

Apogee Biotechnology Corporation

Annual Progress Report: 2011 Nonformula Grant

Reporting Period

July 1, 2013 – May 31, 2014

Nonformula Grant Overview

The Apogee Biotechnology Corporation received \$832,608 in nonformula funds for the grant award period June 1, 2012 through May 31, 2014. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Development of ABC294640 for Combination Chemotherapy of Pancreatic Cancer – This project will complete preclinical studies and regulatory activities to support an Investigational New Drug (IND) application to evaluate ABC294640 in combination with Gemzar and/or Abraxane for the treatment of pancreatic cancer. ABC294640 is currently in single-agent phase I testing, and has received Orphan Drug designation from the FDA for pancreatic cancer. To complete the preclinical studies, we will define the therapeutic efficacy of ABC294640 *in vitro* and *in vivo* using an orthotopic model of pancreatic cancer, both as a single agent and in combination with gemcitabine or *Abraxane*. Concomitantly, we will complete preclinical regulatory tasks required to support an IND application and subsequent clinical testing of ABC294640 in combination with Gemzar and/or Abraxane in patients with advanced pancreatic cancer.

Duration of Project

6/1/2012 – 5/31/2014

Project Overview

The research objectives of this project are to complete a preclinical efficacy and safety data package and all 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 following Specific Aims will be completed:

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, antiangiogenic and antimetastatic 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 all 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.

Principal Investigator

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Expected Research Outcomes and Benefits

The expected outcome of this research project is the enablement of an innovative Phase I/IIa clinical trial of a new drug combination for the treatment of pancreatic cancer. There is a dire need for improvement of therapy for pancreatic cancer. The American Cancer Society estimates that the incidence of pancreatic cancer in the United States in 2010 was 43,140 cases.

Approximately 20% of new pancreatic cancer patients present with localized disease, and for them, surgery alone provides a median survival of 12-14 months. However, there is a very high incidence of locoregional and systemic recurrence in these patients. Additionally, poor response to systemic chemotherapy in patients with more advanced disease results in a dismal, overall 5-year survival rate of <5%. Systemic therapy for pancreatic cancer typically includes the

antimetabolite gemcitabine, which provides palliation of symptoms, but has minimal impact on survival.

New and more effective therapies are needed for pancreatic cancer patients, and significant effort is being focused on the identification of appropriate new targets for pancreatic cancer drugs.

Sphingolipid metabolism is being increasingly recognized as a key pathway in cancer biology. Extensive research on sphingolipid metabolism demonstrates the roles of ceramide, sphingosine and sphingosine 1-phosphate (S1P) in regulating tumor cell apoptosis and proliferation, as well as angiogenesis, inflammation and tumor sensitivity to anticancer drugs. Apogee Biotechnology Corporation has developed the first non-lipid inhibitors of sphingosine kinases (SK1 and SK2), and conducted extensive studies of their therapeutic activities in multiple models of cancer and inflammatory diseases. Studies with pancreatic cancer cells and xenografts support the hypothesis that ABC294640 will be an effective therapeutic agent in pancreatic cancer. The significance of the proposed project is further enhanced by the fact that ABC294640 has entered clinical trials as a single agent. Furthermore, the FDA has granted Orphan Drug status to ABC294640 for the potential treatment of advanced pancreatic cancer.

The completion of this project will enable a promising clinical trial evaluating a new target and drug and its ability to extend life and improve the quality of life, providing new hope for pancreatic cancer patients and their families.

Summary of Research Completed

Specific Aim 1. To evaluate the effects of ABC294640, alone and in combination with gemcitabine or paclitaxel, on pancreatic cancer cells in vitro. 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. We have previously reported mechanistic studies on the combination of ABC294640 with Paclitaxel (PTX), a microtubule-stabilizing drug, showing effects of the combined treatment on cell cycle progression, apoptosis, autophagy, sphingolipid profiles, microtubule structure and certain signaling systems.

In this reporting period, we focused on rigorous analyses of synergy between ABC294640 and paclitaxel or gemcitabine. Figure 2 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). 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 3 and 4. 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 3) or paclitaxel (Figure 4) 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.

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: Figure 5 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. Figure 6 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. Finally, Figure 7 quantifies the dose-response curve for down-regulation of c-Myc, pRb and RRM2 (ribonucleotide 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.

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 8). Interestingly, these cells metastasize, which may allow quantification of the effects of the test drugs on both primary tumor growth and metastasis. The effects of treatment of mice bearing orthotopic pancreatic tumors with ABC294640 alone or in combination with Abraxane were examined. As indicated in Figure 9, 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.

Specific Aim 3. To complete all 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. 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 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/m², weekly) and cohorts will receive increasing doses of oral ABC294640. The starting dosage for ABC294640 will be 1/3 the MTD determined in a previous single-agent phase I trial. It is expected that up to 12 patients will be used to determine the MTD for ABC294640 given 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 impurity 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 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.

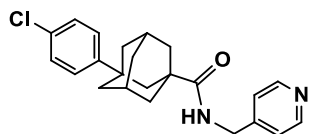


Figure 1. ABC294640

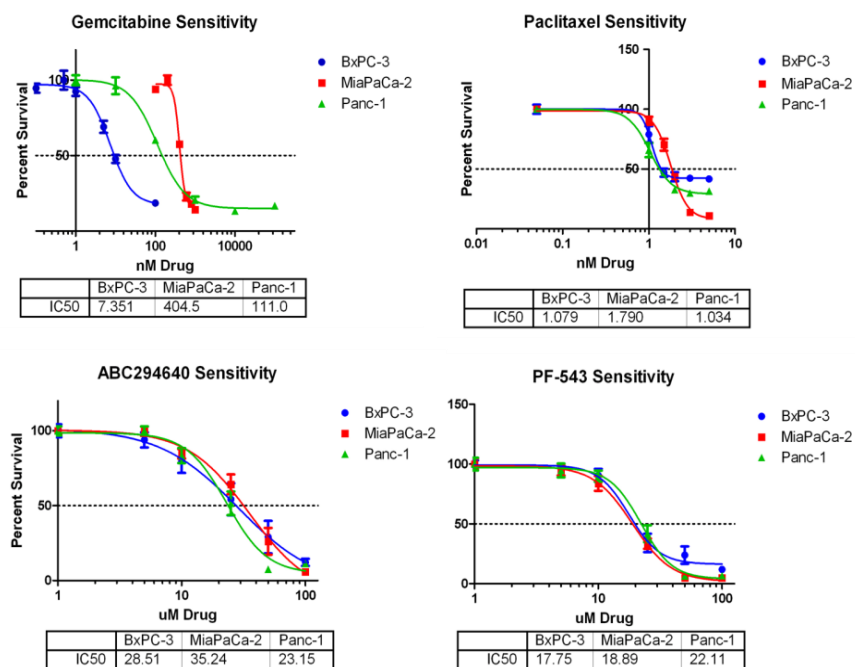


Figure 2. Cytotoxicities of study drugs toward pancreatic cancer cell lines.

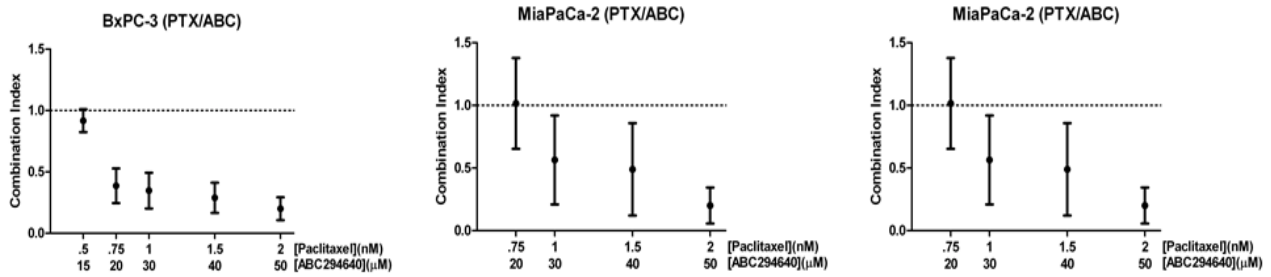


Figure 3. Interactions between ABC294640 and Paclitaxel in pancreatic cancer cell lines.

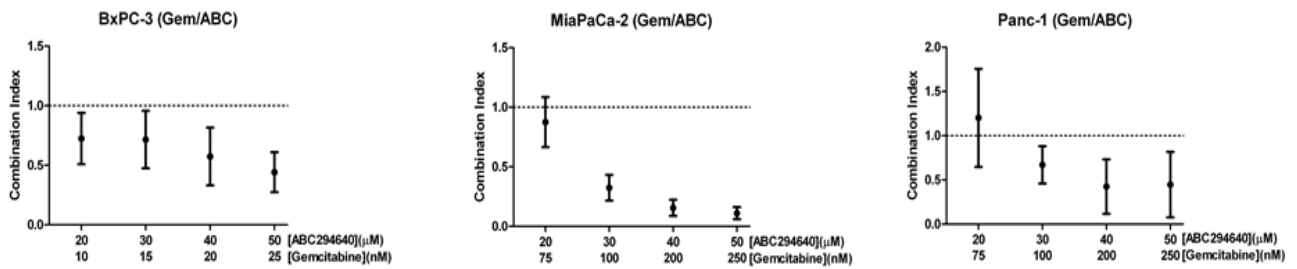


Figure 4. Interactions between ABC294640 and gemcitabine in pancreatic cancer cell lines.

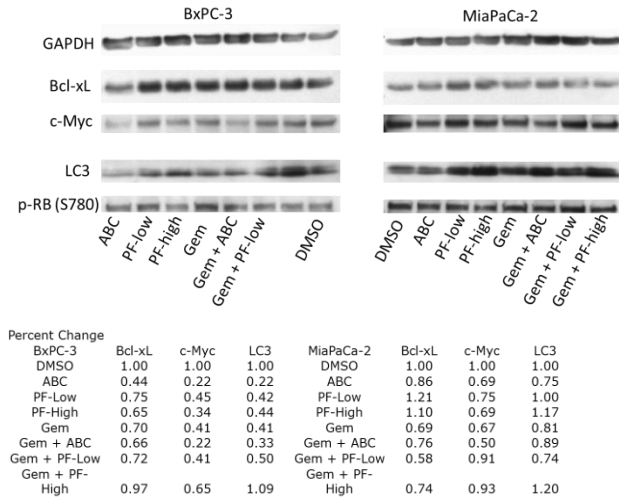


Figure 5. Signaling effects of SphK inhibitors and gemcitabine in pancreatic cancer cells.

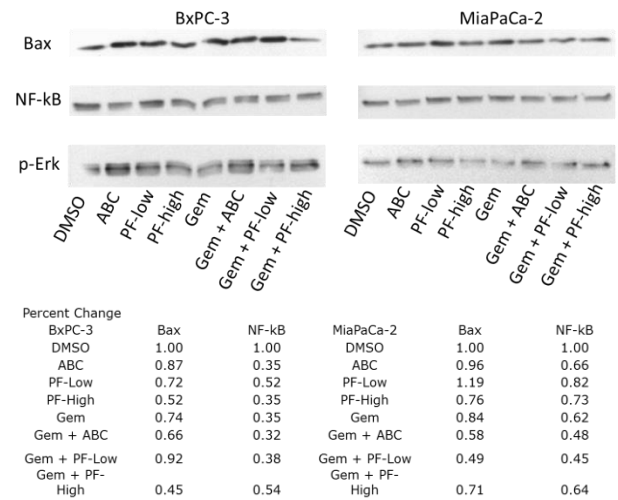


Figure 6. Signaling effects of SphK inhibitors and gemcitabine in pancreatic cancer cells.

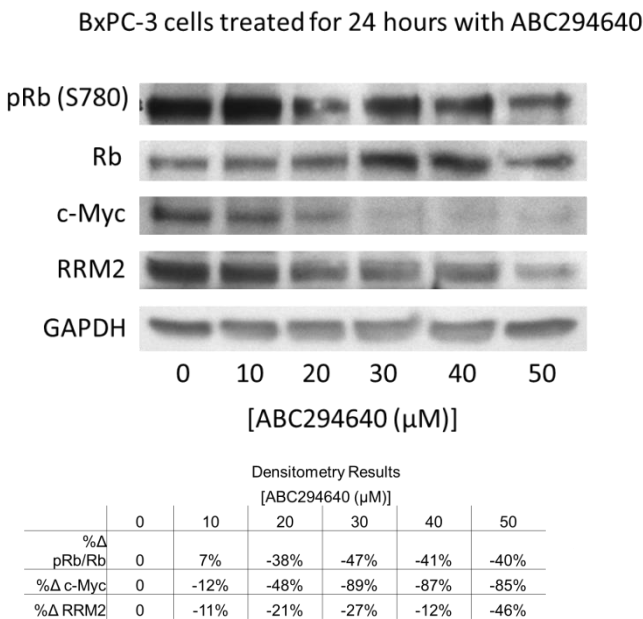


Figure 7. ABC294640 dose-response for signaling inhibition in BxPC-3 cells.

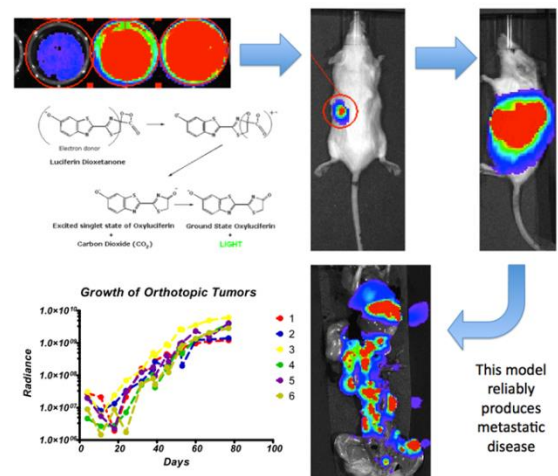


Figure 8. Orthotopic pancreatic cancer model.

Figure 9. Inhibition of pancreatic tumor growth by ABC294640.

