Comparative benefits of Nab-paclitaxel over gemcitabine or polysorbate-based docetaxel in experimental pancreatic cancer

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Gemcitabine has limited clinical benefits in pancreatic ductal adenocarcinoma. The solvent-based traditional taxanes docetaxel and paclitaxel have not shown clinical results superior to gemcitabine or docetaxel. For pancreatic ductal adenocarcinoma cells AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1, gemcitabine IC50 ranged from 494 nM to 23.9 μM; docetaxel IC50 ranged from 5 to 34 nM; nab-paclitaxel IC50 range was from 243 nM to 4.9 μM. Addition of IC25 dose of docetaxel or nab-paclitaxel decreased gemcitabine IC50. Net tumor growth inhibition after gemcitabine, docetaxel or nab-paclitaxel was 67, 31 and 72 %, which corresponded with intratumoral proliferative and apoptotic indices. Tumor stromal density was decreased by nab-paclitaxel and to a lesser extent by docetaxel as measured through reduction in α-smooth muscle actin, S100A4 and collagen 1 expression. Animal survival was prolonged after nab-paclitaxel treatment (41 days, P = 0.002) compared with gemcitabine (32 days, P = 0.005), docetaxel (32 days, P = 0.005) and controls (20 days). Survival in nab-paclitaxel/gemcitabine and docetaxel/gemcitabine sequential treatment groups was not superior to nab-paclitaxel alone. Low-dose combination of gemcitabine with nab-paclitaxel or docetaxel was more effective compared with controls or gemcitabine alone but not superior to regular dose nab-paclitaxel alone. Combination treatment of gemcitabine+nab-paclitaxel or gemcitabine+docetaxel increased gemcitabine concentration in plasma and tumor. The superior antitumor activity of nab-paclitaxel provides a strong rationale for considering nab-paclitaxel as first-line monotherapy in pancreatic ductal adenocarcinoma.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in the Western countries, with an overall 5 year survival rate of <5% (1). Despite some advances in combination chemotherapy regimens, the prognosis of PDAC remains very poor, mostly due to late diagnosis, early and aggressive local and metastatic progression, and high resistance to conventional chemotherapy and radiation therapy. Pancreatectomy remains the single most effective treatment option for PDAC, despite the fact that only 10–20% of patients present with localized locoregional disease extent suitable for resection, and despite postoperative recurrence remaining very common (2–4). Therefore, much attention has been focused toward improving systemic treatment options for PDAC for possible definitive or perioperative therapy benefit. Gemcitabine (Gem), a deoxycytidine nucleoside analog, exerts its cytotoxic effects mainly by inhibiting DNA synthesis and inducing cell death. The use of gemcitabine is still a standard treatment for locally advanced and metastatic PDAC; however, it is only modestly effective with a median survival of ~6 months in randomized clinical trials (5–7). Therefore, other agents or drug combinations have been tested in advanced and metastatic PDAC (8–10). Taxanes, docetaxel and paclitaxel, showed promising antitumor activity in other solid tumors (11,12), which prompted testing in PDAC (13–15). Taxanes exert cytotoxic effects by preventing microtubule depolymerization and stabilizing microtubule causing decrease in free tubulin that leads to mitotic arrest and inhibition in cell proliferation. In a preclinical study, polysorbate-based docetaxel showed effectiveness in a murine PDAC model (16). Cremophor-based paclitaxel is one of the most widely used and effective anti-neoplastic agents with a wide spectrum of antitumor activity, particularly against ovarian cancer, breast cancer, non-small-cell lung cancer, head and neck cancer and kidney cancer (17). Nanoparticle albumin (nab)-bound paclitaxel is a water-soluble, cremophor-free, albumin-bound 130 nm particle formulation of paclitaxel that is widely approved for the treatment of metastatic breast cancer (18). Nab-paclitaxel initially was developed to avoid toxicity associated with solvent cremophor required to solubilize paclitaxel. Preclinical and clinical data have demonstrated superior efficacy and safety of nab-paclitaxel over solvent-based paclitaxel (18,19). Therefore, for advanced and metastatic PDAC, there is some importance to identify the most active single cytotoxic agent with low toxicity that can avoid chemoresistance and serve as a backbone for the exploration of combination with potential targeted agents. This study was undertaken to identify the most effective single agent in an experimental PDAC setting by comparing gemcitabine, docetaxel, a polysorbate-based taxane, and nab-paclitaxel, a water-soluble albumin-bound paclitaxel, and to evaluate effects and mechanisms of gemcitabine–taxane combinations.

Materials and methods

Reagents

Gemcitabine was purchased from Eli Lilly Corporation (Indianapolis, IN). Docetaxel (Taxotere) was purchased from Sanofi-Aventis (Bridge water, NJ). Nab-paclitaxel (Abraxane) was obtained from Abraxis BioScience (Los Angeles, CA). The cell proliferation reagent WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenes disulfonate) was purchased from Roche Diagnostic (Indianapolis, IN).

Cell culture

Human PDAC cell lines AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1 were obtained from the American Type Culture Collection (Rockville, MD). Cell lines were grown in RPMI 1640 medium (Sigma Chemical, St Louis, MO) supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO2 atmosphere. Each cell line was tested and authenticated by American Type Culture Collection. Cell lines were confirmed to be pathogen free.

In vitro cytotoxicity assay

Assays were performed using the colorimetric WST-1 reagent as described previously (20). Briefly, cells were plated in a 96 well plate and treated after 24 h with gemcitabine, docetaxel or nab-paclitaxel. The range of concentrations used was comparable with their clinically achievable concentrations. After 72 h, 10 μl WST-1 reagent was added in each well followed by additional incubation for 2 h. The absorbance at 450nm was measured using a microplate reader. Drug sensitivity curves and IC50 values were calculated using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA).

Abbreviations: IHC, immunohistochemical; PDAC, pancreatic ductal adenocarcinoma; SMA, smooth muscle actin; WST-1, 1-(4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzenes disulfonate).
Immunoblot analysis

Subconfluent monolayers of cells were treated with 10 μM concentrations of gemcitabine, docetaxel or nab-paclitaxel and lysed after 16 h. Tumor tissue lysates were prepared as described previously (21). Briefly, tumor tissues were immediately snap frozen in liquid nitrogen and stored at −80°C. These samples were crushed in liquid nitrogen using a sterilized mortar, resuspended in lysis buffer and extracts were sonicated. Proteins in supernatants were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) followed by blockade for 1 h in Tris-buffered saline-T. Membranes were incubated over-night at 4°C with the following antibodies: phospho-stathmin (Ser38; 4191) (Cell Signaling Technology, Beverly, MA), α-smooth muscle actin (SMA) (A5228), α-tubulin and glyceraldehyde 3-phosphate dehydrogenase (all from Sigma). The membranes were then incubated with the corresponding horse-radish peroxidase-conjugated secondary antibodies (Pierce Biotechnologies, Santa Cruz, CA). Enhanced chemiluminescence reagent (Perkin Elmer Life Sciences, Boston, MA) was used to detect specific bands and was quantitated by densitometry.

Subcutaneous tumor growth study

All animals were housed in a pathogen-free facility and had access to food and water ad libitum. Animal experiments were performed in accordance with the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center (Dallas, TX). Athymic female nude mice (4–6 weeks) were purchased from the National Cancer Institute and used to establish the subcutaneous xenograft model as described previously (22). Two weeks after AsPC-1 (0.75 × 10⁶) cell injection, mice were randomly grouped (n = 6–8 per group) and treated with gemcitabine (100 mg/kg, twice weekly), docetaxel (4 mg/kg, twice weekly) and nab-paclitaxel (10 mg/kg, twice weekly) for 2 weeks. Low-dose combination experiments were performed by treatment with gemcitabine (50 mg/kg, twice weekly), docetaxel (2 mg/kg, twice weekly) or nab-paclitaxel (5 mg/kg, twice weekly) for 2 weeks. The tumor volume (V) was calculated using the formula V = ½ (length × width²). Net growth in tumor size was calculated by subtracting tumor volume on the first treatment day from that on the last day. Mice were killed after the completion of treatment; tumors were dissected, weighed and processed for histological, immunohistochemical (IHC) and immunoblot analysis.

Immunohistochemical analysis

Tumor tissues fixed in 4% paraformaldehyde were embedded in paraffin. Paraffin-embedded tissues were sectioned (5 μm), deparaffinized and rehydrated, whereas heat-mediated antigen retrieval was performed using citrate buffer. After incubation in blocking buffer (CAS-Block; Invitrogen, Carlsbad, CA), tissue sections were incubated with following antibodies: α-SMA (1:200; Sigma), Ki67 (1:200), S100A4 (1:100), collagen 1 (1:200) (all from Abcam, Cambridge, MA) for 1 h followed by 40 min incubation with Cy3-conjugated secondary antibody (1:200) at room temperature. Slides were mounted using mounting solution containing 4′,6-diamidino-2-phenylindole (Invitrogen). Intratumoral apoptotic activity was evaluated by staining tissue sections with ‘Apoptag Apoptosis Detection Kit’ according to the manufacturer’s (Millipore, Temecula, CA) instructions. Fluorescence microscopy was used to detect fluorescent signals using the IX81 Olympus microscope.

Fig. 1. Gemcitabine, docetaxel and nab-paclitaxel inhibit in vitro proliferation of PDAC cells. Cells were plated on 96 well plates and treated with gemcitabine, docetaxel and nab-paclitaxel and incubated for 72 h. Following incubation, WST-1 reagent (10 μl) was added in each well and incubated for two additional hours. The absorbance at 450 nm was measured using a microplate reader. The resulting number of viable cells was calculated by measuring absorbance of color produced in each well. Data are the mean ± SD of triplicate determinations. Drug sensitivity curves and IC₅₀ values were calculated using GraphPad Prism 4.0 software.
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Pharmacokinetic analysis

Gemcitabine concentrations in tumor and plasma were analyzed as described previously (23). Two weeks after subcutaneous injection of $0.75 \times 10^6$ AsPC-1 cells, athymic nu/nu mice were randomly grouped ($n = 6$–8 per group) and treated intraperitoneally with phosphate-buffered saline (control), gemcitabine (50 mg/kg, twice weekly), docetaxel (2 mg/kg, twice weekly) or nab-paclitaxel (5 mg/kg, twice weekly) for 2 weeks. Tumor harvesting and plasma collection were performed at a time point that was 24 h after the final nab-paclitaxel or docetaxel treatment and 4 h after the final gemcitabine treatment. Tumor samples, snap frozen in liquid nitrogen, were homogenized in phosphate-buffered saline containing 100 μg/ml tetrathymuridine to prevent ex vivo metabolism of gemcitabine (2′,2′-difluoro-2′-deoxycytidine) to the inactive metabolite, 2′-2′-difluorodeoxyuridine. Plasma collection was also performed in the presence of 100 μg/ml of tetrathyridouridine. Analytical methods were developed to detect gemcitabine using an Applied Biosystems/MDS Sciex 4000 QTRAP mass spectrometer coupled to a Shimadzu Prominence LC. Chromatography was performed using a Phenomenex C8 column. Pharmacokinetic analysis was performed using the Pharsight WinNonLin software package. Non-compartmental modeling with sparse sampling was utilized.

Animal survival analysis

Animal survival studies were performed using 6- to 8-week-old female non-obese diabetic/severe combined immunodeficient mice (24). The mice were intraperitoneally injected with AsPC-1 ($0.75 \times 10^6$) cells, randomly grouped after 2 weeks and treated intraperitoneally similarly as described above in the subcutaneous tumor growth study. Animals were euthanized when turning moribund according to predefined criteria (25,26). Animal survival time was evaluated from the first day of treatment until death.

Statistical analysis

Data were analyzed using GraphPad Prism 4 Software. In vitro cell proliferation data are expressed as mean ± standard deviation. Statistical analysis for normally distributed in vivo study data was performed by analysis of variance for multiple-group comparison and Student’s t-test for the individual group comparison. Survival study statistics were assessed using log-rank group comparisons (GraphPad Prism 4). P values of < 0.05 were considered to represent statistically significant group differences.

Results

Gemcitabine, docetaxel and nab-paclitaxel inhibit PDAC cell proliferation

In vitro analysis of four PDAC cell lines revealed that the proliferation of these cell lines was differentially inhibited by gemcitabine, docetaxel and nab-paclitaxel. Gemcitabine dose dependently inhibited PDAC cell proliferation, and at 10 μM concentration inhibition in proliferation was 32, 74, 80 and 46% in AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1 cells, respectively (Figure 1). Docetaxel caused a more uniform inhibition in
cell proliferation of PDAC cells, with an inhibition in cell proliferation at 10 μM concentration of 98, 89, 96 and 99% in AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1 cells, respectively (Figure 1). Nab-paclitaxel also inhibited PDAC cell proliferation in a dose-dependent fashion; at 10 μM concentration of nab-paclitaxel, inhibition in cell proliferation was 56, 80, 79 and 63% in AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1 cells, respectively (Figure 1). These data suggest higher in vitro cytotoxic activity of docetaxel compared with gemcitabine or nab-paclitaxel.

To evaluate the effect of combination treatment benefits of gemcitabine with water-soluble and solvent-based taxanes, the IC\textsubscript{25} dose of nab-paclitaxel or docetaxel was combined with increasing doses of gemcitabine. Addition of nab-paclitaxel at IC\textsubscript{25} decreased the gemcitabine IC\textsubscript{50} from 23.9 to 1.6 μM for AsPC-1, 830 to 189 nM for BxPC-3, 494 to 123 nM for MIA PaCa-2 and 9.5 μM to 913 nm for Panc-1 cells (Figure 2A). Addition of the IC\textsubscript{25} dose of docetaxel decreased the gemcitabine IC\textsubscript{50} from 23.9 μM to 436 nm for AsPC-1, 830 to 470 nM for BxPC-3, 494 nm to 124 nM for MIA PaCa-2 and 9.5 μM to 0.2 nM for Panc-1 cells (Figure 2A). These data suggest that docetaxel more efficiently decreased the gemcitabine IC\textsubscript{50} than nab-paclitaxel except for BxPC-3 cells.

**Docetaxel and nab-paclitaxel induce phospho-stathmin and reduce tubulin**

The mechanism of action of docetaxel and nab-paclitaxel on cell proliferation inhibition was investigated by analyzing the expression of stathmin and tubulin, proteins regulating microtubule dynamics. Immunoblot analysis of AsPC-1 cells revealed that both agents, either alone or in combination, significantly increased the expression of phospho-stathmin, an inactive form of stathmin protein. Additionally, both nab-paclitaxel and docetaxel caused a pronounced decrease in α-tubulin expression (Figure 2B).

**Gemcitabine, docetaxel and nab-paclitaxel effects on local tumor growth**

Evaluation of gemcitabine, docetaxel and nab-paclitaxel therapy effects on local tumor growth revealed that all three agents had an inhibitory effect on tumor growth. Treatment of AsPC-1 tumor-bearing mice with gemcitabine resulted in statistically significant net tumor growth inhibition of 68% (P = 0.03) compared with the control group (Figure 3A and B). Docetaxel treatment caused 40% inhibition in net tumor growth. Nab-paclitaxel treatment of AsPC-1 tumor bearing mice was more effective and resulted in 72% inhibition in net tumor growth (P = 0.02) compared with controls (Figure 3A and B).

Tumor weight measurement at completion of therapy revealed that gemcitabine, docetaxel and nab-paclitaxel single-agent treatment groups had decreased mean tumor weight compared with controls. Mean tumor weight in control, gemcitabine, docetaxel and nab-paclitaxel therapy groups were 0.52, 0.23, 0.34 and 0.22 g,
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respectively (Figure 3C). Immunoblot analysis of tumor lysates from AsPC-1 murine xenografts indicated increased phospho-stathmin levels in docetaxel- or nab-paclitaxel-treated mice. Tumors from gemcitabine-treated mice also showed increased phospho-stathmin expression that was not seen in AsPC-1 cells grown in vitro. In contrast to the in vitro data, α-tubulin expression was not changed in tumor lysates from docetaxel- or nab-paclitaxel-treated mice (Figure 3D). No significant change in mice body weight was observed in gemcitabine, docetaxel or nab-paclitaxel therapy groups (Supplementary Figure S1A, available at Carcinogenesis Online).

Gemcitabine, docetaxel and nab-paclitaxel effects on intratumoral proliferation and apoptosis

Investigation of the mechanisms of antitumor activities of gemcitabine, docetaxel and nab-paclitaxel by IHC analysis of tumor tissues revealed that the tumors from these therapy mice presented a decreased tumor cell proliferation and nab-paclitaxel treatment was more effective. Relative to controls, intratumoral proliferation index was 57% (P < 0.001), 48% (P < 0.0002) and 37% (P < 0.0002) in gemcitabine, docetaxel and nab-paclitaxel-therapy groups, respectively (Figure 4A).

Intratumoral apoptosis analysis by Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling staining demonstrated a 3.9-fold increase in apoptotic index in the gemcitabine therapy group (P = 0.04), a 3-fold increase in the docetaxel-treated group (P = 0.03) and a 3.6-fold increase in the nab-paclitaxel-treated group (P = 0.009) compared with controls (Figure 4B).

Gemcitabine, docetaxel and nab-paclitaxel effects on tumor stroma composition

Gemcitabine, docetaxel and nab-paclitaxel treatment effects on stromal marker proteins α-SMA, S100A4 and collagen 1 were analyzed by IHC analysis of tumor tissues. Relative to controls, α-SMA-staining pixel intensity in gemcitabine, docetaxel and nab-paclitaxel treatment group was decreased by 11.6, 32.7 and 71.8%, respectively (Figures 5A and B). Immunoblot analysis of α-SMA protein expression in tumor tissue lysates also revealed a significant decrease in the nab-paclitaxel treatment group compared with control, gemcitabine or docetaxel group (Figure 5C). Other stromal marker protein S100A4 and collagen 1 contents were also significantly decreased by docetaxel and nab-paclitaxel but not by gemcitabine treatment. Nab-paclitaxel caused a greater decrease in the S100A4 and collagen 1 contents than that in docetaxel, but this difference was not statistically significant (Supplementary Figures S2 and S3, available at Carcinogenesis Online).

Gemcitabine, docetaxel and nab-paclitaxel effects on animal survival

An AsPC-1 PDAC xenograft model in non-obese diabetic/severe combined immunodeficient mice was used to evaluate the effects of gemcitabine, docetaxel or nab-paclitaxel on animal survival after treatment for 2 weeks, tumors were dissected and processed for IHC analysis. (A) Tumor tissue sections were stained with Ki67 antibody and slides were photographed under a fluorescent microscope. Ki67-positive cells were counted in five different high-power fields. (B) Intratumoral apoptosis was measured by staining tumor tissue sections with Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling procedure and photographed under a fluorescent microscope. Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling-positive apoptotic cells were counted in five different high-power fields. The data are expressed as the mean ± standard deviation. Gem, gemcitabine; DT, docetaxel; NPT, nanoparticle albumin-bound paclitaxel.

Fig. 4. Effects of gemcitabine, docetaxel and nab-paclitaxel therapy on intratumoral proliferative and apoptotic activities. AsPC-1 tumor-bearing mice were treated for 2 weeks, tumors were dissected and processed for IHC analysis. (A) Tumor tissue sections were stained with Ki67 antibody and slides were photographed under a fluorescent microscope. Ki67-positive cells were counted in five different high-power fields. (B) Intratumoral apoptosis was measured by staining tumor tissue sections with Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling procedure and photographed under a fluorescent microscope. Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling-positive apoptotic cells were counted in five different high-power fields. The data are expressed as the mean ± standard deviation. Gem, gemcitabine; DT, docetaxel; NPT, nanoparticle albumin-bound paclitaxel.
3 weeks (six doses) of therapy. In the untreated control group, the median animal survival was 20 days, which was increased to 32 days by gemcitabine treatment ($P = 0.005$) and 33 days by docetaxel treatment ($P = 0.005$). Nab-paclitaxel treatment was more effective with a resulting median animal survival of 42 days ($P = 0.0004$). Sequential therapy of docetaxel (three doses) followed by gemcitabine (three doses) was more effective than either therapy alone, resulting in a median survival of 43 days ($P < 0.0009$ versus controls or gemcitabine). Both sequential therapy groups did not fare significantly better than nab-paclitaxel single-agent therapy (Figure 5).

**Antitumor response of low-dose combination of gemcitabine with docetaxel or nab-paclitaxel**

Low-dose combination treatment benefits of gemcitabine (50 mg/kg, twice a week) with docetaxel (2 mg/kg, twice a week) or nab-paclitaxel (5 mg/kg, twice a week) on local tumor growth and animal survival was evaluated in AsPC-1 PDAC murine xenograft models. Under these conditions, the local tumor growth reduction by gemcitabine monotherapy was 48%. Inhibition in tumor growth was greater in gemcitabine plus docetaxel (61%) and gemcitabine plus nab-paclitaxel (78%) combination therapy groups (Figure 6A). There was no significant change in mouse body weight during the experiment (Supplementary Figure S1B, available at Carcinogenesis Online).

To investigate the mechanism of docetaxel and nab-paclitaxel-induced gemcitabine response, gemcitabine concentration was analyzed in plasma and tumors. Docetaxel or nab-paclitaxel combination treatments caused a >3-fold increase in gemcitabine plasma concentrations compared with gemcitabine monotherapy. Also, docetaxel or nab-paclitaxel combination treatments caused a >6-fold increase in gemcitabine tumor concentrations compared with gemcitabine monotherapy (Figure 6B).

Low-dose combinations of docetaxel or nab-paclitaxel increased gemcitabine-induced animal survival. In an experiment with 2-week therapy, median survival in the control group was 21 days; gemcitabine, docetaxel and nab-paclitaxel monotherapy increased survival to 25, 27 and 32 days, respectively (Figure 6C). Combination treatment with gemcitabine plus nab-paclitaxel increased animal survival to 44 days ($P < 0.03$ versus control, gemcitabine or nab-paclitaxel). Similarly, the combination treatment with gemcitabine plus docetaxel increased animal survival to 43 days ($P = 0.002$ versus control, gemcitabine or docetaxel) (Figure 6C).
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Discussion

PDAC is characterized by insufficient therapeutic armamentarium, unusual aggressiveness, early distant metastasis, frequent intrinsic or acquired chemoresistance, and thus a generally devastating prognosis. Poor drug delivery, due to the highly desmoplastic and hypoperfused nature of PDAC, increased intratumoral pressures and the short half-life of gemcitabine, may be partly responsible for its limited clinical response (27,28). The use of traditional taxanes, such as docetaxel or paclitaxel, as single-agent chemotherapy for PDAC showed promising preclinical results (16) but only moderate clinical activity (13,14,29–31). Docetaxel showed no clinical activity at lower dose (60 mg/mq) but some activity at higher dose (100 mg/mq) with a median overall survival of 7–8.5 months, but this was accompanied by some significant grade 3–4 toxicity (13,14,29). Paclitaxel therapy yielded a median overall survival of 5 months in a series of 45 patients with advanced PDAC (15). Also, a combination of these taxanes with gemcitabine did not produce better response than gemcitabine alone (32–35). Recently, an intense cytotoxic regimen (FOLFIRINOX) showed significantly better response than gemcitabine alone, but this regimen also has a significant side-effect profile (36). These studies not only showed the general impact of cytotoxic agents in treating PDAC patients but also supported the somewhat urgent need to identify the single most active cytotoxic agent that can be used as core drug and base for more effective combination regimens with better toxicity profiles. The scope of this present study was to evaluate the most active single-agent cytotoxic therapy by comparing treatment benefits of gemcitabine, the traditional standard chemotherapy agent; docetaxel, the most active solvent-based taxane in PDAC; and nab-paclitaxel, a water-based solvent-free taxane, in experimental PDAC. In addition, combination effects were to be elucidated.

Stathmin is a microtubule destabilizing protein that is frequently overexpressed in a number of cancers (37). Taxanes in their known antimicrotubule mechanism increase phosphorylation of stathmin, causing its inactivation that leads to microtubule stabilization and subsequent mitotic cell death (38). Taxanes have also been shown to inhibit tubulin expression (39). In our study, the in vitro mechanism of action of docetaxel and nab-paclitaxel appears very similar as both induce phospho-stathmin and decrease α-tubulin expression to similar extent. We would conclude that phospho-stathmin would not be able to serve as a useful biomarker that correlates with the greater in vivo activity of nab-paclitaxel. Gemcitabine treatment also showed an increase in phospho-stathmin in tumor lysates probably due to an induction in CDK-1 expression that has shown to be increased by gemcitabine and that can cause phosphorylation of stathmin (40). In contrast to the AsPC-1 cells in vitro data, there was no significant change in α-tubulin expression in tumor lysates probably due to the presence of multiple non-epithelial cell types in tumor tissues. Also, nab-paclitaxel did not cause any decrease in α-tubulin expression in endothelial cells such as...
human umbilical vein endothelial cells or WI-38 fibroblast cells (data not shown).

Targeting the desmoplastic tumor stroma, a histological hallmark of PDAC, is emerging as a potentially promising strategy for PDAC treatment. Alpha-SMA, a marker of pancreatic myofibroblast-like stellate cells, is increased in the stroma of PDAC and correlates with aggressive tumor phenotype (41). Other stromal markers S100A4 and collagen 1 are also overexpressed and correlated with poor prognosis in patients with pancreatic cancer (42,43). In our study, docetaxel and nab-paclitaxel reduced stromal density as measured by the decrease in α-SMA, S100A4 and collagen 1 expression compared with controls or after gemcitabine treatment. Importantly, nab-paclitaxel as single agent was more effective than docetaxel in decreasing stromal content. These findings suggest that reduction in tumor stroma density by nab-paclitaxel might support or correlate with its superior antitumor response, potentially supporting a greater concentration of chemotherapeutics within the tumor tissues, as recently proposed (44) and as shown in case of gemcitabine. This increased intratumoral gemcitabine concentration observed after nab-paclitaxel and gemcitabine combination treatment was correlated with decreased cytidine deaminase levels, a gemcitabine metabolizing enzyme (45). Interestingly, low-dose combination treatment of gemcitabine with nab-paclitaxel or docetaxel both increased gemcitabine concentration in tumors and plasma, indicating that increase in gemcitabine concentration may be related to taxanes in general and not specifically to nab-paclitaxel. We also observed a decreasing pattern in cytidine deaminase expression in tumor tissue lysates after docetaxel or nab-paclitaxel treatment (Supplementary Figure S1C, available at Carcinogenesis Online). Our findings suggest that although gemcitabine or docetaxel may show higher in vitro cytotoxic activity, nab-paclitaxel is the most effective single-agent chemotherapy agent in vivo probably due to its effects on tumor stroma and an increased drug delivery (44,46).

The intraperitoneal murine xenograft survival model reflects the rapidly progressive nature of the disease well (47) and revealed that nab-paclitaxel was clearly more effective than gemcitabine or docetaxel monotherapy. Nab-paclitaxel monotherapy appeared as effective as sequential therapy of nab-paclitaxel and gemcitabine or docetaxel and gemcitabine. This corroborates the fact that a recent phase II trial (19 patients) of nab-paclitaxel as second-line therapy in patients with advanced pancreatic cancer, who progressed on gemcitabine-based therapy showed it to be well tolerated, with preliminary evidence of activity in a subset of patients despite the second-line setting (48). Another recent phase I/II nab-paclitaxel and gemcitabine combination protocol has shown some significant increase in antitumor response compared with gemcitabine alone in advanced pancreatic cancer (44). In our study, the combination of gemcitabine at 100 mg/kg with nab-paclitaxel at 10 mg/kg or docetaxel at 4 mg/kg (all doses at their respective 80% of maximum tolerated dose in mice) resulted in severe toxic events. We therefore combined gemcitabine with nab-paclitaxel or docetaxel at half-dose to study potential combination treatment benefits. This low-dose approach for combination of gemcitabine (50 mg/kg) with nab-paclitaxel (5 mg/kg) or docetaxel (2 mg/kg) resulted in an increased antitumor response compared with gemcitabine alone but was very comparable with the nab-paclitaxel single-agent treatment response at the regular 10 mg/kg dose. Thus, combination of gemcitabine and either taxane at feasible concentrations does not outperform nab-paclitaxel monotherapy in this murine experimental setting. These findings provide a strong rationale for evaluating nab-paclitaxel as first-line monotherapy or as a backbone of combination regimens with potential biologic targeting agents in patients with pancreatic cancer.

Supplementary material

Supplementary Figures S1–S3 can be found at http://carcin.oxfordjournals.org/

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