Modified Scheduling of Gemcitabine and Nab-paclitaxel Results in Increased Treatment Efficacy in Pancreatic Cancer *in vitro* and *in vivo*

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Abstract

Objectives of the Study: Gemcitabine and nab-paclitaxel (Abraxane®) is a chemotherapy regimen commonly administered for patients with advanced/metastatic pancreatic cancer. The current clinical approach relies on the simultaneous administration of both drugs. However, we have previously shown that gemcitabine upregulate Caveolin-1 (Cav-1), a protein involved in nutrient endocytosis, including albumin and albumin-bound chemotherapy such as nab-paclitaxel. We hypothesized that pre-treatment of cells with gemcitabine followed by nab-paclitaxel treatment using altered scheduling could enhance the effectiveness of the treatment when compared to simultaneous administration.

Methods Used: In vitro and in vivo models of pancreatic cancer were used to compare the efficacy of the sequential treatment regimen compared to the clinically delivered simultaneous treatment. AlamarBlue, colony forming assays, immunoblotting, flow cytometry analyses, and in vivo tumor growth assays in athymic nude mice were conducted.

Results and Conclusions: Immunoblotting revealed upregulation of Cav-1 expression and albumin uptake in a time-dependent fashion when tumor cells were treated with gemcitabine. AlamarBlue and colony forming assays established that the optimal scheduling resulting in the greatest degree of tumor cell kill was achieved when gemcitabine was administered 48 hrs before nab-paclitaxel. Cell cycle analysis revealed induction of G1 and S phase arrest in gemcitabine-treated cells, followed by re-entry of cells into G2/M at about 48-60 hrs after gemcitabine treatment. In vivo models with AsPC1, HPAFII, and a novel patient-derived pancreatic cancer cell line (G37) confirmed substantial improvement in efficacy of altered scheduling with pre-treatment with gemcitabine on Day 1, followed by nab-paclitaxel on Day 3, compared with concurrent administration on Day 1. Ongoing studies are evaluating induction of apoptosis and post-mitotic death, the dependency of Cav-1, as well as the dependency of cell cycle specific effects on the improved efficacy of this scheduling regimen.

Caveolin-1 depletion leads to reduced nab-paclitaxel uptake

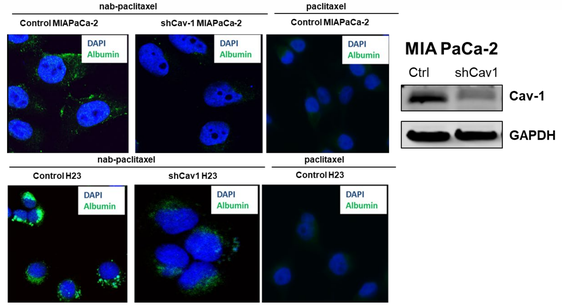

Fig. 1. Immunofluorescence microscopy of albumin in MiaPaCa-2 cells (top panel) and H23 cells (bottom panel) indicates reduced uptake in Cav-1 deficient cells

Gemcitabine treatment upregulates Caveolin-1 in tumor cells

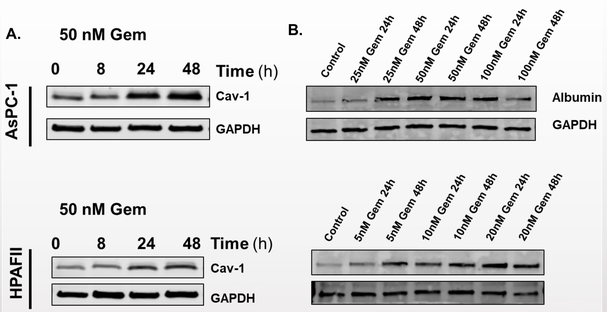

Fig. 2. (A). Western blot looking at time course of Cav-1 expression with 50 nM gemcitabine treatment in AsPC-1 cells (top blot) and HPAFII cells (bottom blot) shows Cav-1 upregulation. **(B).** Western blot staining albumin expression in AsPC-1 (top blot) and HPAFII cells (bottom blot) with increasing gemcitabine doses indicates increased albumin intake with gemcitabine.

Nab-paclitaxel delivery at 48 hours after gemcitabine increases cytotoxic effects

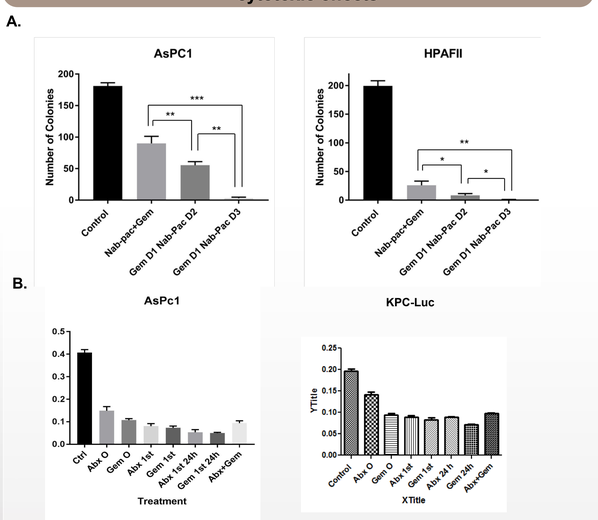

Fig. 3. (A) Colony forming assays were conducted looking at surviving colonies when AsPC-1 cells (left graph) and HPAFII cells (right graph) were treated with gemcitabine and nab-paclitaxel at differing sequences., showing the sequential treatment as more lethal to the cells than the simultaneous treatment. **(B)** An AlamarBlue assay was ran using the IC50 values of nab-paclitaxel (AsPc-1: 10 nM, KPC-Luc: 25 nM) and gemcitabine (AsPC-1: 60 nM, KPC-Luc: 7 nM) derived from cytotoxicity assay. The most efficacious treatment in both cell lines was gemcitabine pretreatment 48h prior to nab-paclitaxel addition

Western for apoptosis supports scheduling confirms efficacy of treatment

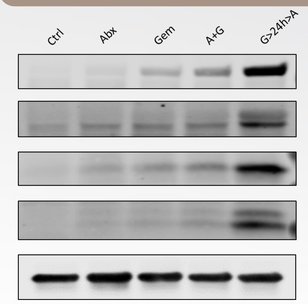

Fig. 4. Western blot staining for apoptotic protein expression between treatment groups reveals sequential treatment to express a greater amount compared to the combined treatment

Cell cycle analysis indicative of dependence on cell cycle for nab-paclitaxel efficacy

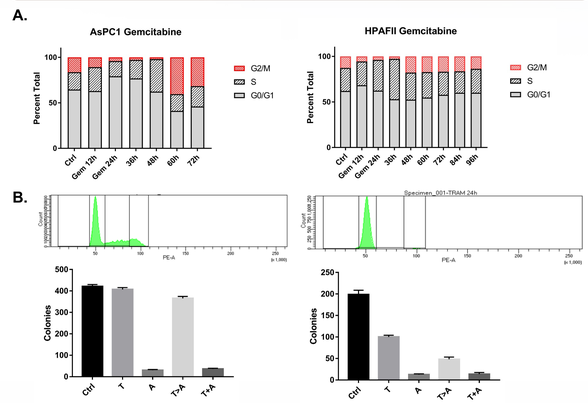

Fig. 5. (A) Flow cytometry analysis following gemcitabine treatment in AsPC-1 (left graph) and HPAFII (right graph) reveals an increase of cells in G2/M 48-60h after treatment. **(B)** Flow cytometry analysis of AsPC-1 cells treated with 10 nM trametinib 24 h prior confirms the drug stalls cells in G0/G1. Colony forming assay in AsPC-1 (left graph) and HPAFII cells (right graph) indicates an antagonistic effect between the two drugs.

shCav-1 knockdown suggestive of dependence of Caveolin-1 for efficacy of treatment

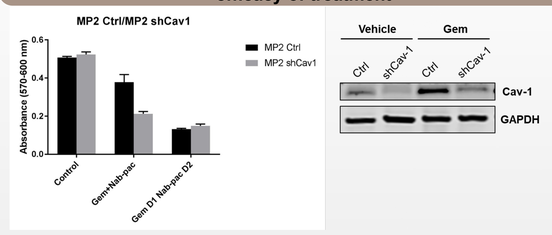

Fig. 6. AlamarBlue assay comparing potency of combination to potency of sequential treatment in Cav-1 proficient/deficient cells shows potential for Cav-1 as marker for efficacy

In vivo treatment

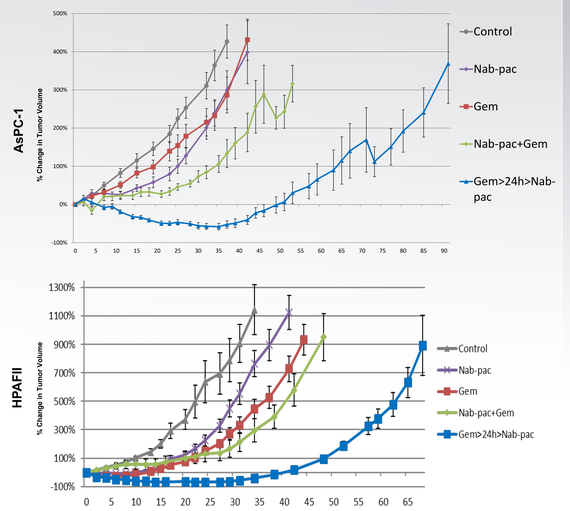

Fig. 7. Athymic nude mice were injected with flank tumors of AsPc-1 cells (top graph) and HPAFII cells (bottom graph), then treated with either gemcitabine and nab-paclitaxel simultaneously or pretreated with gemcitabine 48 hours prior to nab-paclitaxel administration.

Conclusions:

An antagonistic effect may be present between gemcitabine and nab-paclitaxel
Administration of gemcitabine and nab-paclitaxel sequentially can be more efficacious than when done simultaneously

Significance: This simple and alternative treatment regimen could potentially translate into better clinical outcomes for patients with pancreatic cancer, without changing the chemotherapeutic compounds currently administered in the clinic. A phase II trial is being designed on the basis of these preclinical studies.

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