

INTRODUCTION

Recent *in vitro* and *in vivo* studies have indicated that drug ratios can have profound effects on synergistic and antagonistic interactions (Mayer et al., *Mol Cancer Ther* 2006 5: 1854-63). Dissimilar drug pharmacokinetics creates disparate drug exposure rates and therefore even if the drugs are injected at a synergistic ratio they are unlikely to remain at that ratio for a significant amount of time. Thus, dissimilar pharmacokinetics likely contributes to the inability to achieve drug synergy *in vivo*, and may even result in drug antagonism. Dual drug formulations with coordinated drug release is a newly developed application of liposomal delivery technology developed in order to deliver synergistic drug combinations at fixed drug ratios and avoid tumor exposure to antagonistic ratios (Tardi et al., *Biochim Biophys Acta* 2007 1768: 678-87). Co-delivery and coordinated release of synergistic drug combinations facilitates maximum efficacy by maintaining synergy throughout the pharmacodynamic distribution.

In this study, we systematically evaluated drug ratio-dependent synergy of irinotecan and cisplatin against a panel of 20 human and murine tumor cell lines *in vitro*. The tumor cell panel was enriched in lung tumor carcinomas and exhibited a range of irinotecan and cisplatin sensitivities. Following dose-ratio evaluations, we translated our *in vitro* findings *in vivo* by co-formulation of irinotecan and cisplatin in liposomes capable of maintaining the combination at a synergistic 7:1 molar ratio (CPX-571) following intravenous (IV) administration. The efficacy of the CPX-571 dual drug formulation was compared to that of the free drug cocktail as well as each individual liposome-encapsulated drug in a range of human tumor xenograft models implanted in nude mice. The liposomal dual drug formulation showed a high degree of antitumor activity and significantly improved efficacy over the unencapsulated cocktail as well as the individual liposomal drugs in all tumor models tested.

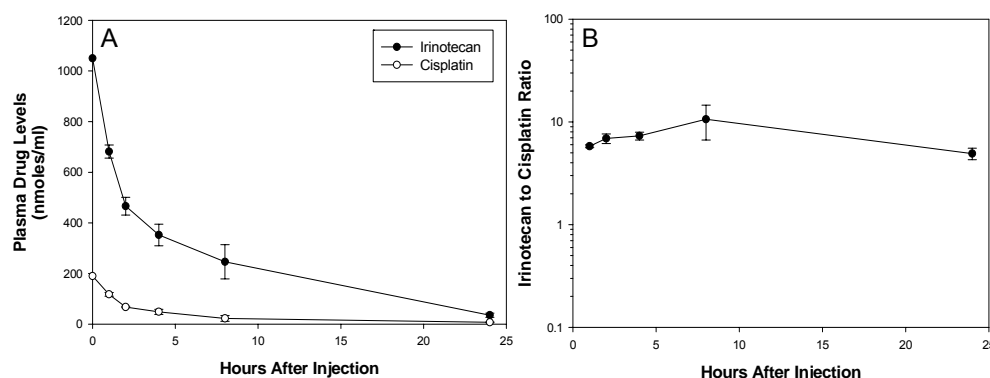
DEVELOPMENT OF CPX-571

1 Median effect analysis of irinotecan:cisplatin molar ratios for synergy

Cell Lines Screened	Tumor	CI @ ED80														
		1:64	1:32	1:16	1:8	1:4	1:2	1:1	1:1	2:1	4:1	8:1	16:1	32:1	64:1	
LCC6	Breast	0.69	0.58	0.71	0.89	0.83	1.01	1.57	1.03	0.65	0.83	0.52	0.54	0.76		
MCF-7	Breast	0.71	0.87	0.88	0.88	0.95	1.15	0.71	0.82	0.71	1.00	0.87	0.83	0.89		
MB 231	Breast	1.02	1.01	0.90	0.79	0.87	1.84	1.34	0.90	1.10	0.76	1.49	0.81			
HCT-116	Colon	0.36	0.38	0.37	0.33	0.82	0.84	1.90	1.73	0.98	0.76	0.82	1.06			
Colon-26	Colon	1.22	1.27	1.40	1.16	1.00	1.24	1.04	1.38	1.10	1.03	1.34	1.25			
HT-29	Colon	1.05	0.86	0.90	0.86	1.23	1.09	1.97	1.17	0.90	0.76	0.82	0.97	1.05		
A549	Lung	0.67	0.61	0.67	0.56	0.44	0.39	0.44	1.65	1.31	0.49	0.37	0.41	0.49		
H460	Lung	0.92	0.81	0.84	0.80	0.75	0.73	0.94	1.14	2.03	0.53	0.36	0.80	0.48		
H322	Lung	0.47	0.52	0.68	0.96	0.57	1.28	1.24	0.95	0.73	0.54	0.44	0.52	0.54		
H1299	Lung	1.07	1.08	1.12	1.09	0.76	0.98	3.01	2.57	1.83	0.85	0.64	1.49	0.82		
H522	Lung	1.15	0.82	1.09	0.84	0.82	0.92	2.26	1.80	0.89	0.48	0.39	0.73	0.47		
Ovcar-3	Ovarian	1.33	1.35	1.08	1.10	0.80	0.75	0.89	0.89	1.84	0.53	0.28	0.33	0.35		
Ovcar-5	Ovarian	1.29	1.64	1.55	1.34	1.30	1.42	1.51	1.30	1.15	0.80	1.05	0.99	1.17		
SK-OV-3	Ovarian	1.33	1.26	1.30	1.51	1.49	1.70	1.75	1.28	1.17	0.55	0.70	0.79	0.76		
IGROV-1	Ovarian	1.16	1.16	1.16	1.00	0.95	0.95	1.23	1.20	1.16	0.77	0.76	0.71	0.75		
A2780	Ovarian	0.93	0.94	0.81	0.75	0.80	0.81	0.87	0.70	0.81	0.81	1.20	1.03	1.25		
Capan-1	Pancreatic	1.46	1.22	1.11	1.25	0.86	0.89	1.12	1.17	0.69	0.89	0.83	0.71	0.99		
BXP-3	Pancreatic	1.00	1.02	0.91	1.10	0.81	0.99	1.04	0.88	0.70	0.60	0.49	0.64	0.61		
N87	Gastric	1.76	1.87	1.35	1.55	1.65	1.09	1.05	0.68	0.51	0.18	0.11	0.06	0.04		
A253	H&N	0.85	0.84	0.81	0.92	0.76	0.83	0.79	0.78	0.86	0.83	0.90	0.82	0.84		

In vitro screening of irinotecan and cisplatin for median-effect combination indices (CI; Chou and Talalay 1984, *Adv. Enzyme Reg.* 22:27-55) based on molar drug ratios. CI screening a human tumor cell line panel results in a fixed-ratio matrix "heat map" shown for drug concentrations resulting in an 80% fraction of cells affected (ED = 80). Green (CI < 1), synergy; yellow (CI ~ 1), additivity; red (CI > 1), antagonism.

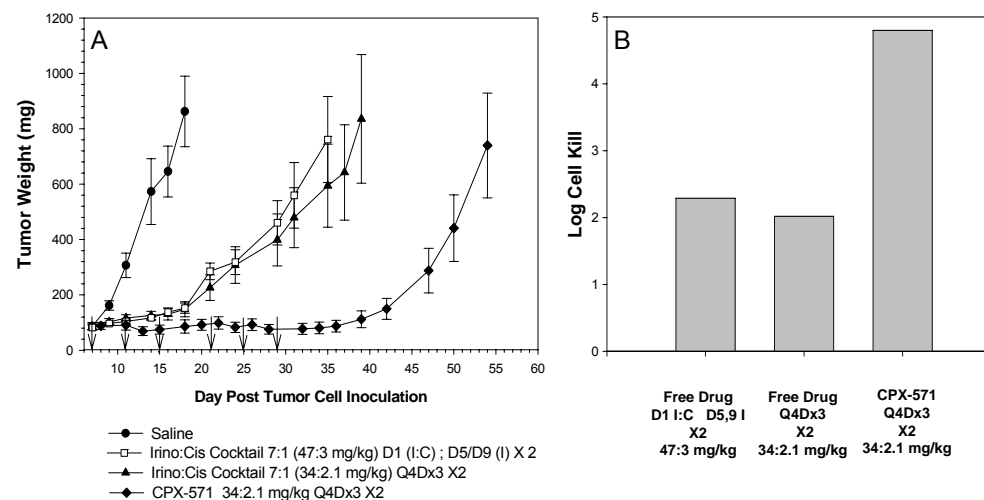
2 Irinotecan and Cisplatin were encapsulated in liposomes that maintain the optimal ratio following systemic administration



Plasma levels of irinotecan and cisplatin were determined at five time points after i.v. administration in female CD-1 nude mice. **A.** Following injection of CPX-571 at an irinotecan dose of 60 μ moles/kg (41 mg/kg) and a cisplatin dose of 8.6 μ moles/kg (2.6 mg/kg), mouse plasma was isolated at the indicated times and assayed for irinotecan (●) and cisplatin (○) by HPLC. **B.** Based on the plasma drug levels, the molar ratio of irinotecan:cisplatin was calculated for each time point. All data points represent the mean values obtained from three mice per time point, and the error bars represent the SE.

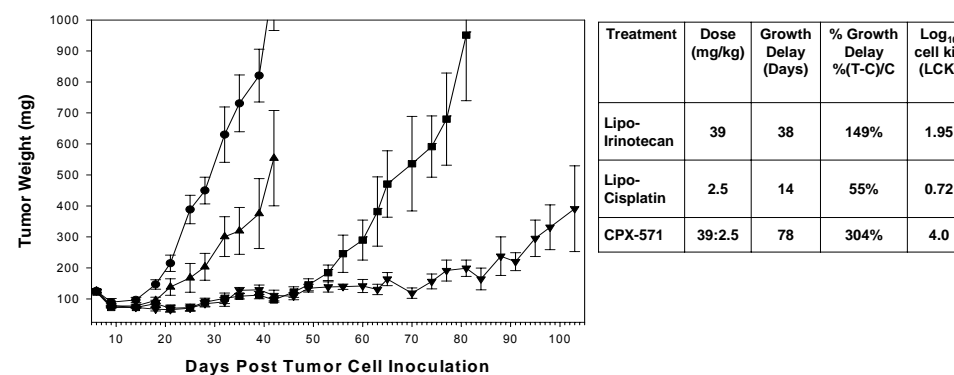
RESULTS

1 CPX-571 is more effective than free drug cocktails against H460 NSCLC



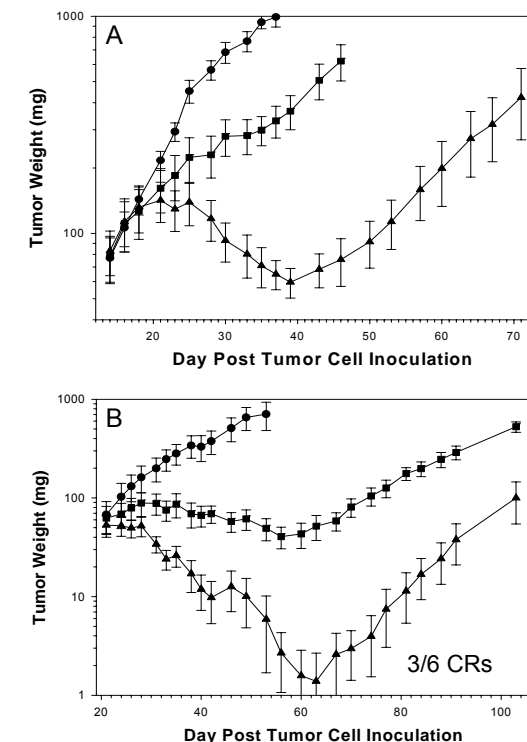
CD-1 nude mice were inoculated s.c. with 2×10^6 H460 human NSCLC tumor cells. Mice (n=6) were treated with MTD doses of free drug combinations optimized for efficacy consisting of either two courses of alternating irinotecan:cisplatin (I:C) on day one followed by irinotecan only (I) on days 5 and 9, or two courses of co-administration of irinotecan and cisplatin on a Q4Dx3 schedule. The free drug schedules were compared with two courses of MTD doses of CPX-571 on a Q4Dx3 schedule. **A.** mean tumor growth curves with error bars representing the SE. **B.** log cell kill calculated from the mean tumor volume using the formula \log_{10} cell kill = (T - C) / (3.32) (Td) where T - C (tumor growth delay) = days to reach a defined mass for the treated animals - days to reach the same mass for control animals; 3.32 is the \log_{10} unit constant; Td is the doubling time for the tumor in days determined using LabCat® (Innovative Programming Associates, Inc., Princeton, NJ).

2 CPX-571 has greater than additive antitumor activity against H69 SCLC



H69 human SCLC tumor cells (1×10^7) in growth factor reduced matrigel were implanted in female Foxn1 nude mice. Mice (n=6) were treated with MTD doses of CPX-571 on a Q7Dx3 schedule. The mean tumor growth curves (left) were used to estimate the log cell kill values shown in the table to the right. Mice received injections of (●) saline, (▲) 8.3 μ moles/kg (2.5 mg/kg) liposomal cisplatin, (■) 58 μ moles/kg (39 mg/kg) liposomal irinotecan or (▼) CPX-571 containing 58 and 8.3 μ moles/kg irinotecan and cisplatin. Both individual liposomal agents were therapeutically active and contributed to the overall antitumor activity. CPX-571 showed greater than additive antitumor activity when compared to the sum of dose matched individual agents.

3 Irinotecan:cisplatin formulations are also more efficacious than the free drug cocktail in HT29 human colon tumor and Capan-1 human pancreatic tumor xenograft models.



Effect of treatment on human tumor xenografts implanted in female Foxn1 nude mice (n=6) and staged until tumors reached 50 to 100 mg. **A.** HT29 human colon tumor xenograft model. **B.** Capan-1 human pancreatic xenograft model. Mice received Q7Dx3 injections of -●- saline, -■- free drug cocktail of irinotecan:cisplatin 42:3.4 mg/kg, or -▲- liposomal irinotecan:cisplatin 28:2.5 mg/kg.

CONCLUSIONS

- Irinotecan:cisplatin combinations display strong drug ratio-dependent synergy *in vitro*
- Liposomal formulations of irinotecan:cisplatin maintained a synergistic ratio in plasma for at least 24 hours following systemic administration
- CPX-571 is superior when compared to free drug cocktails of irinotecan:cisplatin in a range of solid tumor xenograft models
- Log cell kill analysis indicates that CPX-571 exhibits greater than additive antitumor activity
- CPX-571 is a promising candidate for clinical evaluation