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Using a novel NQO1 bioactivatable drug, beta-lapachone (ARQ761), to enhance chemotherapeutic effects by metabolic modulation in Pancreatic Cancer

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Abstract

Novel, tumor-selective therapies are needed to increase the survival rate of pancreatic cancer patients. K-Ras-mutant-driven NAD(P)H:quinone oxidoreductase 1 (NQO1) is over-expressed in pancreatic tumor *vs* associated normal tissue, while catalase expression is lowered compared to levels in associated normal pancreas tissue. ARQ761 undergoes a robust, futile redox cycle in NQO1+ cancer cells, producing massive hydrogen peroxide (H₂O₂) levels; normal tissues are spared by low NQO1 and high catalase expression. DNA damage created by ARQ761 in pancreatic cancer cells 'hyperactivates' PARP1, causing metabolic catastrophe and NAD⁺-keresis cell death. NQO1: catalase levels (high in tumor, low in normal tissue) are an attractive therapeutic

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window to treat pancreatic cancer. Based on a growing body of literature, we are leading a clinical trial to evaluate the combination of ARQ761 and chemotherapy in patients with pancreatic cancer.

Keywords

β -lapachone; pancreatic cancer; experimental therapeutics; cancer metabolism; hyperpolarization

Introduction

Pancreatic cancer has risen in incidence and is a historically recalcitrant disease that is expected to be the second leading cause of cancer-related deaths by 2020^{1,2}. Current therapies against pancreatic cancer lack rationale that exploit cancer-specific targets, are subject to inherent resistance mechanisms and are ineffective against non-cycling cancer cells. Effective treatment for pancreatic cancer will require use of agents that cause tumor-specific cell death, independent of oncogenic driver mutations such as Kras and p53, or apoptotic processes (e.g., caspase-independent). A majority of cancers lack functional p53, have activated or mutant tumor driver mutations, and/or have defects in apoptotic pathways that confer growth advantages and drug resistance^{3,4}. Agents that kill pancreatic cancer cells irrespective of growth state (i.e., effective against stationary and log-phase cells) and target primary and metastatic tumor cells are needed, since resistant, growth-arrested cancer (stem-like) cells can repopulate a tumor or metastasize.

NAD(P)H:quinone oxidoreductase 1 (NQO1) expression is an exploitable, tumor-specific target for pancreatic cancer therapies. Elevated NQO1 expression is noted in many early forms of cancers such as in pancreatic intraepithelial neoplasia (PanINs)⁵, prostatic intraepithelial neoplasia^{6,7} and breast ductal carcinoma in situ^{8,9} and further increase in this enzyme occurs as these cancers progress.¹⁰ NQO1 bioactivatable drugs are a unique class of rare quinones that include β -lapachone (β -lap, ARQ761 in clinical form) and deoxynyboquinones.¹¹ NQO1 catalyzes the two-electron oxidoreduction of β -lap to generate an unstable hydroquinone that spontaneously reacts with two oxygen molecules in a two-step back-reaction to regenerate the original compound.¹² NQO1-dependent futile redox cycling oxidizes ~60 moles of NAD(P)H to create ~120 moles of reactive oxygen species (ROS) in ~2 mins.^{11,13} High levels of superoxide dismutase (SOD) in cancers ultimately generates long-lived and cell membrane-permeable hydrogen peroxide (H₂O₂) that diffuses into the nucleus and causes massive oxidative base and single strand break DNA lesions. Rapid accumulation of DNA lesions overwhelms the cell's DNA repair capacity and causes 'hyperactivation' of poly(ADP-ribose) polymerase-1 (PARP1). Rapid protein PARylation, including PAR-PARP1, severe NAD⁺/ATP depletion, massive DNA lesions and repair inhibition follows.¹¹ ROS (H₂O₂) formation only occurs while pools of NAD(P)H are available for NQO1-driven futile redox cycling of the drug. A lethal dose of β -lap induces caspase-independent programmed necrosis (i.e. NAD⁺-keresis) (Figure 1).^{6,7,11,10}

Concomitant NQO1 overexpression and loss of catalase are a hallmark of many solid cancers. NQO1 is over-expressed in >85% of resected pancreatic cancer samples and is preferentially expressed in pancreatic cancer vs. non-cancer adjacent pancreas.^{14,15} A

majority (>90%) of pancreatic tumors have over-expressed NQO1 levels, believed mainly due to mutant K-Ras driven transcription.^{5,16} We assessed NQO1 mRNA levels in 462 pancreatic adenocarcinoma tissues *vs.* associated normal tissue, where uniformly elevated NQO1 levels were noted, consistent with our prior data¹⁷. As also noted in non-small cell lung cancer, head and neck, prostate, and breast cancers^{6,8,12,18}, we also demonstrated a reciprocal loss of expression of catalase in pancreatic adenocarcinoma tumors, with elevated catalase and low NQO1 levels in associated normal tissue.¹⁶ Thus, NQO1: catalase ratios in pancreatic adenocarcinoma tumor *vs.* associated normal pancreas tissue of patients is predicted to be an important and highly exploitable therapeutic target. Similar NQO1: catalase ratios at the mRNA level have been reported in pancreatic cancer.^{5,16,17,19,20} This distribution of redox cycle-related enzymes in tumor *vs.* normal tissue strongly suggest that NQO1 bioactivatable drugs (i.e., β -lapachone (β -lap) are ideally suited for treatment of pancreatic adenocarcinoma that have a high rate of NQO1 expression. Thus, NQO1: catalase ratios represent an exploitable therapeutic window for treatment of pancreatic adenocarcinomas.^{11,16,21}

β -Lapachone provides synergistic antitumor activity against preclinical human pancreatic cancer xenografts *in vivo* as a single agent, as well as when combined with standard of care chemotherapy. β -Lapachone is highly efficacious against human MiaPaCa2 xenografts in athymic nude mice due to increased drug PK (and tumor-selective PD) effects in the pancreas.²² Efficacy was demonstrated by a high number of cures in mice seen at 20–30 mg/kg HP β CD- β -lap against MiaPaCa2 xenografts. When administered in combination with DNA damaging agents (e.g., ionizing radiation), doses of HP β CD- β -lap as low as 5 mg/kg are highly efficacious where 100% cures were seen at 300 days. Additionally, efficacy was verified by *ex vivo* tumor luciferase activities 14–60 days post-therapy.^{22,23} Significant inhibition of ¹⁸F¹⁸FDG uptake is seen when post-treatment efficacy is evaluated 7 days later, suggesting that glucose metabolism was strongly suppressed during tumor regression in preclinical models.^{23,24} Beta-lapachone synergized with multiple DNA damaging agents^{12,18,25,26}, including gemcitabine^{24,23} and paclitaxel.^{27,28} Mechanistically, combination therapies are predicted to enhance DNA damage to a threshold that triggers PARP1 hyperactivation and cell death at sublethal doses of both the DNA damaging agent(s) and β -lapachone.^{6,11,16,29}

We are leading the clinical development of a novel NQO1 bioactivatable drug, β -lapachone (used as HP β CD- β -lap, ARQ761). The major toxicities of ARQ761 monotherapy have been methemoglobinemia and hemolytic anemia. Other toxicities normally associated with conventional cytotoxic regimens such as nausea, neutropenia and thrombocytopenia were not observed. Single-agent clinical activity has been seen starting at the first dose level studied (195 mg/m²).³⁰

NQO1 expression is being developed as a companion diagnostic with ARQ761 and is used as an integral biomarker on clinical trials. Archival formalin-fixed, paraffin embedded tissue was tested for NQO1 expression using an immunohistochemistry (IHC) assay. In the first in human phase 1 clinical trial of monotherapy ARQ761, NQO1 expression was found to be a principal determinant of disease response. After evaluation of 19 patients with advanced solid tumors, there was a positive trend ($P=0.06$) between clinical benefit and tumor NQO1

expression. NQO1 expression by IHC was subsequently used as an enrichment/enrollment biomarker and only patients with NQO1-positive tumors were enrolled. A histo-score (H-score) was assigned based on intensity of NQO1 expression and prevalence of positive tumor cells. An H-Score of 200 (range 0–300) has been determined to be the cut off in defining NQO1 expression as positive or negative.³⁰ Future studies will validate the NQO1:catalase ratio in patient tumors as a biomarker of response, and determine patterns of expression across multiple tumor types (including pancreas, non-small lung, triple negative breast and bladder cancers), changes in expression with treatment and the relationship of NQO1 expression with molecular biomarkers (e.g., KRAS).

Rationale for Phase 1b clinical trial of ARQ761 with gemcitabine and nab-paclitaxel in advanced pancreatic cancer

The combination of gemcitabine and nab-paclitaxel has demonstrated improved overall survival compared to single agent gemcitabine alone and has emerged as the new standard reference chemotherapy for patients with advanced pancreatic cancer and good performance status³¹. Based on the data above, we demonstrated synergistic anti-tumor effects with beta-lapachone (ARQ761) plus gemcitabine in pre-clinical models. We propose building on gemcitabine plus nab-paclitaxel chemotherapy backbone by combining this doublet with ARQ761. We anticipate this combination will be safe and tolerable and result in substantial synergy and antitumor efficacy in patients with advanced pancreatic cancer.

As drug levels are cleared from circulation rapidly, biweekly dosing is feasible. Such a rapid rise and clearance of drug levels is ideally suited for the ‘kiss of death’ responses of NQO1 bioactivatable drugs, such as β -lapachone, where such treatments take advantage of the therapeutic window of NQO1-mediated burst of H_2O_2 and subsequent PARP1 hyperactivation in tumor and spare these effects on normal tissue. This affords an irreversible cell death mechanism as long as 4–6 μ M doses are achieved for 1–2 h.

Clinical trial: phase 1b clinical trial of ARQ761 treatment with gemcitabine/nab-paclitaxel chemotherapy in pancreatic cancer

This is a single arm, open label, multi-institution phase 1b clinical trial for patients with advanced pancreatic cancer (NCT02514031). The primary objective is to determine the maximum tolerated dose (MTD) of ARQ761 when administered in combination with gemcitabine and nab-paclitaxel. Secondary objectives include determining 1) the safety and tolerability of the combination, 2) clinical activity as defined by overall response rate (ORR), progression free survival (PFS) and time to progression (TTP) and 3) pharmacokinetic profile of ARQ761 when administered in combination with chemotherapy. To further enhance our understanding of this treatment strategy, additional exploratory objectives will be performed. This includes correlation of NQO1 expression with clinical outcomes and determination of change in PAR-PARP1, gamma-H2AX formation, and NAD^+ /ATP losses in patient biopsies.

Inclusion criteria include patients with histologically or cytologically confirmed pancreatic adenocarcinoma that is metastatic, unresectable, or recurrent. Patients may have received, at most, one line of prior non-gemcitabine chemotherapy for metastatic/unresectable disease. Patients must have measurable disease as required per RECIST criteria 1.1³² and good performance status as defined by Eastern Cooperative Group performance status of 0 or 1. Patients with untreated brain metastases are excluded. Other exclusion criteria include pregnant women, laboratory abnormalities, uncontrolled intercurrent illness or social situations that would limit compliance with or tolerability of study requirements or conditions that may confound the ability to interpret data from the study. Although all tumors will be retrospectively analyzed for NQO1 status using the IHC assay, NQO1 status is not used as an enrichment biomarker in this clinical trial. Based on published reports, we expect the rate of NQO1 expression to be greater than 85% in pancreatic adenocarcinoma.^{14,15} Also, by enrolling patients regardless of NQO1 status, the investigators will learn about the predictive effect of NQO1 in the pancreatic cancer population which can help inform future ARQ761 clinical trial designs.

Treatment schema

Treatment will include gemcitabine, nab-paclitaxel, and ARQ761. Patient treatment will consist of gemcitabine and nab-paclitaxel administered intravenously on days 1, 8, 15 -every 28 days, as per the MPACT clinical trial (figure 2).³¹

ARQ761 will be delivered on days 1 and 15 as an intravenous infusion to start 60 mins after completion of gemcitabine and nab-paclitaxel to allow for incorporation of gemcitabine and additional DNA damage created in combination with nab-paclitaxel. Mechanistically, ARQ761 (β -lap) will synergize best (requiring the least levels of both drug within the tumor) at a time when extensive DNA lesions created by gemcitabine and nab-paclitaxel are present, allowing PARP1 hyperactivation.^{12,22,33-35}

Starting dose of gemcitabine (1000 mg/m²) + nab-paclitaxel (125 mg/m²) follow the doses used in the MPACT clinical trial.³¹ The maximum tolerated dose (MTD) of ARQ761 will be determined by dose-titrating using a standard 3+3 method, as highlighted in Table 1.³⁶ Three patients within a dose level must be observed for one cycle (28 days) before accrual to the next higher dose level may begin. If a subject is withdrawn from the study prior to completing 22 days of combination therapy without experiencing a Dose limiting toxicity (DLT) prior to withdrawal, an additional subject may be added to that dose level. If a subject misses 2 doses for reasons other than adverse events, an additional subject may be added to that dose level. For the dose expansion phase of the study, there will be no replacements of subjects made.

DLT will be defined by the occurrence of any of the following toxicities possibly or probably related to drug during cycle 1 as defined by the NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.0)

- a. Grade 3 or 4 hemolysis or hemolytic anemia, except
 - i. Transfusion for hemolysis or hemolytic anemia will be considered a DLT only if Hgb < 8.0 g/dL (Grade 3 anemia)

- b. Any drug related grade 3 or grade 4 non-hematological toxicity, except alopecia, or asymptomatic elevation of hepatic transaminases thought to be secondary to gemcitabine.
- c. Grade 3 diarrhea, vomiting, or nausea that persists for > 3 days despite optimal supportive care.
- d. Grade 4 neutropenia lasting more than 5 days or febrile neutropenia.
- e. Platelets <50,000/ μ l for longer than 5 days or any platelet count <25,000/ μ l.
- f. Grade 3 or 4 hyperbilirubinemia that does not recover to Grade 1 within 7 days
- g. Missing two consecutive weekly doses of gemcitabine and nab-paclitaxel because of unresolved toxicity.
- h. Grade 3 hypoxia observed at the end of infusion of ARQ761 on the pulse oximetry readout without clinically significant symptoms will not be considered as a DLT. Arterial Blood Gas (ABG) will be obtained at the discretion of the investigator. Arterial O₂ saturation levels will be used to evaluate hypoxia if required.
- i. Any other toxicity possibly or probably related to treatment in the view of the investigator that represents a clinically significant hazard to the patient.
- j. Anemia or lymphopenia is not considered a DLT unless as specified above.
- k. Infusion related reaction Grade 4 or Grade 3 infusion-related reaction that persists for more than 24 hours or that precludes administration of the full dose of the investigational drug.

Tumor response and toxicity monitoring

Disease response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee.³² Any patient who receives treatment on this protocol will be evaluable for toxicity. Toxicity will be assessed according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0. The most common adverse events noted with the MPACT study that resulted in a dose reduction, delay or withholding of chemotherapy was neutropenia and thrombocytopenia.³¹ We will follow standard recommended dose modifications for gemcitabine and nab-paclitaxel per the MPACT trial.

Pharmacokinetic and pharmacodynamic sampling

Serial venous blood samples will be drawn from each subject during the first cycle on days 1 and 15 for determination of plasma levels of ARQ761. Serial venous blood samples will be drawn from each subject before and three hours after cycle 1 days 1 and 15 infusions of study drug for biomarker analyses.

Tumor biopsies will be performed at baseline (if not available) and after cycle 1. The goal of the planned laboratory correlative studies is discussed in the exploratory objectives indicated

above. The purpose of the follow up biopsy is to inform the mechanism of action of ARQ761 and chemotherapy and will drive the translational aspect of the study. The planned analysis includes immunohistochemistry (IHC) analysis of NQO1, catalase, TUNEL, Ki-67 and γ -H2AX by IHC. Additional analysis by western blot for μ -calpain and PARP cleavage will be performed.

Conclusion

Rational hypothesis driven treatments for pancreatic cancer that exploit a well-defined mechanism of action are needed. Difficulty in obtaining pancreatic cancer tumor tissue has hampered the ability to develop markers of response and predictors of efficacy. This phase 1 clinical trial explores a novel bioactivatable compound to target pancreatic cancer therapy. Pharmacodynamic markers will validate NQO1: catalase expression as a predictive biomarker of response.

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Synopsis

Using a defined mechanism of action of a unique NAD(P)H:quinone oxidoreductase 1 (NQO1) bioactivatable compound, beta-lapachone (ARQ761), and multiple therapeutic agents we defined specific synergistic modalities for the treatment of pancreatic cancers, which express high levels of this enzyme. We are leading a clinical trial of beta-lapachone plus chemotherapy in patients with advanced pancreatic cancer.

β -Lap cytotoxicity: “noncaspase-mediated programmed necrosis”

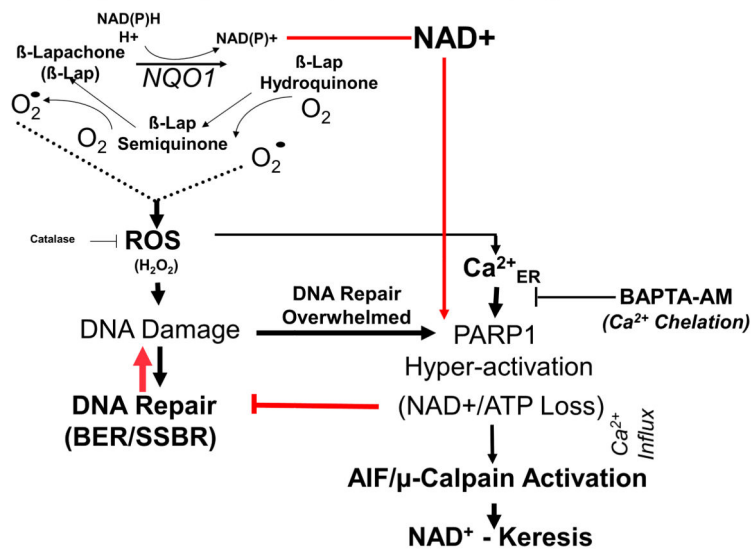


Figure 1. β -Lap causes unique NQO1-specific antitumor lethality irrespective of a wide range of driver or carrier mutations. *A*, NQO1-mediated cell death by β -lap. Note the combined futile cycle mechanism of NQO1-mediated redox cycling and H_2O_2 -induced PARP1 hyperactivation reduce NAD(P)H equivalents that stimulates NAD⁺ elimination by GAPD-SNO-NAD conjugation (glycolysis inhibition) and PARP1 ADP-ribosylation.

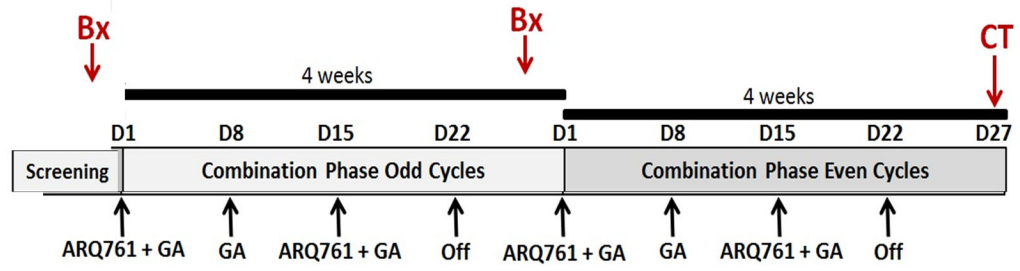


Figure 2. Phase 1b clinical trial of ARQ761 combined with gemcitabine and nab-paclitaxel in patients with advanced pancreatic cancer

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Table 1

Dose escalation schema for the Phase 1b clinical trial of ARQ761 combined with gemcitabine and nab-paclitaxel in patients with advanced pancreatic cancer.

Dose Level	ARQ761 IV D1, 15	Gemcitabine D1, 8, 15	Nab-paclitaxel D1, 8, 15
-3	136 mg/m ²	1000 mg/m ²	125 mg/m ²
-2	156 mg/m ²	1000 mg/m ²	125 mg/m ²
-1	175 mg/m ²	1000 mg/m ²	125 mg/m ²
1	195 mg/m ²	1000 mg/m ²	125 mg/m ²
2	290 mg/m ²	1000 mg/m ²	125 mg/m ²
3	390 mg/m ²	1000 mg/m ²	125 mg/m ²
Expansion	MTD	1000 mg/m ²	125 mg/m ²

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