PK analysis were collected for 24 h following each dose of simvastatin. Plasma samples were assayed for dasatinib, simvastatin and simvastatin acid by validated LC MS/MS. PK parameters were derived from plasma concentration versus time data by noncompartmental methods.
Results: Dasatinib increased the C_{max} of simvastatin by 37% and AUC_{∞} by 20% versus simvastatin alone. Dasatinib also increased the C_{max} of simvastatin acid by 41% and AUC_{∞} by 27% versus simvastatin alone.

Table: Summary statistics for simvastatin pharmacokinetic parameters

Treatment	C_{max} (ng/mL) Geometric mean (CV%)	AUC_{∞} (ng·h/mL) Geometric mean (CV%)	AUC_{0-T} (ng·h/mL) Geometric mean (CV%)	T_{max} (h) Median (min, max)	$t_{1/2}$ (h) Mean (SD)
Simvastatin 80 mg (n = 48)	26.68 (57)	117.95 (80)	108.05 (74)	1.50 (0.50, 8.00)	6.65 (3.00)
Simvastatin 80 mg and dasatinib 100 mg (n = 48)	36.53 (57)	141.29 (68)	132.97 (66)	1.00 (1.00, 5.00)	5.16 (2.85)

C_{max} = maximum plasma concentration; AUC = area under plasma concentration–time curve; T_{max} = time to maximum plasma concentration; $t_{1/2}$ = terminal half-life; SD = standard deviation; CV% = coefficient of variation.

Conclusions: Dasatinib increases exposure to the CYP3A4 substrates simvastatin and simvastatin acid. Due to the small effect size, these findings are not felt to be clinically significant.

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Delivery of indenoisoquinoline using customized releasable PEG linkers

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Background: Indenoisoquinolines are novel topoisomerase I inhibitors with good *in vitro* anti-tumor efficacies but suffer from poor water solubility. Using customized releasable PEG linker technology, we have successfully solublized the lead indenoisoquinoline compound, MJ-III-65 (NSC 706744), to facilitate its administration to animals.
Material and Methods: NSC 706744 and customized releasable PEG linkers were synthesized separately according to previously published methods. The selected PEG linker, PEG-RNL9, was conjugated with NSC 706744 through its secondary amine group. Two different molecular weights of PEG were used, i.e. 20k PEG to give EZN-2087 (NSC 735982) and 40k PEG to give EZN-2088 (NSC 735983). The NSC 735982 was determined to contain 4.4% NSC 706744 by weight while the NSC 735983 was 3% by weight. The *in vivo* hollow fiber assay (HFA) in mice was conducted per previously published methods using NSC 735982 at equivalent active doses of 12 and 18 mg/kg/dose and NSC 735983 at equivalent active doses of 9 and 12 mg/kg/dose.
Results: The PEG conjugates were stable in saline at room temperature for at least 4 hours and the half-lives in rat plasma were about 4 hours. This feature enabled administration of PEG conjugates of NSC 706744 *in vivo*. The *in vivo* studies of both PEGylated compounds, particularly NSC 735983 showed antitumor activity. Using the published scoring comparison, NSC 735982 produced scores of 12/48 IP and 4/48 SC for a total score of 16/96. Of greater note, the doses of NSC 735983 tested resulted in scores of 28/48 IP and 10/48 SC. The NSC 735983 total score of 38/96 places it in the top 3% of the 3604 compounds evaluated in the hollow fiber assay to date.
Conclusions: PEGylation of indenoisoquinoline compound using customized releasable PEG linkers has successfully solubilized the lead compound NSC 706744. This feature enabled *in vivo* evaluation of NSC 706744 in a form that was potentially more bioavailable than the parent compound. The NCI's *in vivo* HFA study revealed that anti-tumor efficacy could be achieved through this modification. In the future, customized releasable PEG linkers with different half-lives can be applied to this compound to further study the relationship between pharmacokinetic profile and efficacy.

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Enhanced antitumor activity and safety of albumin-bound nab-docetaxel versus polysorbate 80-based docetaxel

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Background: Docetaxel (Taxotere®; TAX) is currently formulated in the solvent polysorbate 80. Removal of solvents from taxane formulations, as in the case of albumin-bound *nab*-paclitaxel (Abraxane®), has resulted in significantly higher response rates and greater safety *[Gradishar, JCO 2005;23:7794]*. Polysorbate 80 strongly inhibited the binding of taxanes to albumin, possibly inhibiting albumin-based drug transport through the gp60 endothelial receptor *[Desai, EORTC-NCI-AACR, 2004]* and consequently reducing albumin-binding to tumor-secreted protein (SPARC). We compared the efficacy, toxicity, and pharmacokinetics (PK) of TAX and solvent-free *nab*-docetaxel.
Methods: *nab*-Docetaxel and TAX were tested in nude mice (q4dx3) in HCT-116 human colon carcinoma xenograft (equitoxic doses of 22 and 15 mg/kg, respectively;10/group) and PC3 human prostate xenograft (0, 10, 15, 20, or 30 mg/kg *nab*-docetaxel or 10 mg/kg TAX; 6/group). *nab*-Docetaxel was compared with TAX in rats for single-dose toxicity (25, 50, 75, 100, and 125 mg/kg), multiple-dose toxicity (5, 10, 15, 30, and 50 mg/kg q4dx3), and PK (10, 20, and 30 mg/kg) (all 3/group).
Results: Both drugs were effective in HCT-116 xenograft; at equitoxic doses, *nab*-docetaxel exhibited greater antitumor activity than TAX ($P < 0.0001$, ANOVA). In PC3 xenograft, TAX was toxic (6/6 rats died); *nab*-docetaxel was well tolerated at all doses (1 death [15 mg/kg]). Tumor suppression was observed at all *nab*-docetaxel doses (6/6 complete regressions at 30 mg/kg). In the single-dose study, mortality was more rapid and complete for TAX than for *nab*-docetaxel at all doses. LD_{50} was 63 mg/kg for *nab*-docetaxel and ~12.5 mg/kg for TAX. In the multiple-dose study, mortality was similar for both drugs, with complete survival only at the lowest dose (5 mg/kg), where weight loss, neutropenia, and organ toxicity were substantially less for *nab*-docetaxel than for TAX. PK was similar for *nab*-docetaxel and TAX at 10 mg/kg; however, at 20 and 30 mg/kg, C_{max} and AUC were lower and V_z and V_{ss} were higher for *nab*-docetaxel than for TAX. The AUC-dose relationship was linear for *nab*-docetaxel and exponential for TAX.
Conclusions: *nab*-Docetaxel was less toxic than TAX. *nab*-Docetaxel showed greater antitumor activity than TAX against HCT-116 colon and PC3 prostate tumors. PK results suggest solvent-mediated sequestration of docetaxel in plasma for TAX. These observations are similar to those seen for *nab*-paclitaxel vs solvent-based paclitaxel.

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Pharmacokinetics of IHL-305, a novel PEGylated liposome containing irinotecan, in rats and dogs

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Background: Recently, liposomal formulations of anticancer drugs have been developed to enhance their pharmacologic activity and/or to reduce their toxicity. IHL-305 is a PEGylated liposome containing irinotecan. In human xenografts, IHL-305 showed superior antitumor activity to irinotecan hydrochloride (CPT-11). We compared the plasma pharmacokinetics and excretion after intravenous administration of IHL-305 with those of CPT-11 in rats and dogs.
Materials and Methods: IHL-305 or CPT-11 was injected intravenously (i.v.) to SD rats (3, 10, and 30 mg/kg), and to beagle dogs (1, 3, and 10 mg/kg) in plasma pharmacokinetic studies. Plasma concentrations of irinotecan and its metabolites were analyzed by fluorescence-HPLC. In excretion studies, IHL-305 containing [^{14}C]irinotecan or [^{14}C]CPT-11 was given i.v. (10 mg/kg) to male rats and a dog. Urine, feces, and bile (rats only) were collected and radioactivity (RA) in excreta was analyzed.
Results: Irinotecan decreased monoexponentially with almost linear pharmacokinetics in both animals after IHL-305 dosing at the doses examined. The total clearances of irinotecan after IHL-305 dosing were about 1/500 and 1/100 of those after CPT-11 dosing in rats and dogs, respectively. The distribution volume was about 1/80 to 1/30 of those after CPT-11 dosing. No apparent gender difference was observed in rats. The AUC ratios of lactone to carboxylate forms of irinotecan were about 150 and 1.6 after IHL-305 and CPT-11 dosing in dogs, respectively. IHL-305 increased the AUC and mean residence time of 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of irinotecan, by about 3- and 9-fold compared with those after CPT-11 dosing in rats, respectively. Urinary and fecal excretion of RA after IHL-305 dosing was almost completed at 48 hours, whereas at 24 hours after CPT-11 dosing in rats.

For a dog, there was a similar tendency. Biliary excretion continued up to 48 hours after IHL-305 dosing, whereas it was completed at 24 hours after CPT-11 dosing in rats.
Conclusions: After the administration of IHL-305, irinotecan was cleared very slowly from plasma, and most irinotecan in plasma existed as the lactone form. IHL-305 dosing also retained more plasma SN-38 longer than CPT-11 dosing in rats. These pharmacokinetic profiles of IHL-305 were considered to explain its superior antitumor activity other than enhanced permeability and retention (EPR) effect.

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Novel prodrugs of SN38 generated by Multi-Arm Poly(ethylene glycol)

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Background: SN38 (10-hydroxy-7-ethyl-camptothecin) is the active metabolite of CPT-11 (Camptosar®). SN38 has not been used directly as an anticancer drug due to its poor solubility in any pharmaceutically acceptable excipients. Using multi-arm high molecular weight PEG, we have successfully generated novel water soluble prodrugs of SN38.
Material and Methods: In order to increase drug payload, multi-arm PEG was used. In particular, 40k 4-arm-PEG-OH was first converted to PEG acid, then conjugated with a properly protected SN38 intermediate with different amino acid linkers attached to the 20-hydroxyl position to give the PEG-SN38 conjugates. The aqueous stability and hydrolysis property in rat and human plasma were monitored using UV based HPLC methods. The *in vitro* cytotoxicity of all the PEG conjugates was tested in several different tumor cell lines. The *in vitro* metabolism study of PEG-SN38 conjugates was examined in rat hepatocytes.
Results: Using proper protecting and de-protecting strategies, two different chemistries have been developed to synthesize the PEG-SN38 conjugates in high yields. The process was readily adaptable for scale up development. All four PEG-SN38 conjugates had good solubility in water, with up to 4 mg/mL equivalent solubility of SN38 achieved. All compounds showed good stability in saline and other aqueous medium for up to 24 hrs at room temperature. All conjugates demonstrated potent *in vitro* cytotoxicity against a panel of cancer cell lines. The sensitivity of cells to PEG-SN38 was in the order: COLO205 > HT29 = OVCAR-3 > A549. PEG-SN38 conjugates were equipotent to native SN38 and about 10 to 600 fold more potent than CPT-11. PEG-SN38 conjugates were 8 to16 fold more sensitive than Pegamotecan (a PEGylated prodrug of camptothecin) in COLO 205, HT-29 and OVCAR-3 cells. In human plasma, SN38 was steadily released from the PEG conjugates with a doubling time of 22 to 52 minutes and the release appeared to be pH and concentration dependent. Metabolic study using rat hepatocytes showed SN38 released from conjugates formed a phase II SN38-glucuronide metabolite.
Conclusions: Using multi-arm high molecular PEG, we have successfully prepared several water soluble prodrugs of SN38 for direct parental applications. The payload of the parent drug was almost doubled compared to the traditional straight chain PEGylation. High water solubility was achieved. All PEG-SN38 conjugates showed potent *in vitro* anti-tumor activities which are much more potent than the small molecule prodrug CPT-11 and Pegamotecan. These results warrant further study of these conjugates in animals. PEGylation appears to be a promising approach to deliver SN38, a potent but insoluble cytotoxic agent.

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Mass balance, pharmacokinetics and metabolism of [14C] BMS-354825 in healthy male subjects

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Background: Dasatinib (DAS) – a potent, orally active inhibitor of several oncogenic kinases – has demonstrated clinical efficacy in CML and Ph+ ALL. This study assessed the mass balance, PK, metabolism, and routes/extent of elimination of a single oral dose of 100 mg (120 μCi) [^{14}C] DAS in healthy male subjects.
Methods: This was an open-label, non-randomized, single-dose study involving 8 subjects (21−41 y.o.). All received a single oral dose of 100 mg of [^{14}C] DAS solution containing 120 μCi of total radioactivity (TRA). Vital signs, physical exams, ECGs, clinical labs, and adverse events were conducted/monitored. Blood, urine, and feces were collected to measure DAS, the piperazine N-oxide metabolite of DAS (M5) and TRA, and for biotransformation analyses.

	Cmax (ng/mL), GM[a] (CV)	AUC_{∞} (ng h/mL), GM[a] (CV)	AUC_{0-T} (ng h/mL), GM[a] (CV)	Tmax (h), Med[b] (Min, Max)	T-half (h), Mean (SD)	CLR (mL/h), Mean (SD)	UR (%), Mean (SD)
DAS	104.47 (29)	313.97 (42)	298.8 (44)	0.5 (0.25, 1.5)	3.59 (1.01)	404.83 (168.73)	0.12 (0.05)
M5	2.96 (55)	15.28 (53)	8.88 (74)	1.5 (0.75, 3)	3.15 (1.2)	–	1.2 (0.49)
TRA	224.61 (23)	1231.34 (53)	400.43 (41)	0.5 (0.25, 1.5)	3.96 (2.63)	8636.92 (2651.12)	–

[a]GM, geometric mean. [b]Med, median.

In plasma, DAS AUC(INF) accounted for ~29% of the AUC(INF) of TRA. Multiple metabolites were identified with DAS as the major component. In feces, DAS was a prominent component accounting for 19% of the dose. Metabolites M20 (4-hydroxy-chloromethylphenyl DAS) and M6 (the carboxylic acid derivative of DAS) were detected in significant amounts. No conjugated metabolites were detected in feces.
Conclusions: (1) Radioactivity was primarily eliminated in feces. Mean total recoveries through 9 days post dose were 85% in feces and 4% in urine (total mean = 89%). (2) Negligible amounts of DAS and M5 were excreted in the urine, ~1% of dose. (3) The parent drug was an important drug-related component and M5 a minor metabolite in plasma. (4) A single 100 mg dose of [^{14}C] DAS was safe and tolerable.

156 POSTER

Antitumor activity of IHL-305, a novel PEGylated liposome containing irinotecan, in human xenograft models

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Background: Irinotecan hydrochloride (CPT-11) is an antitumor agent that acts by inhibiting DNA topoisomerase I. CPT-11 is widely used in clinic due to its confirmed evidence of antitumor efficacy. IHL-305 is a preparation of irinotecan encapsulated in PEGylated liposome. Liposome preparations are known to be selectively transported to tumor tissues due to the effect of enhanced permeability and retention (EPR). In this study, antitumor efficacy profiles of IHL-305 were evaluated in comparison with CPT-11 using nude mice subcutaneously transplanted with various human cancer cell lines.
Materials and Methods: After transplanting human cancer cell lines (colon, non-small cell lung, small cell lung, prostate, ovarian, and gastric cancer cells) subcutaneously to the inguinal region of nude mice, the animals were grouped on the day when the estimated tumor volume reached about 60–180 mm^3 (Day 0). IHL-305 or CPT-11 was administered intravenously (i.v.) 1–3 times at 4–14 days intervals (total dose 16.875–135 mg/kg or 18.75–270 mg/kg as irinotecan). Physiologic saline or empty liposomes were administered as negative controls with the same administration schedule. Tumors were excised on Day 21, and tumor growth inhibition (TGI) rates (%) were calculated from tumor weights.
Results: The TGI rates for IHL-305 doses (16.875–135 mg/kg) versus CPT-11 doses (18.75–270 mg/kg) tested were 99.2−99.5% vs 35.5−67.2% on QG-56 (NSCLC), 34.7−93.1% vs 4.8−45.8% on NCI-H460 (NSCLC), 86.7−99.8% vs 74.1−88.0% on NCI-H82 (SCLC), 97.9−99.0% vs 48.0−62.3% on PC-3 (prostate), 24.0−89.9% vs 7.7−42.5% on HT-29 (colon), 62.1−91.9% vs 39.0−87.7% on HCT116 (colon), 77.1−80.8% vs 57.3−69.2% on MKN45 (gastric), and 69.1−97.7% 20.2−64.3% on ES-2 (ovarian) cancer xenografts. In all tested xenograft models, IHL-305 demonstrated superior TGI rates to CPT-11 even in HT-29 colon cancer cell line, which has shown intrinsic resistance to CPT-11. No significant changes of body weight were noted in IHL-305 treated groups.
Conclusions: IHL-305 demonstrated stronger tumor growth inhibition effect than CPT-11 on various human cancer xenografts.

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Combination therapy for liver tumor growth and metastasis by low dose rapamycin and FTY720

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Background: Our previous studies demonstrated that the new immunomodulator FTY720 could suppress liver tumor growth and metastasis through down-regulation of cell survival and invasion pathways. On the