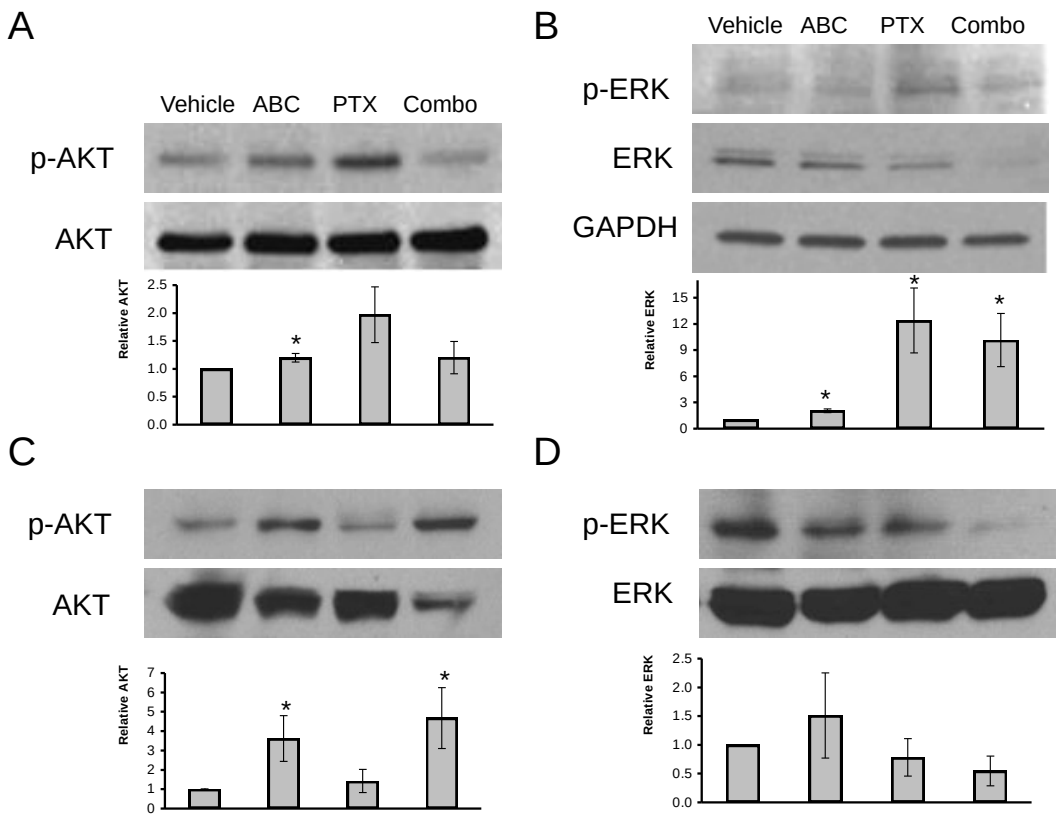
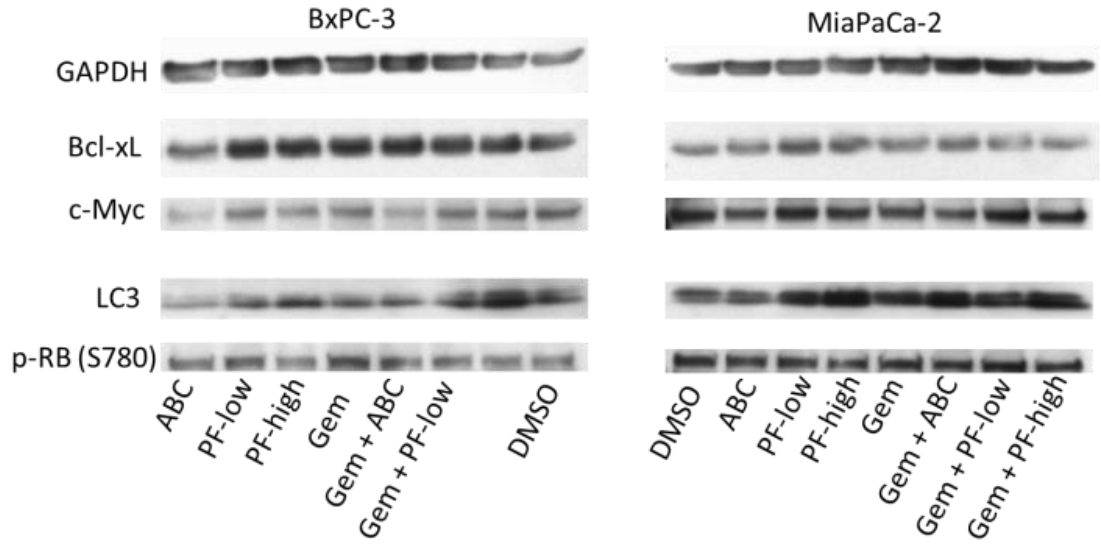


Figure 9. Effects of ABC294640 and PTX on AKT and ERK1/2 in PANC-1 cells. PANC-1 cells were treated with 12.5 μM ABC294640 and/or 10 nM PTX for 72 h (Panels A and B) or 50 μM ABC294640 and/or 100 nM PTX for 24 h (Panels C and D). Total cell protein was then analyzed by immunoblotting. The relative AKT activation is shown as p-AKT (S473) normalized to total AKT for each sample (Panels A and C). The relative ERK activation is shown as p-ERK normalized to total ERK1/2 for each sample (Panels B and D). Histograms for both AKT and ERK activation are expressed relative to the vehicle control as the mean ± standard error of at least 3 independent experiments. *$P < 0.05$ compared with the vehicle controls.

Similar signaling studies in the pancreatic cancer cells lines include:

Figure 10 which demonstrates that ABC294640 and gemcitabine both decrease levels of Bcl-xL, c-Myc and LC3 in both BxPC-3 and MiaPaCa-2 cells. These are key proteins regulating apoptosis, proliferation and autophagy, respectively. Combinations of the drugs tend to further decrease expression of these proteins, and therefore could be involved in the synergistic reduction in cell growth.

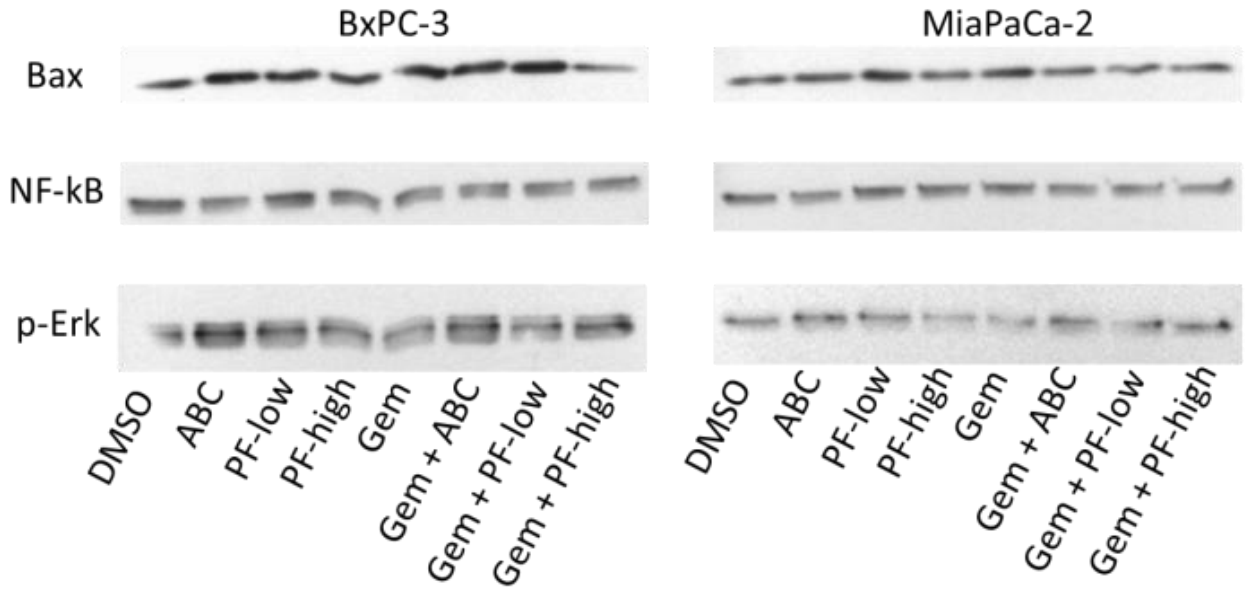


Percent Change

BxPC-3	Bcl-xL	c-Myc	LC3	MiaPaCa-2	Bcl-xL	c-Myc	LC3
DMSO	1.00	1.00	1.00	DMSO	1.00	1.00	1.00
ABC	0.44	0.22	0.22	ABC	0.86	0.69	0.75
PF-Low	0.75	0.45	0.42	PF-Low	1.21	0.75	1.00
PF-High	0.65	0.34	0.44	PF-High	1.10	0.69	1.17
Gem	0.70	0.41	0.41	Gem	0.69	0.67	0.81
Gem + ABC	0.66	0.22	0.33	Gem + ABC	0.76	0.50	0.89
Gem + PF-Low	0.72	0.41	0.50	Gem + PF-Low	0.58	0.91	0.74
Gem + PF-High	0.97	0.65	1.09	Gem + PF-High	0.74	0.93	1.20

Figure 10. Signaling effects of SphK inhibitors and gemcitabine in pancreatic cancer cells

Figure 11 which demonstrates that ABC294640 causes a marked reduction in NFκB levels in both cell lines. This is interesting because others have reported that resistance to gemcitabine is at least partially mediated by elevated signaling through the NFκB pathway. Therefore, ABC294640 may reverse resistance to this drug in pancreatic cancer patients.

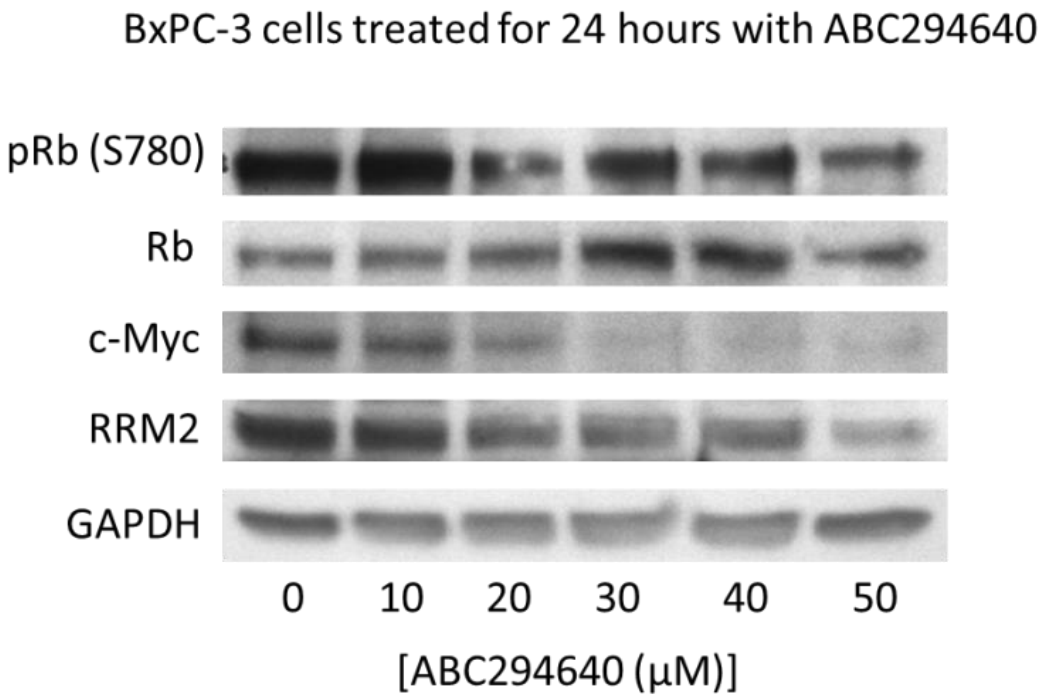


Percent Change

BxPC-3	Bax	NF-kB	MiaPaCa-2	Bax	NF-kB
DMSO	1.00	1.00	DMSO	1.00	1.00
ABC	0.87	0.35	ABC	0.96	0.66
PF-Low	0.72	0.52	PF-Low	1.19	0.82
PF-High	0.52	0.35	PF-High	0.76	0.73
Gem	0.74	0.35	Gem	0.84	0.62
Gem + ABC	0.66	0.32	Gem + ABC	0.58	0.48
Gem + PF-Low	0.92	0.38	Gem + PF-Low	0.49	0.45
Gem + PF-High	0.45	0.54	Gem + PF-High	0.71	0.64

Figure 11. Signaling effects of SphK inhibitors and gemcitabine in pancreatic cancer cells.

Finally, Figure 12 which quantifies the dose-response curve for down-regulation of c-Myc, pRb and RRM2 (ribnucleotide reductase) by ABC294640. RRM2 overexpression is also known to provide resistance to gemcitabine. Overall, these studies establish a mechanistic rationale for the observed synergy between ABC294640 and gemcitabine. This data may also provide biomarkers for predicting patient response to ABC294640 + gemcitabine in future clinical trials.



Densitometry Results

[ABC294640 (μM)]

	0	10	20	30	40	50
%Δ pRb/Rb	0	7%	-38%	-47%	-41%	-40%
%Δ c-Myc	0	-12%	-48%	-89%	-87%	-85%
%Δ RRM2	0	-11%	-21%	-27%	-12%	-46%

Figure 12. ABC294640 dose-response for signaling inhibition in BxPC-3 cells.

Specific Aim 2. To evaluate the effects of ABC294640, alone and in combination with gemcitabine or Abraxane, on pancreatic tumors in vivo. We have generated derivatives of Panc-1 cells that constitutively express luciferase (Panc-1-luc), and shown that they have doubling times equivalent to the parental lines. Luciferase expression is maintained for at least 10 passages in vitro in the absence of continued exposure to G418. Furthermore, these cells efficiently grow in immunodeficient mice and can be analyzed using bioluminescent imaging (Figure 13). Interestingly, these cells metastasize, which may allow quantification of the effects of the test drugs on both primary tumor growth and metastasis.

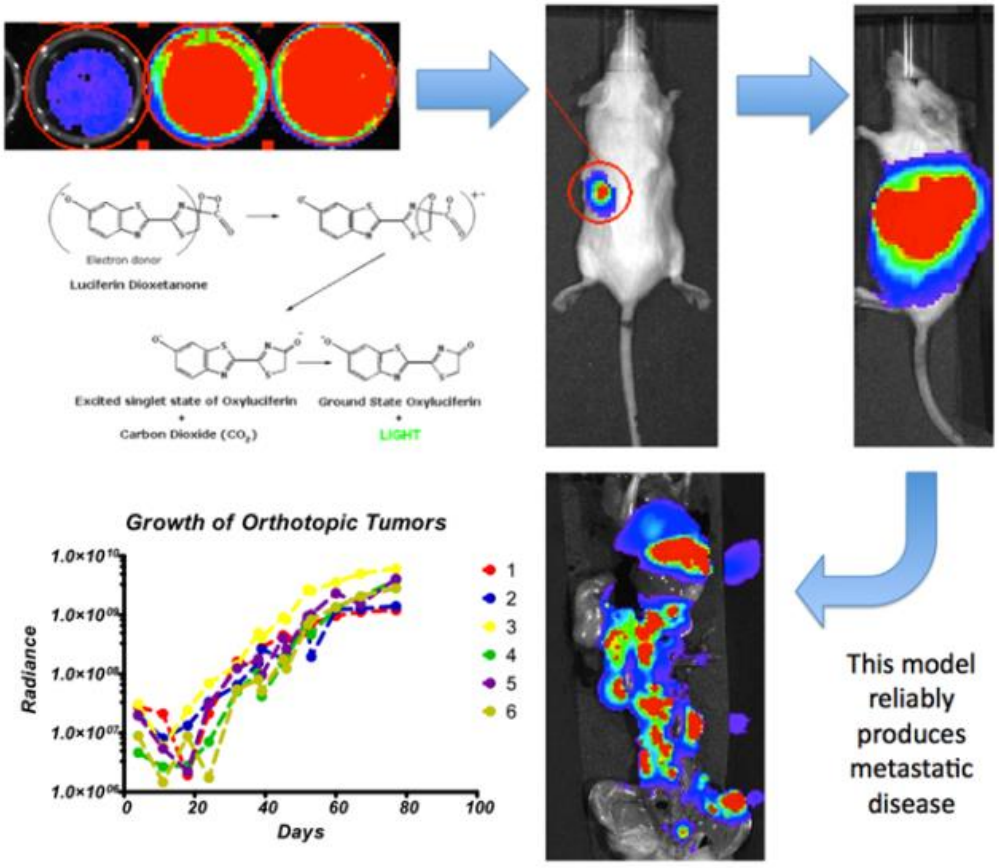


Figure 13. Orthotopic pancreatic cancer model

The effects of treatment of mice bearing orthotopic pancreatic tumors with ABC294640 alone or in combination with Abraxane were examined. As indicated in Figure 14, the pancreatic tumors grew relatively slowly, with a doubling time of approximately 2 weeks. Treatment of the mice with Abraxane alone had no effect on tumor growth, consistent with the lack of effect of single-agent Taxol in this disease. Conversely, treatment with ABC294640 (50 mg/kg, 5 days/week) reduced tumor growth by about 50%. Combination of ABC294640 with Abraxane did not result in further suppression of tumor growth, indicating that this combination, at least at the dose of Abraxane utilized, is not superior to ABC294640 alone. Ongoing experiments are assessing the effects of combination of ABC294640 with gemcitabine in this model.

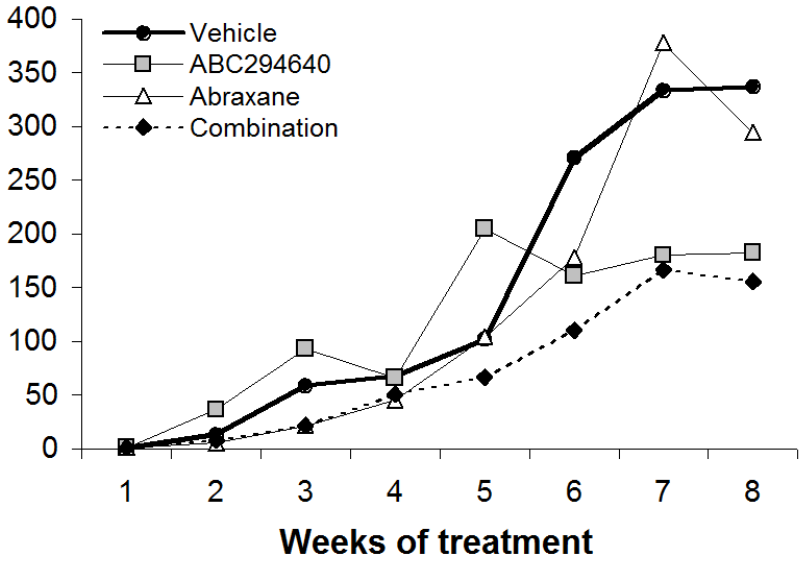


Figure 14. Inhibition of pancreatic tumor growth by ABC294640.

Specific Aim 3. To complete all preclinical tasks necessary to initiate clinical testing of ABC294640 in combination with Gemzar and/or Abraxane.

The preclinical data to date does not support the use of a combination of ABC294640 plus Abraxane in pancreatic cancer patients, and therefore we did not proceed with developing a clinical trial for this combination. However, in anticipation of improved antitumor activity when ABC294640 is combined with gemcitabine, we have written a phase 1b/2 clinical trial protocol for the use of this combination in pancreatic cancer patients who are refractory to standard chemotherapy. The Principal Investigator for this trial will be Dr. Melanie Thomas, who was the PI on the first-in-human phase 1 trial of ABC294640 in patients with advanced solid tumors.

Briefly, the study design for the planned clinical trial is: This is a Phase I/II safety and efficacy trial of ABC294640 in combination with gemcitabine. In Phase I, patients with advanced pancreatic cancer will be given standard dose gemcitabine (1000 mg/m^2, weekly) and cohorts will receive increasing doses of oral ABC294640. The starting dosage for ABC294640 will be 1/2 the MTD determined in a previous singe-agent phase I trial. It is expected that up to 12 patients will be used to determine the MTD for ABC294640given in combination with gemcitabine. In Phase II, up to 40 patients with advanced pancreatic cancer will be dosed with the MTD of ABC294640 determined from phase I of the current trial in combination with the standard dose gemcitabine.

The primary objectives of phase I are to evaluate the safety and determine the maximum tolerated dose (MTD) for the drug combination. In the phase II component, similar pancreatic cancer patients will be randomized into Arms that will receive gemcitabine alone or gemcitabine plus ABC294640 at the MTD determined in phase I. The primary objectives of the phase II component will be to compare the efficacy of the ABC294640 plus gemcitabine combination to that of gemcitabine alone using RECIST criteria for tumor response. The secondary objectives for both components will be to determine the effects of ABC294640-treatment on the pharmacodynamic marker, plasma S1P levels and to assess the correlation of S1P alteration with tumor response. We are currently seeking funding to conduct the phase 1b/2 clinical trial.

In preparation for this next clinical trial, Apogee met with the FDA in June, 2014, to review data on the manufacturing of ABC294640, its safety profile in the single-agent phase 1 clinical trial, and a proposed plan for the development of the drug for mitigating gastrointestinal acute radiation syndrome (a separate project from this PA CURE project). Important to the proposed pancreatic cancer clinical trial, the FDA confirmed that the current manufacturing process is sufficient for clinical trials with both cancer patients and normal volunteers, i.e. the purity profile of the drug and supporting toxicology data are appropriate for its use in future clinical studies. Furthermore, the FDA agreed with Apogee's plan to study plasma levels of S1P as a pharmacodynamic marker of ABC294640 action in patients. The ongoing studies on the ability of ABC294640 to prevent GI toxicity from exposure to ionizing radiation provide strong support for testing the combination of ABC294640 + radiation for the treatment of pancreatic cancer patients. The intensity of radiotherapy in this patient population is limited by GI toxicity, so inclusion of ABC294640 in the treatment of unresectable pancreatic cancer could substantially improve treatment response in this disease. We are currently seeking funding to clinically test this hypothesis.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed

acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We are completing a mechanism-focused manuscript that describes the effects of ABC294640 on pancreatic cancer cells and the orthotopic tumor studies.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None. This project was for preclinical studies only. We expect that the project WILL have an impact on therapeutic approaches to pancreatic cancer, but it will be several more years before definitive clinical trials are completed.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This project provides essential preclinical validation of a new approach toward the treatment of pancreatic cancer. There are several important outcomes from the research relating to pancreatic cancer.

First, the studies establish for the first time that pharmacologically inhibiting SphK2 in human pancreatic cancer cells blocks their ability to replicate and promotes cancer cell death. This fundamental information is a critical factor in "validation" of the target, i.e. providing experimentally-supported rationale for using ABC294640 as a drug in pancreatic cancer patients.

Second, the studies demonstrate several key aspects of how ABC294640 blocks pancreatic cancer cell function – of particular importance is the unexpected finding that a primary mechanism for blocking cancer cell replication is through down-regulation of c-Myc expression and activity. This oncoprotein is known to be a major driver is several other types of cancer, and so these studies are expected to have a broad impact on both solid and hematopoietic cancers.

Third, the data from the orthotopic tumor studies contradicts our original hypothesis that combinations of ABC294640 with other anticancer drugs will be more effective for pancreatic cancer chemotherapy. Instead, the studies shown that ABC294640 is optimally effective by itself since the addition of Abraxane did not improve the antitumor activity of ABC294640. This finding will greatly simplify, streamline and accelerate clinical trials in pancreatic cancer patients.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:

Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes___X____ No_____

If yes, please describe your plans:

ABC294640 is protected by patents owned by Apogee (issued in 2008). These studies and the follow-on clinical trials will "de-risk" the product, thereby giving us a much greater chance of out-licensing the drug for advanced clinical development and commercialization.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

This is a nonformula grant. Biosketches for Drs. Maines and Smith were provided in the original grant application.