Material and Methods: An *in vitro* model of the BBB and *in situ* brain perfusion were used to assess the brain uptake of Taxol-conjugate. Xenograft models of glioblastoma (U87) and non-small cell lung carcinoma (NCI-H460) were established by subcutaneous (s.c.) injections of cancer cells in immunodeficient mice nu/nu. Moreover, glioblastoma as well as lung brain metastasis models were also established by intracranial stereotaxic injections of U87 and NCI-H460 cells, respectively.
Results: We demonstrate here the transcytosis ability of peptides derived from kunitz domain called Angiopeps using an *in vitro* model of the BBB and *in situ* brain perfusion. Angiopep transcytosis across bovine brain capillary endothelial cell (BBCEC) monolayers is at least 19-fold higher than that of holo-transferrin. In addition, Taxol, has been conjugated to these vector-peptides (Angiopeps). Importantly, Taxol-Angiopep conjugate has a similar effect than free Taxol on cancer cell proliferation *in vitro*. However, the conjugation of Taxol to Angiopep allows to bypass P-gp leading to a higher accumulation of Taxol in the brain parenchyma. We also found that Taxol-Angiopep conjugate caused a stronger inhibition of the s.c. tumor growth of U87 and NCI-H460 than free Taxol. Moreover, Taxol-Angiopep conjugate significantly increased survival of mice implanted with intracranial NCI-H460 and U87 cells by 27 and 24%, respectively.
Conclusion: Overall, these results indicate that the conjugation of Taxol with Angiopep-vector increases the effect of Taxol on tumor growth as well as Taxol accumulation in brain. Furthermore, in primary and secondary brain tumor models, Taxol-Angiopep conjugate administration prolonged mice survival.

148 POSTER

Self-assembling nanoparticles targeting G-protein coupled receptors and ABC transporters

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We have previously shown that synthetic analogs of the transmembrane domains (TMs) of G-protein coupled receptors (J Biol Chem 1999; 274(49): 34911–5) and of the multiple drug resistance protein, P-gp (J Med Chem 2005; 48(11): 3768−75) efficiently and specifically inhibit the function of the target protein. The remarkable feature of these inhibitors is that they can be designed rationally solely on the basis of the primary structure of the protein.
A transmembrane antagonist of CXCR4 incorporated in lipid liposomes was shown to completely inhibit metastasis in a mouse model of breast cancer. We have found recently that liposomes may not be needed for the delivery of TM antagonists.

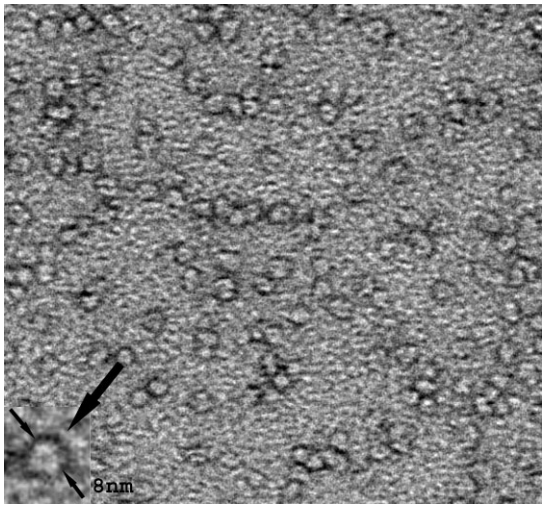

Fig. 1. Cryo-elecron microscopy of the transmembrane antagonist of CXCR4 receptor CXCR4−2–2 demonstrated self-assembly of the compound into nanoparticles with a diameter about 8 nm.

Experiments utilizing multiple angle light scattering have shown that TM peptides self-assemble into stable nanoparticles in aqueous solutions. Cryo-elecron microscopy has revealed formation of uniform particles with a diameter of about 8 nm, which is within the presumed optimal range for intra-tumor delivery. Electron microscopy studies of numerous “mutant” peptides have shown that negative charges added to the C-terminal end of the peptides were critical for formation of small uniform particles. Many TM peptide micelles aggregated further forming rings and strings. Addition of short polyethylene glycol chains reduced aggregation, while PEG chain consisting of 39 repeats provided for non-aggregating and uniform nanoparticles. NMR, light scattering and calorimetry studies have demonstrated remarkable stability of TM peptide micelles with CCM in low micromolar range.
Nanoparticular forms of transmembrane peptides retained full biological activity. CXCR4-targeting TM micelles inhibited signaling through the receptor, while ABCG2 and P-glycoprotein TM nanoparticles inhibited drug efflux mediated by the corresponding transporter. Nanoparticles formed by TM peptides also efficiently encapsulated poorly soluble hydrophobic drugs, thus providing a unique delivery system with dual anti-tumor activity. Self-association of transmembrane peptides in aqueous solutions is a novel phenomenon. It enables the development of new methods of specific and rational targeting of integral membrane proteins *in vivo* and provides for a new paradigm in drug development where the drug itself self-assembles in a nanostructure that has the desired size and surface properties for intra-tumor delivery.

149 POSTER

Pharmacokinetics of irinotecan and its metabolites after i.v. administration of IHL-305, a novel PEGylated liposome containing irinotecan, to tumor-bearing mice

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Background: IHL-305 is a preparation of irinotecan encapsulated in PEGylated liposome. IHL-305 demonstrated stronger tumor growth inhibition effect than irinotecan hydrochloride (CPT-11) on various human cancer xenografts such as the colon, lung, gastric, ovarian, and prostate cancer cell lines. Liposome preparations are selectively transported to tumor tissues by the enhanced permeability and retention (EPR) effect. In this study, we compared the pharmacokinetics after i.v. administration of IHL-305 with CPT-11 in tumor-bearing mice.
Materials and Methods: Female BALB/c mice transplanted with Meth A tumors were i.v. administered with IHL-305 or CPT-11 at a dose of 16.7 mg/kg, and were exsanguinated at 8 time points until 96 hours after dosing, followed by extirpation of the tumor, liver and kidney. The concentrations of irinotecan and its metabolites, SN-38, and SN-38 glucuronide (SN-38G), in plasma and these tissues were measured.
Results: The plasma irinotecan, SN-38, and SN-38G concentrations peaked at 0.167, 6, and 12 hours after IHL-305 dosing, respectively, whereas those after CPT-11 dosing peaked at 0.167 hours. The AUC_{0-inf}, C_{max}, and $t_{1/2}$ of irinotecan after IHL-305 dosing were higher than those after CPT-11 dosing (302-, 55-, and 2.9-fold, respectively). The C_{max} of SN-38 and SN-38G after IHL-305 dosing were lower than those after CPT-11 dosing (0.13- and 0.23-fold), however, the AUC_{0-inf} were higher (2.5- and 1.8-fold), because of their much slower disappearance than CPT-11 dosing. Irinotecan and SN-38 concentrations in tumor tissue after CPT-11 dosing peaked at 0.167 and 0.5 hours, followed by a relatively rapid decrease. Concentrations after IHL-305 dosing remained nearly constant up to 12 hours for irinotecan and 48 hours for SN-38 and then decreased gradually. Their AUC_{0-inf} were higher than those after CPT-11 dosing (9.02- and 3.89-fold). The AUC_{0-inf} of irinotecan and SN-38 in the liver and kidney tissues after IHL-305 dosing were also higher than those after CPT-11 dosing (15- and 2.3-fold for the liver, and 4.1- and 2.4-fold for the kidney, respectively).
Conclusion: The irinotecan and SN-38 concentrations in plasma, tumor, liver, and kidney tissues after IHL-305 administration to tumor-bearing mice were markedly higher than those after CPT-11 dosing. The AUC ratio of SN-38 between CPT-11 and IHL-305 administration in tumor tissue was greater than in the liver and kidney, suggesting that targeting was improved by its liposomal formulation.

150 POSTER

Effects of dasatinib on the pharmacokinetics of simvastatin, a cytochrome P450 3A4 substrate, in healthy subjects

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Background: Dasatinib – an oral inhibitor of multiple oncogenic kinases – has demonstrated clinical efficacy in CML and Ph+ ALL. *In vitro* dasatinib inhibits CYP3A4 (IC_{50} =1.9 μM). The objective of this study was to assess the effect of dasatinib on simvastatin plasma concentration–time profiles in healthy subjects.
Methods: Healthy subjects (N = 48) were treated in this open-label, randomized, two-period, two-treatment, balanced, crossover study. The treatments were: A) 80 mg simvastatin (single dose) (control); and B) 80 mg simvastatin plus 100 mg dasatinib (both single doses). Blood samples for

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.

151 POSTER

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.

152 POSTER

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.

153 POSTER

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.