Improved effectiveness of nanoparticle albumin-bound (*nab*) paclitaxel versus polysorbate-based docetaxel in multiple xenografts as a function of HER2 and SPARC status

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Nanoparticle albumin-bound (*nab*)-paclitaxel (Abraxane) is an albumin-bound 130-nm particle form of paclitaxel that demonstrated higher efficacy and was well tolerated compared with solvent-based paclitaxel (Taxol) and docetaxel (Taxotere) in clinical trials for metastatic breast cancer. *Nab*-paclitaxel enhances tumor targeting through gp60 and caveolae-mediated endothelial transcytosis and the association with the albumin-binding protein SPARC (secreted protein, acidic and rich in cysteine) in the tumor microenvironment. The overexpression of human epidermal growth factor receptor-2 (HER2) in breast cancer has been shown to correlate with resistance to paclitaxel. To evaluate the importance of HER2 and SPARC status in determining the relative efficacy of *nab*-paclitaxel compared with polysorbate-based docetaxel, nude mice bearing six different human tumor xenografts were treated with *nab*-paclitaxel (MX-1: 15 mg/kg, once a week for 3 weeks; LX-1, MDA-MB-231/HER2+, PC3, and HT29: 50 and 120 mg/kg, every 4 days three times ; MDA-MB-231: 120 and 180 mg/kg, every 4 days three times) and polysorbate-based docetaxel (15 mg/kg). HER2 and SPARC status were analyzed by RT-PCR and immunohistochemical staining. MDA-MB-231 and MX-1 breast and LX-1 lung cancers were HER2 negative and low in SPARC expression. *Nab*-paclitaxel at submaximum-tolerated dosage was significantly more effective than polysorbate-based docetaxel at its maximum-tolerated dosage in these three HER2-negative tumors. The HER2-positive tumors had variable SPARC expression, with MDA-MB-231/HER2+ <PC3 <HT29. In these HER2-positive tumors, *nab*-paclitaxel was equal to or better than polysorbate-based docetaxel in tumors with medium to high SPARC levels (PC3 and HT29), but not in MDA-MB-231/HER2+ tumors with low SPARC expression. These results demonstrated that the relative efficacy of *nab*-paclitaxel was significantly higher compared with polysorbate-based docetaxel in HER2-negative tumors (three of three) and in HER2-positive tumors with high levels of SPARC. HER2 and SPARC expression may be useful biomarkers in determining antitumor effectiveness for taxanes. *Anti-Cancer Drugs* 19:899–909 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The taxane family drugs, paclitaxel and docetaxel, are widely used to treat breast, ovarian, lung, and other cancers. Both paclitaxel and docetaxel cause G_2/M cell cycle arrest by promoting microtubule assembly from tubulin and preventing microtubule depolymerization [1]. In addition, both drugs induce apoptosis of cancer cells by promoting the phosphorylation and downregulation of antiapoptotic protein Bcl-2 [2,3]. It has been reported that compared with paclitaxel, docetaxel is more effective in inducing Bcl-2 phosphorylation and apoptosis, and has greater cellular uptake and longer tumor retention time [4,5]. In addition, the sensitivity of tumor cell lines and tumors *in vivo* toward paclitaxel and docetaxel is different [6]. In a phase II clinical trial, docetaxel was shown to be active in some patients with paclitaxel-resistant breast cancer [7]. Both paclitaxel and docetaxel are highly hydrophobic and are conventionally formulated with synthetic solvents: paclitaxel formulated as Taxol with polyethylated castor oil (Cremophor EL, BASF, Ludwigshafen, Germany) and ethanol; docetaxel formulated as Taxotere with polysorbate 80 (Tween 80, Uniqema/Croda, Yorkshire, England) and ethanol. In a randomized phase III study in patients with metastatic breast cancer, polysorbate-based docetaxel displayed improved overall survival compared with Cremophor-based paclitaxel (15.4 vs. 12.7 months; $P = 0.03$) and longer time to progression (5.7 vs. 3.6 months; $P < 0.0001$). The incidence of treatment-related hematologic and nonhematologic toxicities, however, was

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greater for polysorbate-based docetaxel than for solvent-based paclitaxel, forcing a higher number of treatment discontinuations for the docetaxel cohort [8].

The solvents used to formulate paclitaxel and docetaxel directly contribute to severe toxicities of the drugs, including severe hypersensitivity reactions and peripheral neuropathy [6,9–12]. In addition, active drugs can be entrapped by the solvents in micelles formed in the plasma compartment, resulting in increased systemic drug exposure and decreased overall efficacy [13]. Paclitaxel protein-bound particles for injectable suspension [nanoparticle albumin-bound (*nab*)-paclitaxel, also known as Abraxane or ABI-007; Abraxis Bioscience, LLC., Los Angeles, California, USA] are an albumin-bound form of paclitaxel free of solvents or ethanol with a mean size of approximately 130 nm. Our in-vitro and in-vivo drug dissolution studies have shown that once injected into circulation, *nab*-paclitaxel nanoparticles dissolve quickly into smaller soluble albumin-bound paclitaxel complexes with a size of about 10 nm, very similar to the size of endogenous albumin molecules in blood as measured by laser light scattering [14]. The transcytosis of albumin-bound paclitaxel across the endothelial barrier is facilitated by the binding of albumin to the gp60 receptor and caveolar transport [15]. After entering the interstitial space adjacent to tumors, the accumulation of albumin-bound paclitaxel is possibly mediated by 'secreted protein, acidic and rich in cysteine' (SPARC), an albumin-binding protein with significant homology to gp60. The binding of paclitaxel to endothelial cells and albumin is inhibited by surfactants such as Cremophor, polysorbate 80 and tocopheryl polyethylene glycol stearate [15]. In an earlier study with tumor xenografts, *nab*-paclitaxel showed increased antitumor activity, enhanced endothelial cell transport and 33% higher intratumor paclitaxel concentration compared with equal dose of solvent-based paclitaxel [15]. In a phase I trial, the lower toxicities of *nab*-paclitaxel allowed the administration of 70% higher dose than the approved dose of solvent-based paclitaxel (300 vs. 175 mg/m^2) over a shorter infusion time (30 min vs. 3 h), without the need for corticosteroid premedication [16]. In a randomized phase III study in patients with metastatic breast cancer, *nab*-paclitaxel had a significantly higher overall response rate (ORR) compared with solvent-based paclitaxel (33.2 vs. 18.7%; $P = 0.001$), significantly longer time to tumor progression (23.0 vs. 16.9 weeks; $P = 0.006$), and longer survival time in patients receiving *nab*-paclitaxel as second-line treatment (56.4 vs. 46.7 weeks; $P = 0.016$). Compared with solvent-based paclitaxel, *nab*-paclitaxel had markedly lower incidence of treatment-related grade 4 neutropenia (9 vs. 22%; $P < 0.001$). Grade 3 neuropathy was 10% for *nab*-paclitaxel compared with 2% for solvent-based paclitaxel ($P < 0.001$), consistent with the higher dose of paclitaxel administered and was easily managed and improved quickly, which was different from that typically observed with solvent-based paclitaxel. No hypersensitivity reaction occurred with *nab*-paclitaxel [17]. As a result, *nab*-paclitaxel has been approved by FDA in January 2005 for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. It received approval from European Union in January 2008 for the treatment of metastatic breast cancer in patients who have failed first-line treatment for metastatic disease and for whom standard, anthracycline-containing therapy is not indicated.

As *nab*-paclitaxel has greater antitumor activity and is well tolerated compared with solvent-based paclitaxel, it was hypothesized that *nab*-paclitaxel would have better therapeutic potential than polysorbate-based docetaxel [18]. In a recent randomized phase II study of first-line treatment of 300 metastatic breast cancer patients, *nab*-paclitaxel (at 100 mg/m^2 weekly, 3/4 weeks; and 150 mg/m^2 weekly, 3/4 weeks) demonstrated significantly higher ORR than polysorbate-based docetaxel at the highest dose (100 mg/m^2 every 3 weeks) for metastatic breast cancer (63 and 74% vs. 39%, $P = 0.002$ and < 0.001, respectively). *Nab*-paclitaxel at 150 mg/m^2 weekly showed significantly longer progression-free survival (PFS) than docetaxel (14.6 vs. 7.8 months, $P = 0.012$), whereas a numerical increase in PFS was observed for *nab*-paclitaxel 300 mg/m^2 every 3 weeks (10.9 vs. 7.8 months, $P =$ NS). All three arms of *nab*-paclitaxel resulted in lower rates of grade 3/4 neutropenia, febrile neutropenia, and fatigue than polysorbate-based docetaxel [19]. In this study, we compared the antitumor efficacy of *nab*-paclitaxel and polysorbate-based docetaxel in six xenograft tumor models including breast, lung, prostate, and colon cancers. The overexpression of human epidermal growth factor receptor-2 (HER2) in breast cancer has been shown to correlate with resistance to paclitaxel [20,21], and its overexpression in breast cancer cells led to resistance to paclitaxel but not to docetaxel [5]. SPARC has been shown to be an indicator for poor prognosis and aggressive phenotype in breast, lung, gastric, bladder, and many other cancer types [22–26]. Overexpression of the albumin-binding protein SPARC has been postulated to increase tumor distribution of *nab*-paclitaxel in breast tumors and improve tumor response to *nab*-paclitaxel in head-and-neck cancer [27,28]. Therefore, we evaluated HER2 and SPARC expression for their impact on the antitumor efficacy of *nab*-paclitaxel compared with polysorbate-based docetaxel.

Methods

Study drugs

Nab-paclitaxel (Abraxane) was obtained from Abraxis BioScience; and polysorbate-based docetaxel (Taxotere; Sanofi Aventis, Bridgewater, New Jersey, USA) was purchased from a hospital pharmacy. Both drugs were

prepared fresh daily and given within 1 h after reconstitution in physiological saline.

Dose ranging study

Nontumor-bearing female athymic mice were administered *nab*-paclitaxel at 15, 30, 60, 120, and 240 mg/kg and polysorbate-based docetaxel at 7, 15, 22, 33, and 50 mg/kg intravenously on a every 4 days three times (q4dx3) schedule (10 animals per dose group). Vehicle group [phosphate-buffered saline (PBS)] was used as control. Overall toxicity was defined as a decrease in body weight and/or mortality, with nude mice being monitored three times weekly for 20 days. Maximum-tolerated dosage (MTD) was defined as the dose level causing either 20% weight loss or one of 10 mortality.

Animals and human tumor xenografts

All animals were maintained in compliance with the American Association for Laboratory Animal Care guidelines and 'Principles of Laboratory Animal Care' (NIH publication no. 85–23, revised 1985). Female and male athymic NCr-nu mice between 5 and 6 weeks of age weighing approximately 20 g were purchased from Harlan, Inc. (Madison, Wisconsin, USA). Human tumor cell lines MDA-MB-231/HER2 + (breast), PC3 (prostate), and HT29 (colon) were obtained from the American Type Culture Collection (Manassas, Virginia, USA). MX-1 and MDA-MB-231 (breast) tumor cell lines and LX-1 lung carcinoma tumor fragment were obtained from the NCI-Frederick Cancer Research Facility, DCT Tumor Repository (Frederick, Maryland, USA). Cells were propagated *in vivo* as solid tumors. Tumor fragments were implanted subcutaneously into female (for MX-1, LX-1, MDA-MB-231, MDA-MB-231/HER2 + , and HT29) or male (for PC3 prostate tumor) nude mice and allowed to grow to approximately 60–100 mm^3 before treatment was initiated.

Antitumor study

The antitumor activity of polysorbate-based docetaxel (15 mg/kg) was compared with *nab*-paclitaxel in MX-1 (equidose level of 15 mg/kg, once a week for 3 weeks), LX-1, PC3, HT29, MDA-MB-231/HER2 + (50 and 120 mg/kg, q4dx3), and MDA-MB-231 xenografts (120 and 180 mg/kg, q4dx3). Control animals for each xenograft were administered PBS. Numbers of mice in each dose group per tumor xenograft were: eight for MX-1 and LX-1, 10 for MDA-MB-231, seven for MDA-MB-231/HER2 + , eight for PC3, and nine for HT29. The longest (length) and shortest (width) tumor diameters (millimeter) were measured twice weekly. The tumor volume was calculated with the formula: tumor volume (mm^3) = width $(mm)^2 \times$ length $(mm) \times 0.52$. Tumor growth inhibition (TGI) was defined as the percentage of tumor volume reduction compared with the control group at the time of euthanasia for the control animals. Tumor doubling time was defined as the time required for the tumor volume to double twice. The relative efficacy of *nab*-paclitaxel to polysorbate-based docetaxel was determined as the ratio of the mean tumor volume of polysorbate-based docetaxel (15 mg/kg)-treated animals over the mean tumor volume of *nab*-paclitaxel (15 mg/kg for MX-1, 120 mg/kg for LX-1, MDA-MB-231, MDA-MB-231/HER2 + , PC3, and HT29)-treated animals at a fixed time period after the start of the study for both groups and across all tumor types. Animal weights were measured twice weekly. Statistical analysis was performed using the Prism program (GraphPad, San Diego, California, USA). Analysis of variance (ANOVA) statistic was used to compare tumor growth curves.

Antibodies and immunohistochemistry

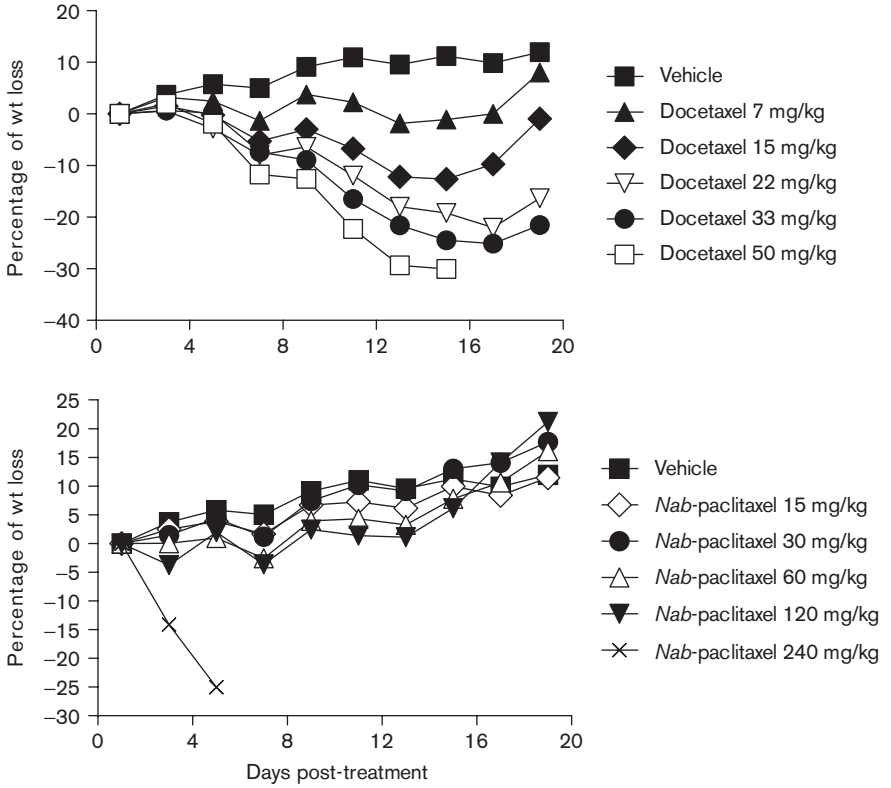
Immunohistochemical staining of slides was performed as described previously [29]. For visualization of SPARC, the slides were incubated with 10 μg/ml of polyclonal anti-human SPARC antibody (R&D Systems, Minneapolis, Minnesota, USA) in PBS–Tween. After 1 h, the slides were washed twice in PBS–Tween for 5 min each, and then incubated for an additional hour with a 1 : 100 dilution of anti-mouse or anti-rabbit horseradish peroxidase conjugated antibody (Pierce, Rockford, Illinois, USA). Sections were washed in PBS–Tween and incubated for 10 min in the substrate solution (100 μl of a stock solution of 3-amino-9-ethylcarbazole in *N,N*-dimethyl formamide at 2.4 mg/ml, 1 ml of acetate buffer, pH 5.2, and 5 μl of 30% wt/wt hydrogen peroxide). The slides were counterstained with Mayer's hematoxylin and mounted with Crystal Mount (BioMeda Corp., Foster City, California, USA). The slides were scored for HER2 and SPARC levels using a 0–4 scale, with 0 being negative and 4 being strongly positive. A HER2 or SPARC score of 0 and 1 was considered negative and a score of 2–4 was considered positive. The subjective scores were given by a single individual reading the slides blinded.

Results

Comparative toxicology of *nab*-paclitaxel and polysorbate-based docetaxel

Nab-paclitaxel exhibited higher MTD than polysorbate-based docetaxel in mice in a dose-finding study with nontumor-bearing athymic mice, in which *nab*-paclitaxel was administered at 15, 30, 60, 120, and 240 mg/kg, whereas polysorbate-based docetaxel was administered at 7, 15, 22, 33, and 50 mg/kg (q4dx3, intravenously). Overall toxicity was defined as a decrease in body weight or mortality. As shown in Fig. 1, *nab*-paclitaxel was nontoxic (no appreciable weight loss) at 15, 30, 60, and 120 mg/kg. Toxicity was observed only at the next dose level of 240 mg/kg, resulting in 100% mortality by day 7. Thus, the MTD of *nab*-paclitaxel was defined as between 120 and 240 mg/kg. A dose of 120 mg/kg *nab*-paclitaxel (sub-MTD) was used in the initial four tumor xenograft studies (LX-1, MDA-MB-231/HER2 + , PC3, and HT29).

Fig. 1



Safety of nanoparticle albumin-bound (*nab*)-paclitaxel compared with polysorbate-based docetaxel in mice. Nontumor-bearing nude mice were treated with *nab*-paclitaxel or polysorbate-based docetaxel at indicated levels and body weights were monitored. Data were represented by percentage of weight loss (or gain) relative to body weight at day 1.

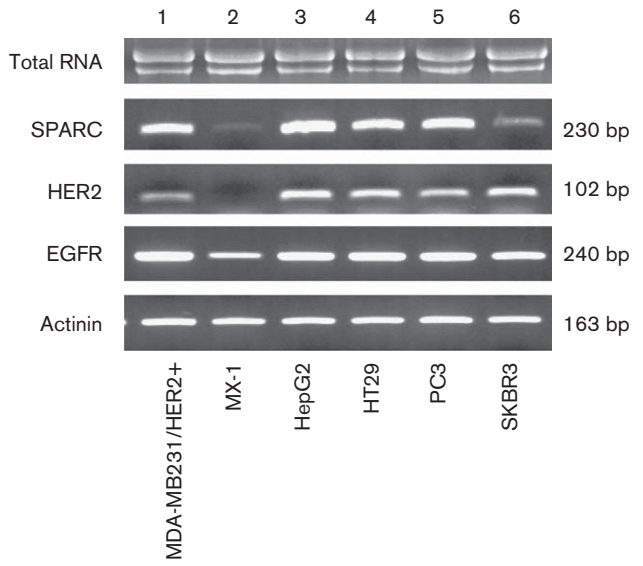
In the final MDA-MB-231 study, a dose of 180 mg/kg was used based on the demonstrated safety of *nab*-paclitaxel, the weight loss analysis of the median lethal dose experiment, and published data [30] showing that *nab*-paclitaxel is well tolerated even at 200 mg/kg. Polysorbate-based docetaxel exhibited a dose-dependent weight loss with no associated mortality at dose levels tested (15–50 mg/kg). Polysorbate-based docetaxel at 22, 33, and 50 mg/kg levels caused severe weight loss of more than 20% (Fig. 1); therefore, the MTD of polysorbate-based docetaxel was defined as 15 mg/kg.

Human epidermal growth factor receptor-2 and secreted protein, acidic and rich in cysteine status among tumor xenografts

The HER2 and SPARC status of each tumor xenograft was examined by RT-PCR and/or immunohistochemistry (IHC). By RT-PCR, MX-1 (breast) cells were HER2 negative, whereas MDA-MB-231/HER2 + (breast), HepG2 (liver), HT29 (colon), PC3 (prostate), and SKBR3 (breast) were HER2 positive (Fig. 2). RT-PCR controls of epidermal growth factor receptor and actinin expression were similar among the various cell lines tested. In addition, the same amount of RNA was used per reaction as demonstrated by the RNA gel of each sample. The MDA-MB-231 acquired from American Type Culture Collection was found to be HER2 positive, by both RT-PCR and IHC, and was defined here as MDA-MB-231/HER2 + to differentiate it from the HER2-negative MDA-MB-231 obtained from NCI repository.

By IHC, MX-1, LX-1, and MDA-MB-231 were HER2 negative (IHC score = 0, Fig. 3a, c, and e) with IHC score for SPARC of 2, 1, and 0, respectively (Fig. 3b, d, and f). MDA-MB-231/HER2 + , PC3, and HT29 xenografts were HER2 positive (IHC score = 3, 2, and 2, respectively, with a score of 2–4 being positive, Fig. 3g, i, and k). These HER2-positive tumors, however, had variable SPARC expression: MDA-MB-231/HER2 + was very weakly positive for SPARC (score = 2, Fig. 3h); PC3 was moderately positive for SPARC (score = 3, Fig. 3j); and HT29 stained strongly positive for SPARC (score = 4 + , Fig. 3l).

Fig. 2



Presence of human epidermal growth factor receptor-2 (HER2) and secreted protein, acidic and rich in cysteine (SPARC) mRNA in tumor cell lines. The presence of SPARC, HER2, and epidermal growth factor receptor (EGFR) mRNA in various tumor cell lines was analyzed by RT-PCR. The quality and amount of total RNA were checked by electrophoresis and the bands shown are 28S and 18S ribosomal RNA. RT-PCRs were performed with gene-specific primers for human SPARC, HER2, EGFR, and actinin. The sizes of PCR products are indicated.

Antitumor activity of *nab*-paclitaxel versus polysorbate-based docetaxel at equal dose in human epidermal growth factor receptor-2-negative MX-1 breast tumor xenografts

At equidose level of 15 mg/kg, *nab*-paclitaxel was more effective than polysorbate-based docetaxel in suppressing the growth of HER2-negative MX-1 tumor ($P < 0.0001$, ANOVA) (Fig. 4a and Table 1). The group receiving *nab*-paclitaxel exhibited a TGI of 80% compared with a TGI of 29% for the polysorbate-based docetaxel group. On the schedule of once a week for 3 weeks, both treatments were nontoxic, as measured by the criteria of weight loss (Fig. 4a).

Antitumor activity of *nab*-paclitaxel (submaximum-tolerated dosage) versus polysorbate-based docetaxel (maximum-tolerated dosage)

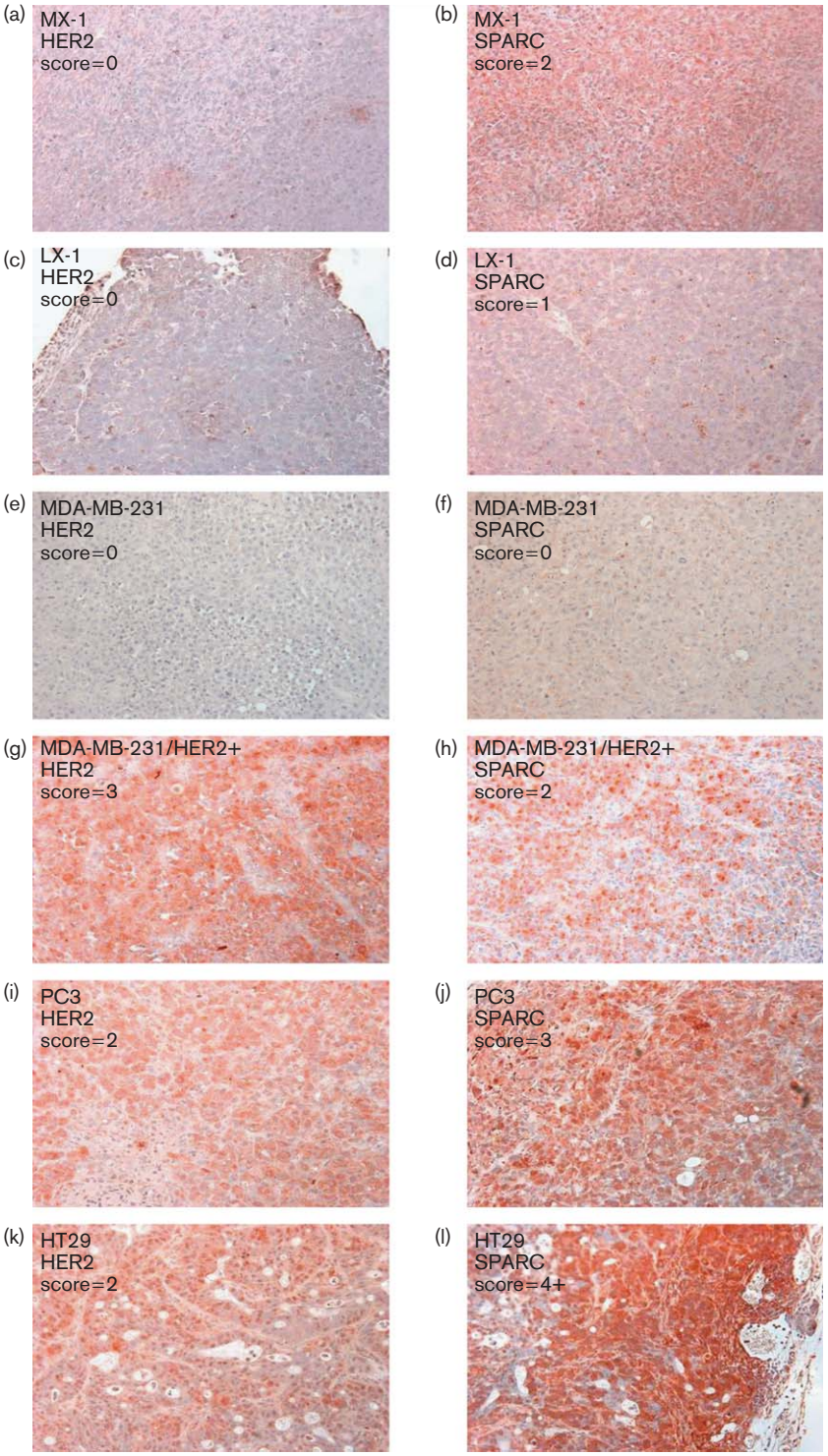
The antitumor activity of *nab*-paclitaxel dosed at levels below its MTD was compared with polysorbate-based docetaxel at its MTD. In the HER2-negative LX-1 xenograft, both 50 and 120 mg/kg dose levels of *nab*-paclitaxel were more effective than 15 mg/kg polysorbate-based docetaxel in suppressing tumor growth ($P = 0.0001$ for *nab*-paclitaxel 50 mg/kg and $P < 0.0001$ for *nab*-paclitaxel 120 mg/kg, ANOVA). TGI was 84 and 98%, respectively, for 50 and 120 mg/kg *nab*-paclitaxel, in comparison with a 61% TGI for polysorbate-based docetaxel administered at 15 mg/kg (Fig. 4b and Table 1). Both *nab*-paclitaxel treatment groups showed longer tumor doubling time (Table 1) than the docetaxel group. In addition, on the schedule of q4dx3, both 50 and 120 mg/kg *nab*-paclitaxel dose groups showed significantly less toxicity than polysorbate-based docetaxel at 15 mg/kg (Fig. 4b, $P = 0.0003$, ANOVA). The *nab*-paclitaxel groups experienced only modest and dose-independent weight loss (nadir = −8.1% for 50 mg/kg group on day 60; nadir = −5.4% for 120 mg/kg group on day 11), whereas the polysorbate-based docetaxel group showed significant weight loss (nadir = −19.9% on day 15).

In the HER2-negative MDA-MB-231 xenograft, *nab*-paclitaxel at both 120 and 180 mg/kg were more effective in tumor suppression than polysorbate-based docetaxel at 15 mg/kg ($P < 0.001$, Fig. 4c and Table 1), with respective TGI of 99 and 98% compared with 78% for docetaxel. There was no significant weight loss with either *nab*-paclitaxel dose group, even at 180 mg/kg (nadir = −1.5% for the 120 mg/kg group on day 4; nadir = −5.8% for the 180 mg/kg group on day 14), and the 15 mg/kg polysorbate-based docetaxel group (nadir = −2.4% on day 14, Fig. 4c).

In contrast, for the HER2-positive/low SPARC expression MDA-MB-231/HER2 + xenograft, *nab*-paclitaxel at both 50 and 120 mg/kg was less effective in tumor suppression than polysorbate-based docetaxel at 15 mg/kg ($P < 0.001$, Fig. 5a and Table 1). The groups receiving *nab*-paclitaxel exhibited TGI of 94 and 99%, respectively, compared with TGI of 96% for the docetaxel group (Fig. 5a and Table 1). The polysorbate-based docetaxel group showed a longer tumor doubling time than both *nab*-paclitaxel groups (Table 1). Both *nab*-paclitaxel dose groups experienced only slight weight loss (nadir = −1.1% for the 50 mg/kg group on day 4; nadir = −3.8% for the 120 mg/kg group on day 11), whereas the polysorbate-based docetaxel group at 15 mg/kg showed moderate weight loss (nadir = −14.4% on day 15, Fig. 5a). These differences in weight loss were not statistically significant (P = NS, ANOVA).

In the HER2-positive/moderate SPARC expression PC3 prostate tumor xenograft, *nab*-paclitaxel at 50 mg/kg was less effective in tumor suppression than polysorbate-based docetaxel at 15 mg/kg ($P < 0.001$), whereas *nab*-paclitaxel at 120 mg/kg was equally effective as polysorbate-based docetaxel (15 mg/kg, P = NS, Fig. 5b and Table 1). The groups receiving *nab*-paclitaxel at either dose-exhibited TGI of 94 and 99%, respectively, compared with a TGI of 97% for the docetaxel group (Fig. 5b and Table 1). Although *nab*-paclitaxel at 50 mg/kg only delayed tumor doubling time, *nab*-paclitaxel at 120 mg/kg and polysorbate-based docetaxel at 15 mg/kg produced

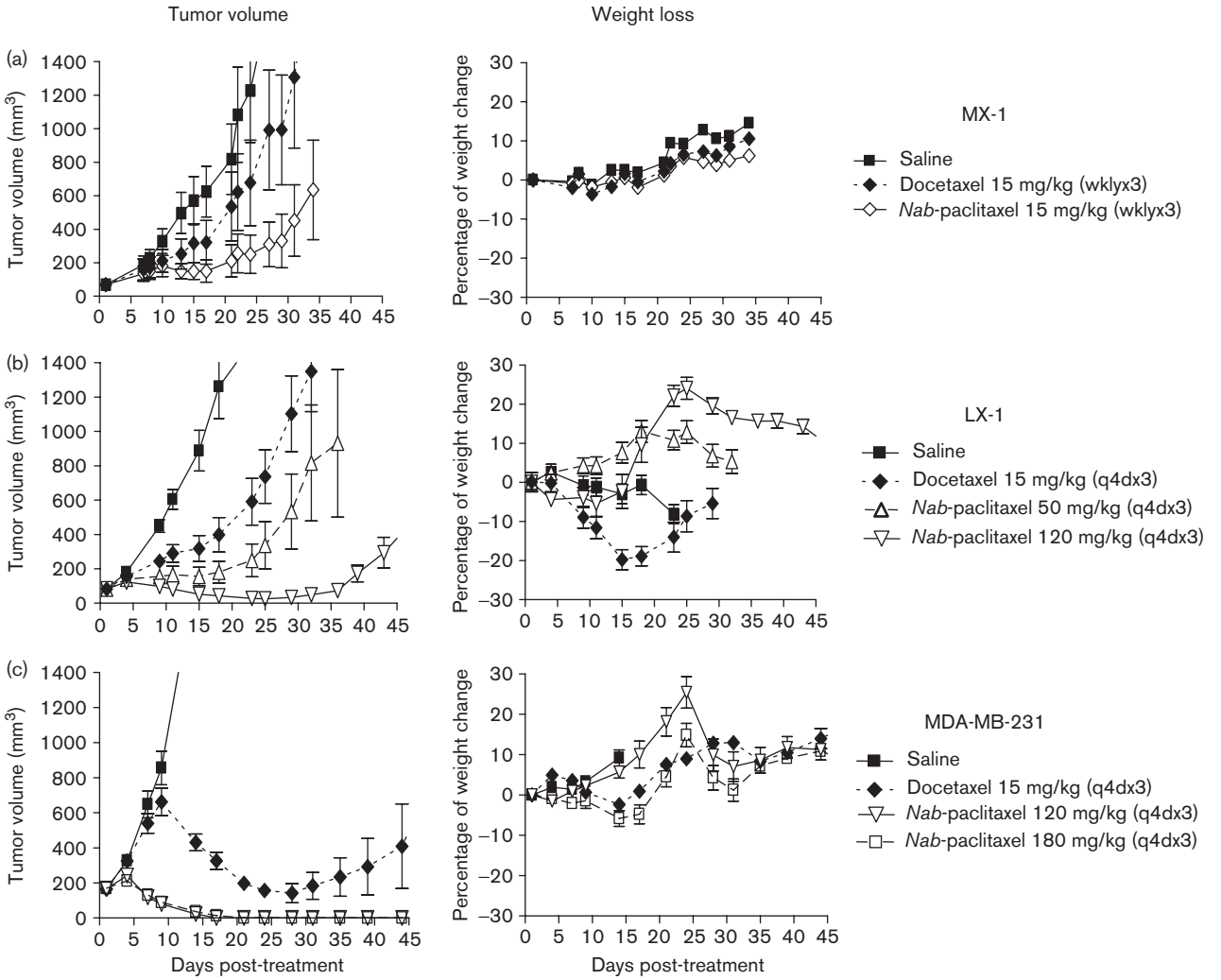
Fig. 3



Human epidermal growth factor receptor-2 (HER2) and secreted protein, acidic and rich in cysteine (SPARC) expression levels in tumor xenografts. HER2 and SPARC expression levels were analyzed by immunohistochemical staining in MX-1 (a and b), LX-1 (c and d), MDA-MB-231 (e and f), MDA-MB-231/HER2+ (g and h), PC3 (i and j), and HT29 (k and l) tumors.

some complete responses: four complete responses among eight mice treated with 120 mg/kg of *nab*-paclitaxel and one complete response in polysorbate-based docetaxel group (Table 1). No significant weight loss for 50 mg/kg *nab*-paclitaxel (nadir = –4.3% on day 12) was observed, whereas weight loss at 120 mg/kg of

Fig. 4



Efficacy and toxicity of *nab*-paclitaxel compared with polysorbate-based docetaxel in human epidermal growth factor receptor-2 (HER2)-negative tumor xenografts. Nude mice bearing different HER2-negative tumor xenografts were treated with nanoparticle albumin-bound (*nab*)-paclitaxel and polysorbate-based docetaxel at indicated levels. Tumor volume was measured for MX-1 (a), LX-1 (b), and MDA-MB-231 (c). Overall toxicity of *nab*-paclitaxel versus polysorbate-based docetaxel was measured by weight loss after treatment. Data were represented by percentage of weight loss (or gain) relative to body weight at day 1. The error bars represented the standard error of the mean. q4dx3, every 4 days three times; wklyx3, weekly three times.

nab-paclitaxel (nadir = −17.7% on day 15) was lower than polysorbate-based docetaxel at 15 mg/kg (nadir = −25.1% at day 20) but not significantly different (Fig. 5b, P = NS, ANOVA).

In the HER2-positive/high SPARC expression HT29 xenograft, both sub-MTD levels of *nab*-paclitaxel were more effective than polysorbate-based docetaxel at its MTD (15 mg/kg) in suppressing tumor growth ($P = 0.0057$ for *nab*-paclitaxel 50 mg/kg and $P < 0.0001$ for *nab*-paclitaxel 120 mg/kg, ANOVA). The two groups receiving *nab*-paclitaxel had TGI of 50 and 65%, respectively, compared with a TGI of 36% for the docetaxel group (Fig. 5c and Table 1). Tumor doubling time was longer for both *nab*-paclitaxel treatment groups compared with the polysorbate-based docetaxel group (Table 1). Furthermore, on the schedule of q4dx3, both *nab*-paclitaxel groups showed significantly less toxicity (Fig. 5c, $P = 0.0004$ for 50 mg/kg *nab*-paclitaxel and $P < 0.0001$ for 120 mg/kg *nab*-paclitaxel vs. 15 mg/kg polysorbate-based docetaxel, ANOVA), with *nab*-paclitaxel groups showing only slight weight loss (nadir = −2.9% for the 50 mg/kg group on day 8; nadir = −5.3% for the 120 mg/kg group on day 11) and the polysorbate-based docetaxel group showing significant weight loss (nadir = −24.4% on day 15).

Table 1 Efficacy in tumor suppression for *nab*-paclitaxel and polysorbate-based docetaxel in tumor xenografts

Tumor	HER2	SPARC	Treatment	# Tumor-free survivors	Tumor growth inhibition (%)	Tumor doubling time (days)
MX-1	0	2	Saline	0/8	0	10
			Nab-paclitaxel (15 mg/kg)	1/8	80	27
			Docetaxel (15 mg/kg)	0/8	29	15
LX-1	0	1	Saline	0/8	0	9
			Nab-paclitaxel (50 mg/kg)	0/8	84	29
			Nab-paclitaxel (120 mg/kg)	0/8	98	46
			Docetaxel (15 mg/kg)	0/8	61	18
MDA-MB-231	0	0	Saline	0/10	0	7
			Nab-paclitaxel (120 mg/kg)	10/10	99	CR
			Nab-paclitaxel (180 mg/kg)	10/10	98	CR
			Docetaxel (15 mg/kg)	5/10	78	9
MDA-MB-231/ HER2+	3	2	Saline	0/7	0	8
			Nab-paclitaxel (50 mg/kg)	0/7	94	30
			Nab-paclitaxel (120 mg/kg)	1/7	99	37
			Docetaxel (15 mg/kg)	0/7	96	49
PC3	2	3	Saline	1/8	0	12
			Nab-paclitaxel (50 mg/kg)	0/8	94	43
			Nab-paclitaxel (120 mg/kg)	4/8	99	CR
			Docetaxel (15 mg/kg)	1/8	97	CR
HT29	2	4+	Saline	0/9	0	11
			Nab-paclitaxel (50 mg/kg)	1/9	50	29
			Nab-paclitaxel (120 mg/kg)	0/9	65	32
			Docetaxel (15 mg/kg)	0/9	36	25

Nude mice bearing different tumor xenografts were treated with *nab*-paclitaxel and polysorbate-based docetaxel at the indicated doses. Tumor growth inhibition was calculated by tumor volume reduction relative to control group when the control animals were euthanized. Tumor doubling time was defined as the time required for tumor volume to double twice.

Relative tumor response as function of human epidermal growth factor receptor-2 and secreted protein, acidic and rich in cysteine status

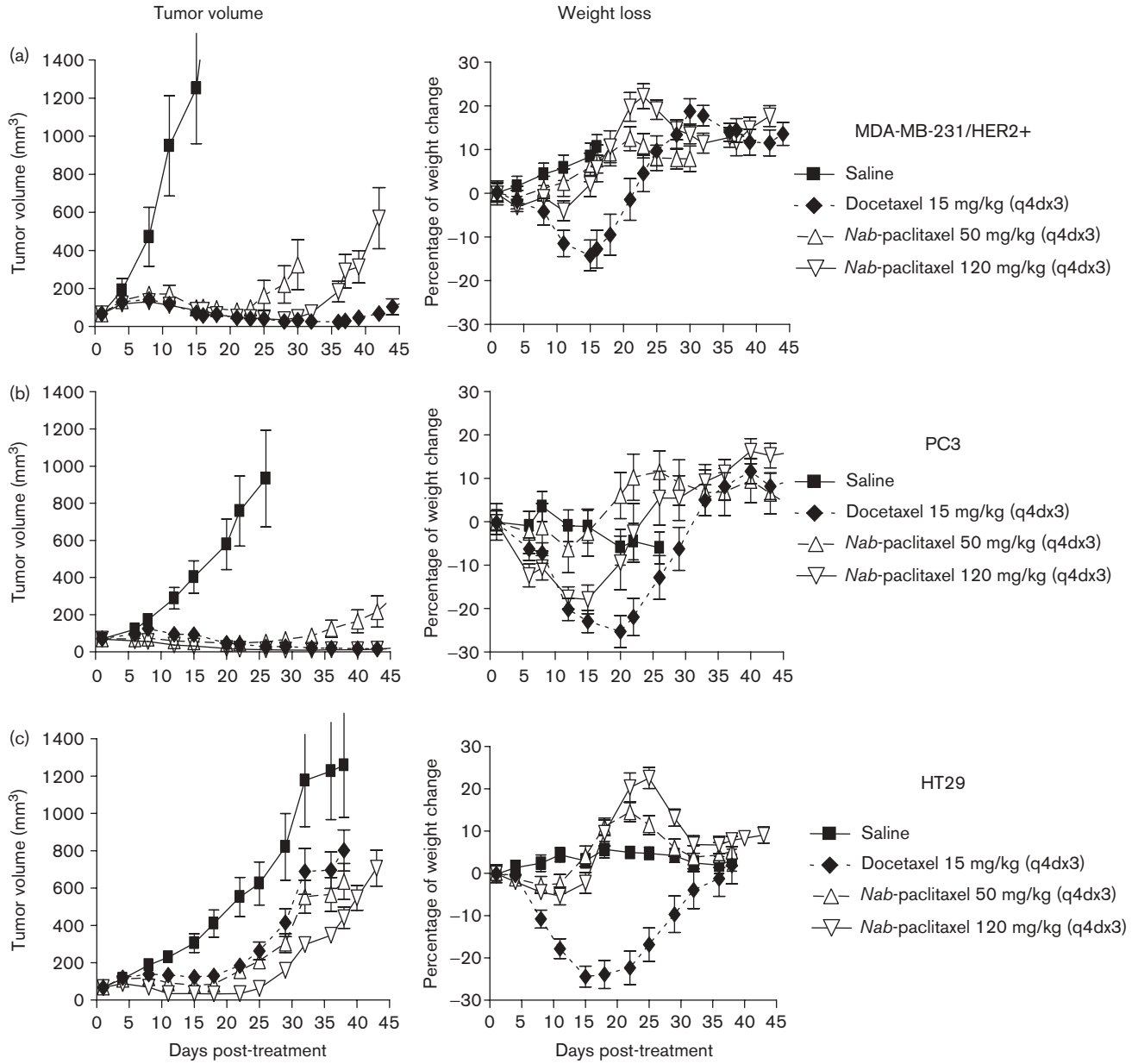
The relative tumor responses of *nab*-paclitaxel to polysorbate-based docetaxel as a function of HER2 and SPARC status are summarized in Fig. 6. To keep the analysis consistent across different tumor xenografts, response to *nab*-paclitaxel relative to polysorbate-based docetaxel was calculated by comparing tumor volume for 120 mg/kg *nab*-paclitaxel group and 15 mg/kg polysorbate-based docetaxel group on day 32 posttreatment (day 31 for MDA-MB-231) after start of treatment for all xenografts. In HER2-negative MDA-MB-231 and LX-1 lung tumors, response to *nab*-paclitaxel was greatly superior. The relative *nab*-paclitaxel efficacy with LX-1 tumor was calculated as 27.8-fold the polysorbate-based docetaxel response, whereas the relative efficacy could not be calculated for MDA-MB-231 tumors as *nab*-paclitaxel treatment resulted in complete response in all 10 mice. More importantly, in the HER2-negative MX-1 breast tumor, *nab*-paclitaxel at the equidose level was also more effective, with a relative response 2.9-fold higher than polysorbate-based docetaxel. Among HER2-positive tumors, there was an improved response to *nab*-paclitaxel in tumors positive for SPARC expression, with relative tumor response to *nab*-paclitaxel being 0.4-fold, 1.0-fold, and 2.3-fold that of polysorbate-based docetaxel for MDA-MB-231/HER2+, PC3, and HT29, respectively.

Discussion

In this study, we demonstrated increased relative antitumor activity of *nab*-paclitaxel versus polysorbate-based docetaxel among various tumor xenografts. In five of the six human tumor xenograft models tested, doses of *nab*-paclitaxel less than its MTD showed antitumor activity superior (in MX-1, LX-1, MDA-MB-231, and HT29 xenografts) or equal (in PC3 xenograft) to the MTD level of polysorbate-based docetaxel. The exception was the MDA-MB-231/HER2+, a HER2-positive/low SPARC expression MDA-MB-231 line, in which polysorbate-based docetaxel at MTD was more effective in tumor suppression than *nab*-paclitaxel at a sub-MTD dose. *Nab*-paclitaxel displayed lower toxicity compared with polysorbate-based docetaxel in all the xenografts as demonstrated by lower weight loss among the *nab*-paclitaxel-treated groups. The reduced toxicity of *nab*-paclitaxel was possibly because of the absence of surfactants such as polysorbate 80, which could account for significant toxicity by itself. In addition, the surfactants can increase systemic drug exposure by sequestering drug in micelles in plasma [13].

HER2-negative status in MX-1, LX-1, and MDA-MB-231 xenografts was associated with better antitumor responses to *nab*-paclitaxel versus polysorbate-based docetaxel, with relative responses to *nab*-paclitaxel of 20-fold or higher for LX-1 and MDA-MB-231 tumors. Surprisingly, at equidose on a milligram per milligram basis, *nab*-paclitaxel exhibited a better antitumor response than polysorbate-based docetaxel in the MX-1 breast tumor. This was unexpected as it is generally considered that docetaxel is more potent than paclitaxel. The improved efficacy at equal dose could be a result of more efficient

Fig. 5

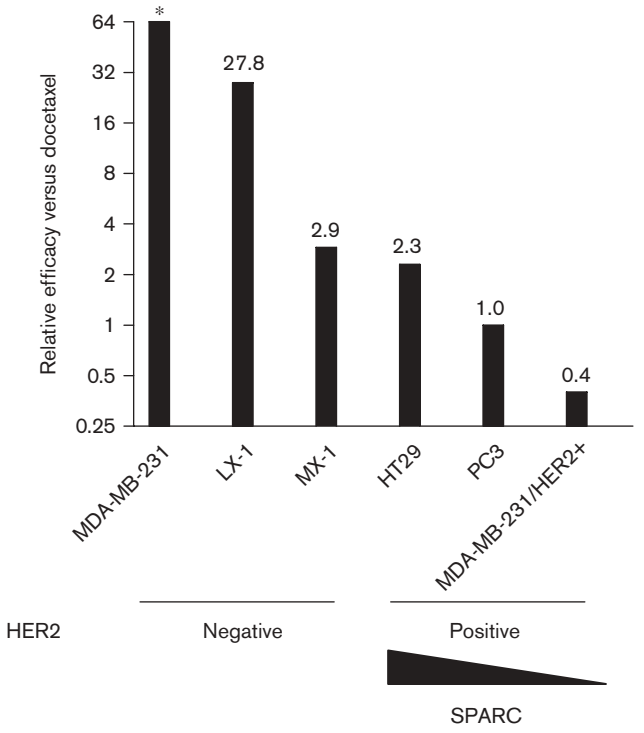


Efficacy and toxicity of nanoparticle albumin-bound (*nab*)-paclitaxel compared with polysorbate-based docetaxel in human epidermal growth factor receptor-2 (HER2)-positive tumor xenografts. Nude mice bearing different HER2-positive tumor xenografts were treated with *nab*-paclitaxel and polysorbate-based docetaxel at indicated levels. Tumor volume was measured for MDA-MB-231/HER2+ (a), PC3 (b), and HT29 (c). Overall toxicity of *nab*-paclitaxel versus polysorbate-based docetaxel was measured by weight loss after treatment. Data were represented by percentage of weight loss (or gain) relative to body weight at day 1. The error bars represented the standard error of the mean. q4dx3, every 4 days three times.

transport of *nab*-paclitaxel to the tumor because of the previously described gp60 and SPARC pathways [15]. In contrast, sequestration in polysorbate-80 micelles may impair transport of docetaxel. In the HER2-negative LX-1 lung and MDA-MB-231 breast tumor xenografts, *nab*-paclitaxel at doses below its MTD was more effective than polysorbate-based docetaxel at its MTD.

In HER2-positive tumors MDA-MB-231/HER2 + , HT29, and PC3, the relative response to *nab*-paclitaxel was lower than in the HER2-negative tumors. In addition, in these tumors the response to *nab*-paclitaxel seemed to exhibit a dependency on SPARC status. Among these tumors, the expression level of SPARC, an albumin-binding protein overexpressed in various cancer types [22,31], was a

Fig. 6



Relative efficacy of nanoparticle albumin-bound (*nab*)-paclitaxel compared with polysorbate-based docetaxel in tumor xenografts. The relative antitumor efficacy (polysorbate-based docetaxel tumor volume/*nab*-paclitaxel tumor volume) in different tumor xenografts was calculated using data from 120 mg/kg *nab*-paclitaxel group (15 mg/kg for MX-1) and 15 mg/kg polysorbate-based docetaxel group on day 32 posttreatment. *Relative efficacy could not be calculated for MDA-MB-231 tumors as *nab*-paclitaxel treatment resulted in complete response in all 10 mice at day 31 posttreatment. HER2, human epidermal growth factor receptor-2; SPARC, secreted protein, acidic and rich in cysteine.

predictor for sensitivity to *nab*-paclitaxel versus polysorbate-based docetaxel. The importance of SPARC among HER2-expressing xenografts can be explained by its ability to serve as a depot for the intratumoral accumulation of *nab*-paclitaxel because of the recently demonstrated affinity of SPARC for albumin [32]. High levels of SPARC expression could potentially facilitate the accumulation of *nab*-paclitaxel in tumors [15], making it more effective than the polysorbate-based docetaxel. This potential advantage of *nab*-paclitaxel was lost in HER2-expressing tumors with low SPARC levels (e.g. MDA-MB-231/HER2 + xenograft). Our results were consistent with previous findings that SPARC expression may correlate with increased tumor distribution of *nab*-paclitaxel compared with Cremophor-based paclitaxel in breast tumors [27] and correlates with response to *nab*-paclitaxel in human head-and-neck cancer [28].

It was interesting to note that the switch in the MDA-MB-231 cell line from HER2 negative to positive resulted in a dramatic change in the relative response of the tumors to *nab*-paclitaxel. Similar behavior has been noted previously in the breast cancer cell line MCF-7 where HER2 overexpression was shown to cause a switch from paclitaxel-sensitive to paclitaxel-resistant phenotype, while having no impact on sensitivity to docetaxel [5].

Our data suggest that HER2 and SPARC status would be useful to predict relative tumor responses to *nab*-paclitaxel versus polysorbate-based docetaxel. HER2 exhibited a good correlation between mRNA expression in cultured cells and protein expression in tumor xenografts, consistent with reports showing that HER2 overexpression is primarily because of gene amplification [33,34]. SPARC mRNA levels in cultured tumor cells *in vitro* as measured by RT-PCR did not correlate with SPARC protein expression in tumor xenografts *in vivo*. Downregulation of SPARC expression in various neoplasms, including multiple myeloma, lung, pancreatic, colon, ovarian, endometrial, and invasive cervical carcinomas, has been shown to be the result of epigenetic silencing through hypermethylation of the SPARC promoter [35–37]. The tumoral SPARC expression is stimulated by intratumoral hypoxia and acidity [24], and stromal cells juxtaposing the tumor cells can also express high levels of SPARC [22,24]. Therefore, HER2 and SPARC status probably can be best determined by IHC of the tumor biopsy before undergoing therapy for either *nab*-paclitaxel or polysorbate-based docetaxel. HER2 amplification occurs only in 25–30% of early-stage breast cancers [38] and SPARC overexpression occurs in over 50% of breast cancers [39]. HER2-positive/SPARC-negative patients are thus expected to be about 15% or less of the breast cancer patient population.

The current preclinical data indicate that *nab*-paclitaxel may have a clinical advantage over polysorbate-based docetaxel in the roughly 70% of breast cancer patients that are HER2-negative and HER2-positive patients with medium-to-high levels of SPARC expression. This, however, remains to be confirmed in clinical trials. In a recent randomized phase II study of first-line treatment of metastatic breast cancer patients, *nab*-paclitaxel (Abraxane) demonstrated significantly improved ORR and PFS and significantly lower rates of neutropenia, febrile neutropenia, and fatigue compared with polysorbate-based docetaxel (Taxotere) [19]. An ongoing retrospective analysis of SPARC and HER-2 expression in these patients will provide interesting insight into whether these preclinical results hold true in the clinic. In addition, it remains to be seen whether these results of *nab*-paclitaxel superiority over polysorbate-based docetaxel can be confirmed in a phase III setting for metastatic breast cancer as well as in other tumor types such as lung cancer and prostate cancer.

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