Increased Antitumor Activity, Intratumor Paclitaxel Concentrations, and Endothelial Cell Transport of Cremophor-Free, Albumin-Bound Paclitaxel, ABI-007, Compared with Cremophor-Based Paclitaxel

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Abstract ABI-007, an albumin-bound, 130-nm particle form of paclitaxel, was developed to avoid Cremophor/ethanol-associated toxicities in Cremophor-based paclitaxel (Taxol) and to exploit albumin receptor-mediated endothelial transport. We studied the antitumor activity, intratumoral paclitaxel accumulation, and endothelial transport for ABI-007 and Cremophor-based paclitaxel. Antitumor activity and mortality were assessed in nude mice bearing human tumor xenografts [lung (H522), breast (MX-1), ovarian (SK-OV-3), prostate (PC-3), and colon (HT29)] treated with ABI-007 or Cremophor-based paclitaxel. Intratumoral paclitaxel concentrations (MX-1-tumored mice) were compared for radiolabeled ABI-007 and Cremophor-based paclitaxel. *In vitro* endothelial transcytosis and Cremophor inhibition of paclitaxel binding to cells and albumin was compared for ABI-007 and Cremophor-based paclitaxel. Both ABI-007 and Cremophor-based paclitaxel caused tumor regression and prolonged survival; the order of sensitivity was lung > breast ≅ ovary > prostate > colon. The LD_{50} and maximum tolerated dose for ABI-007 and Cremophor-based paclitaxel were 47 and 30 mg/kg/d and 30 and 13.4 mg/kg/d, respectively. At equitoxic dose, the ABI-007-treated groups showed more complete regressions, longer time to recurrence, longer doubling time, and prolonged survival. At equal dose, tumor paclitaxel area under the curve was 33% higher for ABI-007 versus Cremophor-based paclitaxel, indicating more effective intratumoral accumulation of ABI-007. Endothelial binding and transcytosis of paclitaxel were markedly higher for ABI-007 versus Cremophor-based paclitaxel, and this difference was abrogated by a known inhibitor of endothelial gp60 receptor/caveolar transport. In addition, Cremophor was found to inhibit binding of paclitaxel to endothelial cells and albumin. Enhanced endothelial cell binding and transcytosis for ABI-007 and inhibition by Cremophor in Cremophor-based paclitaxel may account in part for the greater efficacy and intratumor delivery of ABI-007.

Paclitaxel is a naturally occurring complex diterpenoid product extracted from the bark of the western yew, *Taxus brevifolia* (1). The unique mechanism of paclitaxel of stabilizing tubulin polymer and promoting microtubule assembly effectively inhibits mitosis, motility, and intracellular transport within cancerous cells and results in antineoplastic activity against a wide variety of malignancies (2–4). Paclitaxel is widely used for the treatment of breast, lung, and advanced ovarian cancers (5).

Because paclitaxel has very little aqueous solubility, Cremophor-based paclitaxel uses a Cremophor EL/ethanol vehicle. The amount of Cremophor EL necessary to deliver the requisite doses of paclitaxel is significantly higher than that given with any other marketed drug containing Cremophor EL, reaching plasma concentrations up to 0.4% and remaining >0.1% for 24 hours following a dose of 175 mg/m^2 (6). The Cremophor EL–containing paclitaxel formulation causes severe allergic, hypersensitivity, and anaphylactic reactions in animals and humans (4, 7–14). Although premedication with steroids, antihistamines, and H_2 receptor blockers before administration of Cremophor-based paclitaxel has reduced the severity of hypersensitivity reactions, fatalities associated with drug administration have still occurred. To address this problem, a variety of formulations and delivery systems are being investigated to administer paclitaxel in a more safe and convenient manner (15–27).

ABI-007 is an albumin-bound, 130-nm particle formulation of paclitaxel, which is devoid of any solvents or ethanol (28). The lyophilized formulation comprising albumin and paclitaxel is reconstituted in 0.9% NaCl and forms a colloidal suspension. The human serum albumin (HSA)–stabilized paclitaxel particles have an average particle size of ~130 nm, which allows for i.v. administration without risk of capillary

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blockage. When given i.v. every 3 weeks, the maximum tolerated dose (MTD) in humans for ABI-007 was 300 mg/m^2, considerably higher than the standard dose used for Cremophor-based paclitaxel (29). A randomized, phase III study compared equitoxic doses of ABI-007 (260 mg/m^2) and Cremophor-based paclitaxel (175 mg/m^2) in 454 patients with metastatic breast cancer (30). Response rates were significantly higher for ABI-007 than for Cremophor-based paclitaxel for all patients (33% versus 19%; $P = 0.001$) as well as for those who received study drug as first-line therapy (42% versus 27%; $P = 0.029$).

In this study, we investigated the comparative antitumor activity of ABI-007 and Cremophor-based paclitaxel in xenograft tumor models, compared the intratumoral accumulation of paclitaxel for ABI-007 and Cremophor-based paclitaxel, and investigated the possible mechanisms of increased intratumoral accumulation by studying endothelial cell transport and inhibitory activity of the Cremophor component in Cremophor-based paclitaxel.

Materials and Methods

Study drugs

ABI-007 (Abraxane, American BioScience, Inc., Santa Monica, CA) was prepared by a proprietary process. Cremophor-based paclitaxel (Taxol, Mead Johnson, Evansville, IN) was purchased from a hospital pharmacy. Both drugs were reconstituted in normal saline, prepared fresh daily as required, and given within 1 hour of preparation.

Animals and human tumor xenografts

Female athymic NCr-nu nude mice were obtained from Charles River Laboratories (Raleigh, NC) and held in quarantine for 7 days. At the start of treatment, body weights ranged from 21 to 25 g and ages ranged from 6 to 8 weeks. The human tumors used in this study (in order of decreasing sensitivity to paclitaxel) were H522 (lung), MX-1 (breast), SK-OV-3 (ovarian), PC-3 (prostate), and HT29 (colon). Cell lines were obtained initially from the National Cancer Institute and propagated *in vivo* as solid tumors. Tumors were implanted s.c. as 30 to 40 mg fragments and allowed to increase to a median size of ~160 mg before treatment was initiated.

Study design

Non-tumor-bearing mice were given ABI-007 (2 or 4 animals per dose group) or Cremophor-based paclitaxel (2 mice per dose group) at 13.4, 20, 30, 45, 57, and 100 mg/kg/d for the purpose of dose finding. Dose levels for tumored animals were 13.4, 20, 30, and 45 mg/kg/d for ABI-007 and 13.4, 20, and 30 mg/kg/d for Cremophor-based paclitaxel (pilot studies indicated that Cremophor-based paclitaxel doses >30 mg/kg/d were lethal to mice). Control groups for each xenograft were given saline only. Study drugs were given into the tail vein once daily for 5 consecutive days so that adequate amounts of Cremophor-based paclitaxel could be given; injection volume was 0.1 mL for each 10 g of mouse body weight. Ten mice were treated per dose group per tumor xenograft, except for the SK-OV-3/ABI-007 group ($n = 30$) and the MX-1/Cremophor-based paclitaxel group ($n = 5$).

Tumor evaluation

Caliper measurements of the longest (L) and shortest (W) tumor diameters (mm) were obtained twice weekly. The formula for an ellipsoid sphere $[(L \times W^2) / 2]$ was used to calculate the tumor volume. The volume was converted to tumor weight assuming unit density (i.e., 1 mm^3 = 1 mg). For humane reasons, animals were sacrificed when the implanted tumor volume became >4,000 mm^3. The time required for a tumor to double in mass was calculated based on the initial tumor weight at the beginning of the treatment period. Tumor recurrence was defined as the first observation of increased tumor size following tumor regression.

Intratumor paclitaxel concentrations

Radiolabeled paclitaxel. [^{3}H]Paclitaxel of >99% purity was obtained from Moravek Biochemicals (Brea, CA). The majority of the tritium was in the *m*- and *p*-positions of the aromatic rings, with minor amounts in the 10-, 3′-, and 2-positions of the taxane ring system. To prepare [^{3}H]ABI-007, [^{3}H]paclitaxel was diluted isotopically with unlabeled paclitaxel to a final specific activity of 25 μCi/mg paclitaxel and incorporated into the albumin-bound form using a proprietary process. [^{3}H]Cremophor-based paclitaxel was prepared by spiking Taxol injection concentrate, containing paclitaxel at 6 mg/mL in a mixture of Cremophor and 50% ethanol USP, with [^{3}H]paclitaxel to a specific activity of 25 μCi/mg paclitaxel. The radiochemical purity both [^{3}H]ABI-007 and [^{3}H]Cremophor-based paclitaxel were confirmed by extraction with acetonitrile/0.9% NaCl (2:1) and analysis of the extract by high-performance liquid chromatography in conjunction with a radioactive detector.

Animal treatment. Athymic mice ($n = 126$) bearing MX-1 tumors with size of 600 mm^3 were used in two studies. In total, there were 9 animals per time point and 7 time points (5, 15, and 30 minutes and 1, 3, 8, and 24 hours) for each arm ($n = 63$ for Cremophor-based paclitaxel arm and $n = 63$ for ABI-007 arm). The [^{3}H]ABI-007 and [^{3}H]Cremophor-based paclitaxel dosing solutions were prepared by placing a weighed amount of [^{3}H]ABI-007 powder or measured volume of Taxol injection concentrate and [^{3}H]paclitaxel solution in ethanol into a glass scintillation vial, respectively. Normal saline for injection was added to each vial to achieve final concentrations of ~3 mg/mL paclitaxel. The drugs were given by bolus tail vein injection at a dose of 20 mg/kg paclitaxel (~500 μCi/kg total radioactivity). To quantitate the radioactivity in the dosing preparations, three 100-μL aliquots of each solution were diluted and analyzed for radioactivity by liquid scintillation counting.

Tumor harvesting and radioactivity measurements. After dosing, tumors were removed at the various time points following euthanization by CO_2 asphyxiation. Tumors were homogenized in ~5 volumes of distilled water, and duplicate aliquots representing ~100 mg tissue were weighed directly into combustion cones. The samples were combusted in a sample oxidizer, and the amount of radioactivity was determined by liquid scintillation counting. Tritiated water liberated from combustion samples was trapped in Monophase S (Packard Instrument Co., Meriden, CT), which was contained in liquid scintillation vials. The combustion efficiency was checked daily before the combustion of samples; ^{3}H recovery was always 95% to 105%. Total radioactivity measurements were done using a Beckman LS6500 liquid scintillation spectrometer (Beckman Instruments, Fullerton, CA). Counting time was for a maximum of 10 minutes or to a statistical accuracy of ±2%, whichever occurred first. The spectrometer was programmed to subtract background values and to convert counts per minute to disintegrations per minute automatically.

In vitro binding and transport studies

Fluorescent-labeled ABI-007 and Cremophor-based paclitaxel. Fluorescent-labeled paclitaxel (Oregon Green paclitaxel conjugate, abbreviated as Flutax) was obtained from Molecular Probes (Eugene, OR). Fluorescent-labeled ABI-007 was prepared using a Flutax to unlabeled paclitaxel ratio of 1:50 using a proprietary process. Fluorescent-labeled Cremophor-based paclitaxel was prepared by spiking Flutax into Taxol (Bristol-Myers Squibb, New York, NY) to obtain the same ratio of labeled to unlabeled paclitaxel as in fluorescent ABI-007.

Inhibition of paclitaxel binding to HSA by Diluent 12 (Cremophor EL/ethanol). Costar sterile 96-well flat-bottomed special optics plate 3614 (Corning, Inc., Corning, NY) was coated with 20% albumin

Table 1. Nonspecific deaths

Dose* (mg/kg/d)	H522 (lung)		MX-1 (breast)		SK-OV-3 (ovarian)		PC-3 (prostate)		HT29 (colon)		Nontumor-bearing		Total	
	ABI-007	CBP	ABI-007	CBP	ABI-007	CBP	ABI-007	CBP	ABI-007	CBP	ABI-007	CBP	ABI-007	CBP
100	—	—	—	—	—	—	—	—	—	—	4/4	2/2	4/4	2/2
57	—	—	—	—	—	—	—	—	—	—	3/4	2/2	3/4	2/2
45	6/10	—	0/10	—	8/30	—	10/10	—	7/10	—	2/4	2/2	33/74	2/2
30	0/10	7/10	0/10	1/5	0/30	7/10	2/10	6/10	1/10	2/10	0/2	0/2	3/72	23/47
20	0/10	2/10	0/10	1/5	1/30	2/10	0/10	2/10	0/10	1/10	0/2	0/2	1/72	8/47
13.4	0/10	0/10	0/10	0/5	1/30	0/10	0/10	1/10	0/10	1/10	0/2	0/2	1/72	2/47

NOTE: A treated, normal animal was presumed to be a nonspecific death if its day of death was <14 days after the last day of treatment. A treated, tumored animal was presumed to be a nonspecific death if its day of death was significantly less ($P < 0.05$) than the corresponding day of death in the untreated control group and its tumor volume was <400 mg or if it died with a tumor of ≤400 mg within 40 days after the last day of treatment or with a regressing tumor within 15 days after the last day of treatment.

Abbreviation: CBP, Cremophor-based paclitaxel.

*Administered i.v. once daily for 5 consecutive days.

(Grifols, Miami, FL) at room temperature for 1 hour and washed thrice with PEM buffer (50 mmol/L PIPES, 2 mmol/L EGTA, 2 mmol/L $MgCl_2$). The immobilized HSA was reacted with Flutax at final concentration of 0.5 μg/mL in the presence of increasing concentration of Diluent 12 in PEM buffer for 1 hour at room temperature, washed thrice with PEM buffer, and then read using the Fluoroskan microplate reader. Diluent 12 is the Cremophor EL/ethanol used in Taxol.

Binding of ABI-007-Flutax and Cremophor-based paclitaxel-Flutax to live human umbilical vascular endothelial cells. Human umbilical vascular endothelial cells were grown to confluence on 96-well microplates (Costar 3614). ABI-007-Flutax and Cremophor-based paclitaxel-Flutax were added to human umbilical vascular endothelial cells at final concentrations of 20, 40, 80, 160, 320, and 640 μg paclitaxel/mL PEM for 1 hour at 37°C, washed with thrice with PEM, and read using the Fluoroskan microplate reader (Thermo Labsystems, Helsinki, Finland).

Transport of ABI-007-Flutax and Cremophor-based paclitaxel-Flutax across a monolayer of human lung microvessel vascular endothelial cells. Human lung microvessel vascular endothelial cells in EBM-PRF medium (Cambrex, East Rutherford, NJ) were seeded at 84,000 per insert on top of the permeable membrane separating the upper and lower chamber of the Transwell apparatus (Falcon HTS FluoroBlok Inserts, BD Biosciences, San Jose, CA). After 24 hours, the cells were incubated with EBM-PRF medium supplemented with 5% HSA and either 0 or 10 mmol/L methyl β-cyclodextrin at 37°C. After 15 minutes, transport was initiated with addition of ABI-007-Flutax or Cremophor-based paclitaxel-Flutax at final concentration of 20 μg paclitaxel/mL medium supplemented with 5% HSA to the upper chamber. The movement of Flutax across the endothelial barrier into the bottom chamber was continuously monitored by Fluoroskan microplate reader.

Data analysis for LD_{50}, tumor xenografts, tumor accumulation kinetics, and *in vitro* binding studies

Nonspecific deaths from all groups were pooled. Doses that resulted in 50% mortality (LD_{50}) were calculated using the fitted mortality curves (GraphPad Prism, San Diego, CA). MTD was defined as the highest dose level with <10% mortality. Tumor doubling time and time to tumor recurrence were analyzed by Kaplan-Meier techniques (StatView, SAS Institute, Inc., Cary, NC). Tumor volume, body weight, and mortality were compared using ANOVA for repeated measures (StatView). Median time to tumor recurrence, tumor doubling time, and number of tumor-free survivors (up to 103 days postimplant) were reported for equal doses (30 mg/kg/d) and equitoxic doses (MTD, 13.4 and 30 mg/kg/d for ABI-007 and Cremophor-based paclitaxel, respectively) for the five human tumor xenografts; tumor volumes and Kaplan-Meier analysis of tumor recurrence were compared for the equitoxic doses. Kinetic variables (i.e., area under the curve and maximum concentration) of tumor accumulation were generated using Win NonLin (Pharsight, Mountain View, CA). IC_{50}s for the binding studies were calculated using Prism software.

Results

Antitumor activity. ABI-007 was significantly less toxic than Cremophor-based paclitaxel (Table 1; Fig. 1; $P = 0.017$, ANOVA). The LD_{50} for ABI-007 and Cremophor-based paclitaxel with this dose schedule were 47 and 30 mg/kg/d, respectively. Because of the large number of deaths at the 30 mg/kg Cremophor-based paclitaxel dose, the MTDs for each drug were used for subsequent efficacy comparisons. At the 30 mg/kg/d dose, mortality for ABI-007 and Cremophor-based paclitaxel were 4% (3 of 72) and 49% (23 of 47), respectively ($P < 0.0001$, Fisher's exact test). MTDs were 30

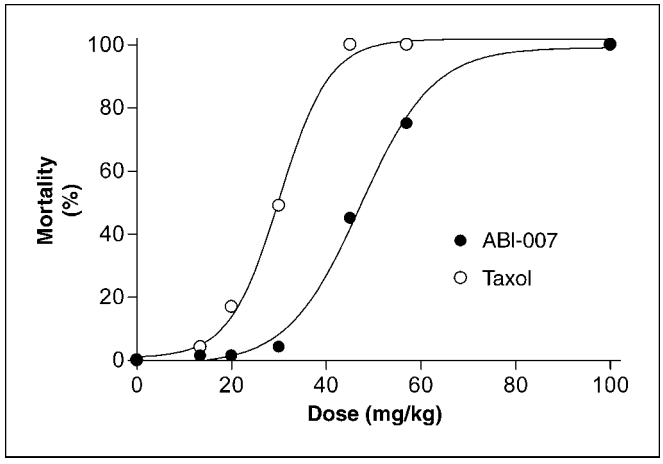

Fig. 1. LD_{50} for Cremophor-based paclitaxel and ABI-007. Mortality data from all tumor models as well as from non-tumor-bearing animals were plotted versus dose and curve-fitted using the Boltzmann sigmoidal equation. ABI-007 was significantly less toxic than Cremophor-based paclitaxel. $P = 0.017$ (ANOVA).

and 13.4 mg/kg/d for ABI-007 and Cremophor-based paclitaxel, respectively; these doses were also equitoxic doses (4% mortality for both).

The lung tumor xenograft was the most sensitive to both drugs as indicated by the proportion of tumor-free survivors, tumor doubling times, and tumor volumes (Table 2; Fig. 2). No statistically significant differences were observed between the ABI-007 and Cremophor-based paclitaxel groups in any measure of antitumor activity, although the mean tumor volumes were lower for ABI-007 than Cremophor-based paclitaxel.

The breast and ovarian tumor xenografts showed the greatest differences between the two treatments. At equitoxic doses (30 and 13.4 mg/kg/d for ABI-007 and Cremophor-based paclitaxel, respectively), the proportion of tumor-free survivors was higher for the ABI-007 groups compared with Cremophor-based paclitaxel for the breast (10 of 10 versus 1 of 5, respectively) and ovarian (7 of 29 versus 0 of 10, respectively) tumor xenografts (Table 2). ABI-007 showed greater antitumor activity than Cremophor-based paclitaxel as measured by median time to tumor recurrence [breast, >103 versus 22 days, respectively ($P = 0.004$); ovarian, 63 versus 26 days, respectively ($P < 0.001$)], tumor doubling time [breast, >95 versus 31.2 days, respectively ($P = 0.0015$); ovarian, >51 versus 44.2 days, respectively ($P < 0.0001$)] (Table 2), and tumor volume [breast ($P = 0.009$); ovarian ($P < 0.0001$)] groups (Fig. 2).

For the prostate tumor xenograft (Table 2; Fig. 2) at the equitoxic doses, the ABI-007 group showed a trend toward slower tumor growth ($P = 0.06$) and longer median tumor doubling times (52.9 versus 40.2 days). Median time to tumor recurrence was significantly longer in the group treated with ABI-007 (48 versus 26 days; $P = 0.04$).

In the colon tumor xenograft (Table 2; Fig. 2) at the equitoxic doses, complete regression was not observed with either ABI-007 or Cremophor-based paclitaxel (Table 2). The ABI-007 group showed a trend toward slower tumor growth group ($P = 0.06$) and longer median tumor doubling times (44.9 and 29.4 days for ABI-007 and Cremophor-based paclitaxel, respectively; $P = 0.01$). Median time to tumor recurrence was significantly greater in the group treated with ABI-007 than in the Cremophor-based paclitaxel group (36 versus 26 days; $P = 0.003$).

Table 2. Antitumor activity of ABI-007 and Cremophor-based paclitaxel at equal and equitoxic doses (administered i.v. once daily for 5 consecutive days)

Xenograft treatment (mg/kg/d)	*n*	Tumor-free survivors	Recurrence (d), median (range)*	*P* †	Tumor doubling (d), median (range) ‡	*P* †
H522 (lung)						
Control	10	0	—	—	6.6 (6-10.4)	—
ABI-007 (30)	10	9	>78 (61->78)	—	>63 (>63->63)	—
CBP (13.4)	10	7	>78 (26->78)	0.2	>63 (41.8->63)	NS
CBP (30)	3	3	>78 (>78->78)	NA§	>63 (>63->63)	NA§
MX-1 (breast)						
Control	10	0	—	—	6.3 (3.9-8.5)	—
ABI-007 (30)	10	10	>103	—	>95 (88->95)	—
CBP (13.4)	5	1	22 (22->103)	0.004	31.2 (26.3->95)	0.0015
CBP (30)	4	2	47 (22->103)	NS	48.9 (25.6->95)	NS
SK-OV-3 (ovarian)						
Control	10	0	—	—	15.5 (10.9-24.3)	—
ABI-007 (30)	29	7	63 (26->77)	—	>51 (41.3->51)	—
CBP (13.4)	10	0	26 (26-63)	<0.0001	44.2 (15.0->51)	<0.0001
CBP (30)	3	0	63 (26-63)	NA§	>51 (26.2->51)	NA§
PC-3 (prostate)						
Control	10	0	—	—	13.5 (6.7-28.3)	—
ABI-007 (30)	8	1	48 (26->80)	—	52.9 (45.2->67)	—
CBP (13.4)	9	1	26 (26->80)	0.04	40.2 (29.6->67)	NS
CBP (30)	4	0	41 (26-48)	NA§	39.6 (32.3-63.7)	NA§
HT29 (colon)						
Control	10	0	—	—	8.8 (6.9-17.4)	—
ABI-007 (30)	9	0	36 (26-61)	—	44.9 (23.5->49)	—
CBP (13.4)	9	0	26 (26-29)	0.003	29.4 (22.9-43.7)	0.01
CBP (30)	8	0	29 (26-33)	0.01	38.1 (33.7-45.7)	NS

Abbreviations: NS, not significant; NA, not assessed.

*The first observation of increased tumor size following a tumor regression.

†Compared with ABI-007 at 30 mg/kg/dose.

‡Time required for tumor to reach two volume doubling from initial dosing day.

§The lethal toxicity associated with Cremophor-based paclitaxel at 30 mg/kg/d prohibited equal-dose comparisons of antitumor activity for the lung, ovary, and prostate tumor xenografts.

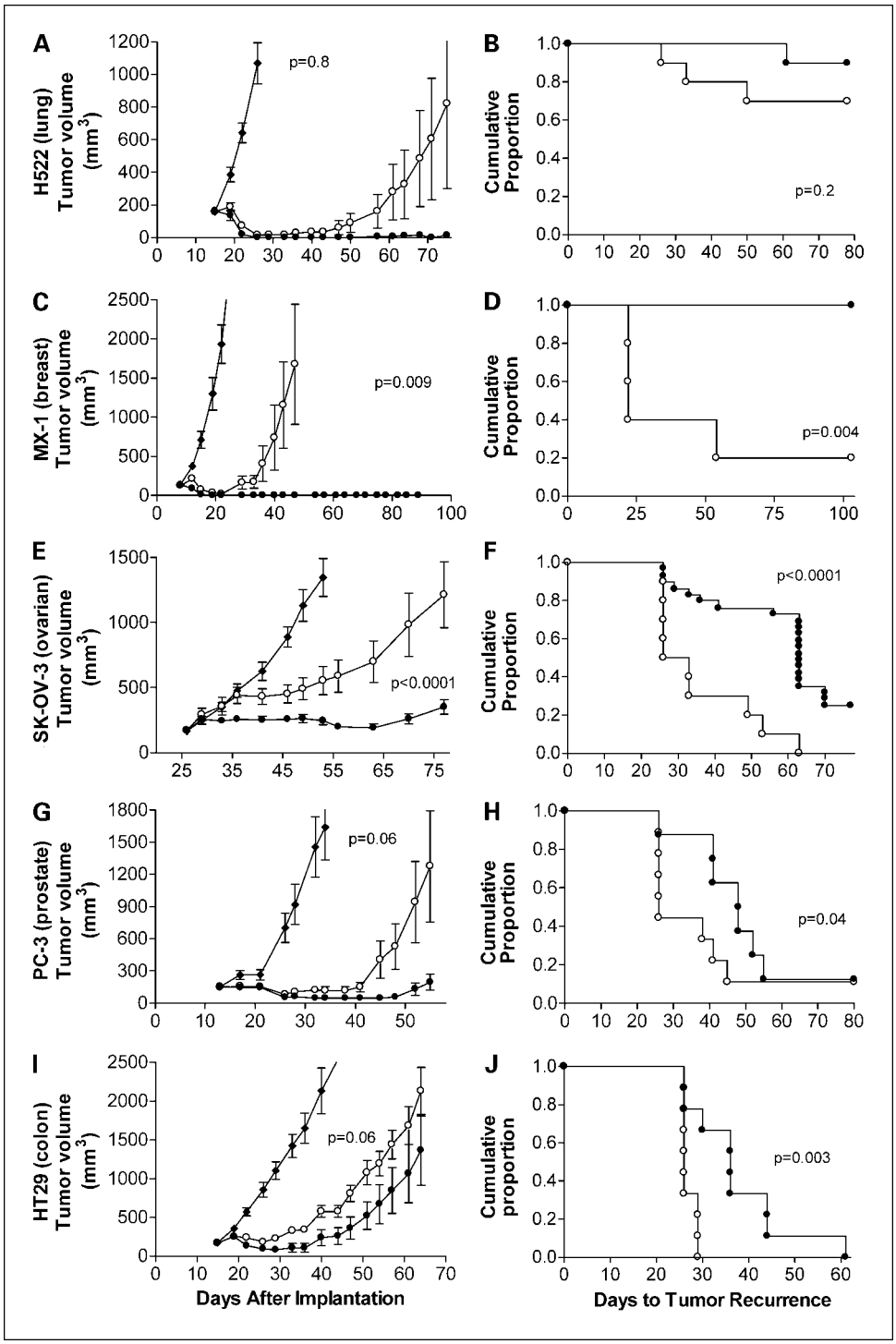

Fig. 2. Tumor volume (*A, C, E, G,* and *I*) and Kaplan-Meier analysis of tumor recurrence (*B, D, F, H*, and *J*) in five tumor xenografts treated with equitoxic doses of ABI-007 (30 mg/kg/d, ●) or Cremophor-based paclitaxel (13.4 mg/kg/d, ○). ♦, untreated controls. *A* and *B*, H522 (lung tumor xenograft); *C* and *D*, MX-1 (breast tumor xenograft); *E* and *F*, SK-OV-3 (ovarian tumor xenograft); *G* and *H*, PC-3 (prostate tumor xenograft); *I* and *J*, HT29 (colon tumor xenograft).

Mean body weights were not significantly different (ANOVA) between ABI-007 and Cremophor-based paclitaxel groups treated at the MTD for the lung, breast, ovarian, colon, and prostate tumor xenografts (data not shown).

Intratumor paclitaxel accumulation. Following equal doses of paclitaxel (20 mg/kg i.v.), the intratumor paclitaxel accumulation was significantly higher for ABI-007 than for Cremophor-based paclitaxel in two independent experiments (Fig. 3; $P < 0.0001$, ANOVA). ABI-007 exhibited a rapid partitioning into tumor tissue with an absorption constant (K_a) 3.3-fold greater than Cremophor-based paclitaxel (0.43 and 0.13 h^{-1} for ABI-007 and Cremophor-based paclitaxel, respectively). The difference in intratumor paclitaxel concentrations was already apparent at the first time point sampled (5 minutes; Fig. 3) and was maximal at 3 hours. Overall, tumor area under the curve of paclitaxel was 33% higher for ABI-007 versus Cremophor-based paclitaxel (3632 versus 2739 nCi hour/g).

In vitro *binding and transport studies.* To investigate potential mechanisms responsible for increased intratumor

concentrations of paclitaxel, we compared the endothelial binding and transport of paclitaxel when formulated as ABI-007 or Cremophor-based paclitaxel. Endothelial binding of paclitaxel increased 9.9-fold ($P < 0.0001$; Fig. 4A) and transport of paclitaxel across an endothelial cell monolayer increased 4.2-fold ($P < 0.0001$; Fig. 4B), in the ABI-007 formulation compared with Cremophor-based paclitaxel. Endothelial transcytosis of ABI-007 was completely suppressed by methyl β-cyclodextrin (Fig. 4B), an inhibitor of caveolar mediated transcytosis (31). Cremophor EL inhibited paclitaxel endothelial cell binding in a dose-dependent manner (IC_{50}, 0.010%; Fig. 4C) with complete inhibition occurring at a concentration of 0.1%. Similar inhibition was observed for paclitaxel binding to albumin (IC_{50}, 0.0017%; Fig. 4D), well below clinically relevant Cremophor concentrations.

Discussion

This study in five human tumor xenograft models showed that the Cremophor-free, albumin-bound particle form of paclitaxel (ABI-007) has increased antitumor activity compared with equitoxic doses of Cremophor-based paclitaxel. Both paclitaxel formulations showed antitumor activity against the xenografts studied here, and the order of sensitivity was the same for both: lung > breast ≅ ovary > prostate > colon. Differences between treatments were evident for breast and ovarian tumor xenografts assessed at equitoxic doses (ABI-007, 30 mg/kg/d; Cremophor-based paclitaxel, 13.4 mg/kg/d), with all antitumor assessments showing greater activity for ABI-007. In the other tumor xenografts, ABI-007 resulted in delayed time to tumor recurrence (colon and prostate) and increased tumor doubling time (colon) compared with equitoxic doses of Cremophor-based paclitaxel; tumor volume was not statistically significantly different between the treatments (colon, prostate, and lung), but tumor volumes were lower at each time point for ABI-007.

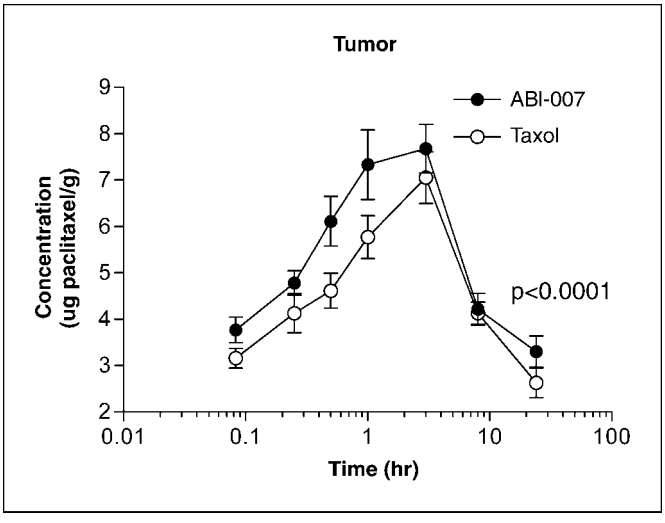

Fig. 3. Intratumor paclitaxel concentrations following equal doses of ABI-007 and Cremophor-based paclitaxel. MX-1 tumor-bearing nude mice were dosed at 20 mg/kg paclitaxel and tumor paclitaxel was monitored by radioactivity. Paclitaxel accumulation was 33% higher for ABI-007 (●) than that of Cremophor-based paclitaxel (○). $P < 0.0001$.

Equitoxic doses were the primary comparison of the antitumor activity for the two paclitaxel formulations because the mortality associated with Cremophor-based paclitaxel at 30 mg/kg/d reduced the number of evaluable animals, thus prohibiting equal-dose comparisons of antitumor activity for the lung, ovary, and prostate tumor xenografts. Equal-dose comparisons (30 mg/kg/d) in the breast and colon tumor xenografts showed that tumor doubling times and times to tumor recurrence were greater in the ABI-007 groups than in Cremophor-based paclitaxel groups (Table 2, not statistically significant, except for time to tumor recurrence in the colon tumor xenograft group). Because chemotherapy is generally given at the highest tolerated dose, we consider the comparison of equitoxic doses (which, in this study, were also MTDs), rather than equal doses, to be more clinically relevant. Indeed, these data have been borne out in the recent phase III clinical study, which showed statistically improved efficacy for ABI-007 compared with equitoxic doses of Cremophor-based paclitaxel (30).

Intratumor concentrations of paclitaxel were 33% higher following administration ABI-007 compared with equal doses of Cremophor-based paclitaxel in the MX-1 xenograft model. This observed increase in intratumor accumulation of paclitaxel with ABI-007 was supported by data showing a 9.9-fold increase in endothelial cell binding for ABI-007, a 4.2-fold increase in endothelial transcytosis for ABI-007, inhibition of endothelial cell binding of paclitaxel in presence of Cremophor, and inhibition of albumin binding of paclitaxel in presence of Cremophor. Further studies in other tumor models and *in vivo* mechanistic studies are indicated to confirm and extend these observations.

Transendothelial cell transport of albumin is mediated by the gp60 (albondin) receptor and caveolar transport (32, 33). Albumin binding to gp60 activates caveolin-1 resulting in the formation of caveoli, which transport albumin and other plasma constituents across the endothelial cell to the interstitial space. Therefore, to identify potential mechanisms for the increased intratumor concentrations that occurred with ABI-007, we compared paclitaxel binding and transport in endothelial cells for ABI-007 and Cremophor-based paclitaxel. The increased binding and transport of albumin-bound paclitaxel across endothelial cells was striking and inhibited by cotreatment with β-methyl cyclodextrin, a known functional inhibitor caveolar transport (34). An unexpected but potentially important finding was the inhibition of this pathway at clinically relevant concentrations of Cremophor EL, which are achieved following administration of Cremophor-based paclitaxel (6). Conventional thinking in drug transport to tumors has been focused on passive transport via the leaky vasculature aspects of tumor microvessels (35). The present results may suggest that tumor microvessel endothelial cells could play an active role in transport of ABI-007 from the vasculature to the tumor interstitium via an albumin-based receptor mediated pathway that is inhibited by Cremophor.

It is known that Cremophor EL in Cremophor-based paclitaxel can sequester paclitaxel in Cremophor micelles at clinically relevant concentrations of Cremophor (36). Cremophor concentrations in blood 24 hours following a Cremophor-based paclitaxel infusion have been reported to be in the range of 0.1% (6). In our hands, Cremophor EL/ethanol suppressed paclitaxel binding to endothelial cells and to albumin with Cremophor IC_{50}s of 0.010% and 0.0017%, well below the reported prevalent

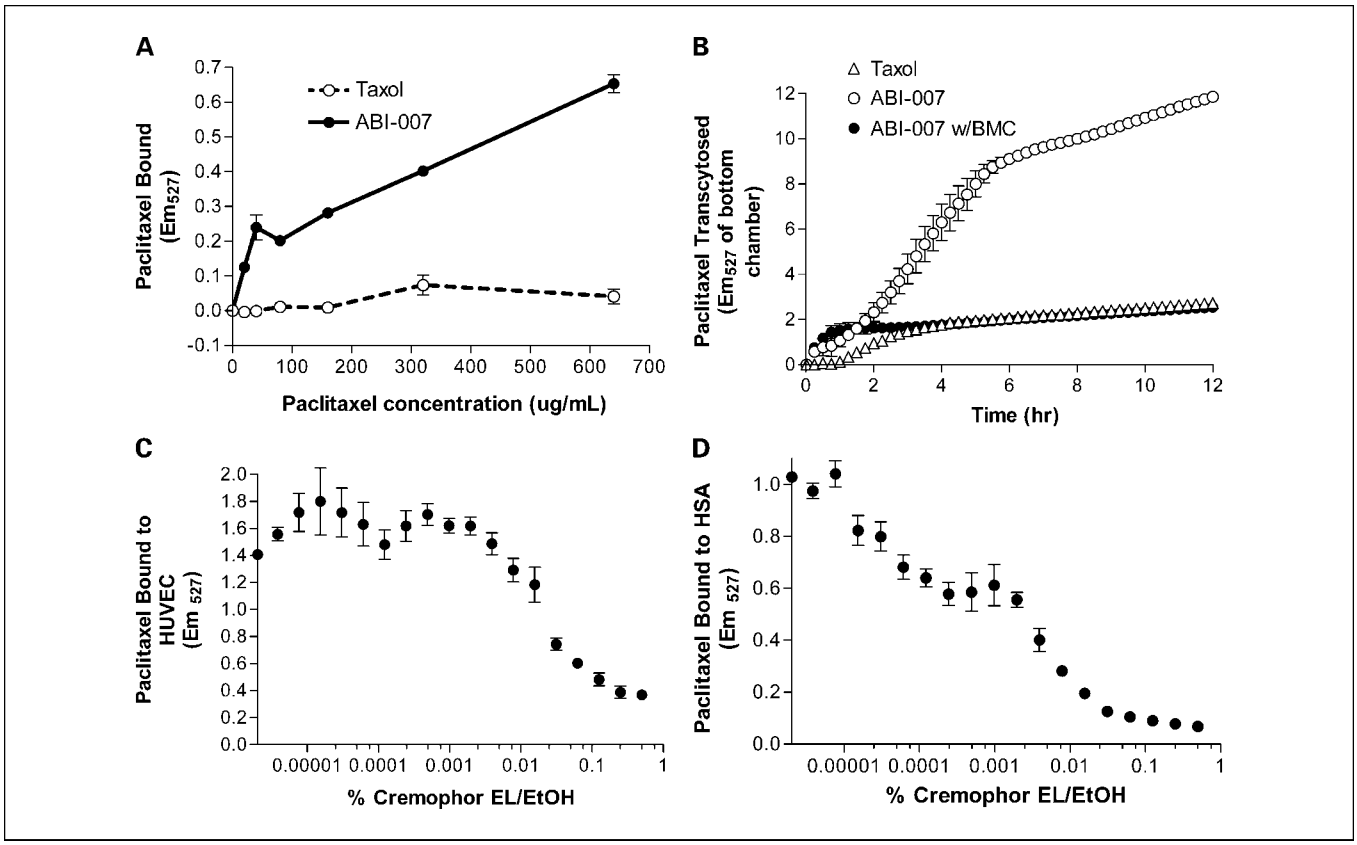

Fig. 4. *A*, binding of paclitaxel formulated as ABI-007 and Cremophor-based paclitaxel to endothelial cells. Binding of ABI-007 (●) and Cremophor-based paclitaxel (○) to live human umbilical vascular endothelial cells. There was a 9.9-fold increase in paclitaxel binding for ABI-007 versus Cremophor-based paclitaxel. $P < 0.0001$. *B*, transcytosis across human umbilical vascular endothelial cells of paclitaxel formulated as ABI-007 or Cremophor-based paclitaxel. Endothelial transcytosis of ABI-007 (●) and Cremophor-based paclitaxel (○). Cremophor-based paclitaxel transport was all paracellular, whereas ABI-007 transport was both paracellular and transcellular. The transcellular component of ABI-007 was inhibited by methyl β-cyclodextrin (*BMC*), a known inhibitor of the gp60/caveolar transport. *C*, inhibition of paclitaxel binding to endothelial cells (human umbilical vascular endothelial cells) by Cremophor. Paclitaxel binding to endothelial cells in the presence of increasing concentration (%v/v) of Cremophor EL/ethanol. There was a dose-dependent inhibition of paclitaxel binding by Cremophor EL/ethanol. *D*, inhibition of paclitaxel binding to HSA by Cremophor. Paclitaxel binding to HSA in presence of increasing concentration (%v/v) of Cremophor EL/ethanol. There was a dose-dependent inhibition of paclitaxel binding by Cremophor EL/ethanol.

blood concentrations of Cremophor at 24 hours following a Cremophor-based paclitaxel infusion. Therefore, based on these data, one would expect the potent inhibitory effect of Cremophor to suppress albumin and cellular binding of paclitaxel *in vivo* and potentially inhibit gp60/caveolar-mediated transport. Thus, the lack of Cremophor-micelle sequestration of paclitaxel and/or increased albumin-mediated transport may explain the increased intratumoral accumulation of paclitaxel for ABI-007. Furthermore, the increased endothelial cell binding seen for ABI-007 could also lead to increased antiangiogenic activity resulting in better tumor response. In fact, increased antiangiogenic activity *in vitro* for ABI-007 versus Cremophor-based paclitaxel has been shown recently.[3]

In summary, ABI-007 is a Cremophor-free, albumin-bound 130-nm particle form of paclitaxel that showed an improved efficacy and therapeutic index in multiple animal models. ABI-007 also exhibited increased endothelial cell transport of paclitaxel *in vitro*, a process that was inhibited by Cremophor. Although the ability to give higher doses of paclitaxel in the absence of Cremophor is clinically important, the increased antitumor activity of ABI-007 may also be related to enhanced intratumor delivery of paclitaxel.

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[3] S.S.W. Ng, et al. Influence of formulation vehicles on metronomic taxane chemotherapy: nanoparticle of albumin-bound versus Cremophor EL-based paclitaxel, submitted for publication to Clinical Cancer Research, 2005.

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