

The Therapeutic Activity of Fixed Ratio Formulations of Irinotecan and Floxuridine are Ratio Dependent: Therapeutic Assessment in the Capan-1 Human Pancreatic Tumour Xenograft Model

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Introduction

Combination chemotherapy regimens historically have been developed by escalating the dose of individual agents based on toxicity. However, we have observed *in vitro* that the antitumor activity of drug combinations is often dependent on the drug:drug ratio. Specifically, while some ratios are synergistic, others can be additive or antagonistic. This has important implications on the *in vivo* application of drug combinations since the individual agents will exhibit independent biodistribution properties, resulting in exposure of tumor cells to drug ratios that may have inferior therapeutic activity. We have developed an approach whereby synergistic drug ratios identified by *in vitro* screening are fixed in drug carriers that maintain this ratio *in vivo*. CPX-1, a liposomal formulation with a fixed 1:1 molar ratio of Irinotecan and Floxuridine exhibiting superior antitumor activity over free drug cocktails was developed based on this approach (see Poster #550 for further details). The studies described here focused on comparing the effects of delivering synergistic vs. antagonistic ratios of Irinotecan:Floxuridine on efficacy in the Capan-1 human pancreatic carcinoma model.

Methods

MTT-viability assays done in triplicate were completed for each individual agent and for combinations of agents at fixed molar ratios (72-hour drug exposure). The averaged data was entered into the CalcuSyn program, developed from the quantitative analysis of dose-effect relationships developed by Chou & Talalay (Adv. Enzyme Reg. 22:27-55, 1984) for analysis of combined effects of multiple drugs. The median-effect analysis linearized the sigmoidal viability curves and provided calculated effective doses for individual and combined drugs, and assigned a combination index (CI) derived from the CI equation used to determine synergy. CI=1 is additive, CI>1 is antagonistic and CI<1 is synergistic. For the MTT assay, CI-values are most reliable between f_a 0.2 and 0.8.

CPX-1 was prepared by passively encapsulating Floxuridine inside 100 nm diameter liposomes containing copper gluconate, pH 7.4. Unencapsulated Floxuridine and copper gluconate was removed by tangential flow against 300 mM Sucrose, 20 mM phosphate, 10 mM EDTA. Irinotecan was subsequently loaded into the liposomes by heating at 50 °C for 1 hour. In the final formulation >95% of the drugs were inside the liposomes, the Irinotecan:Floxuridine molar ratio was 1:1 (equal to 2.7:1 wt. ratio). Drug treatments were diluted in sterile 5% dextrose/water for IV administration.

For PK analysis the formulation was administered IV into female Scid/Rag2 mice at 37 µmol/kg of Irinotecan:Floxuridine. Blood was collected at indicated times in EDTA-treated tubes. Plasma was analyzed for total Irinotecan, Floxuridine, and liposomal lipid utilizing radiolabelled drug and lipid tracers. SCID/Rag2 mice bearing solid tumors (6 mice per group) were administered treatments IV on the indicated days. Tumor measurements and body weights were taken 3x weekly. Study endpoints were determined when control tumors reached 1000 mg or when tumors became ulcerated. Determination of surviving tumor fraction was based on the treatment induced time delay for tumors to reach 500 mg and the tumor doubling time, which yielded the Log Cell Kill. Statistical analyses to compare treatment groups utilized one-way ANOVA (Student Newman-Keuls Method) programs.

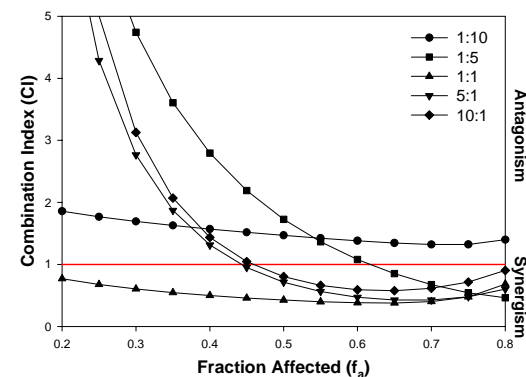


Figure 1: The *in vitro* Combination Index for Irinotecan:Floxuridine combinations is dependent on the Fraction Affected (f_a , fraction of cells killed) and the Irinotecan:Floxuridine ratio.

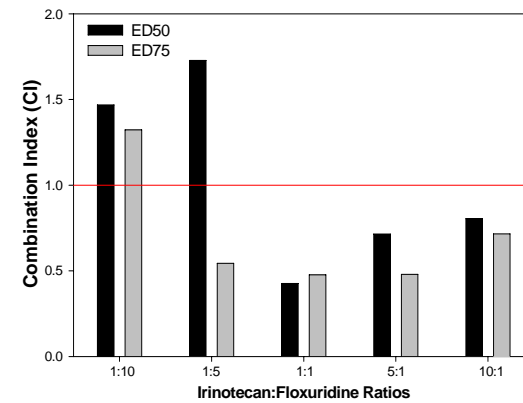


Figure 2: The Combination Index for Irinotecan:Floxuridine combinations at 50% and 75% cell kill in the Capan-1 *in vitro* screen are ratio dependent. Antagonism is observed at a molar ratio of 1:10 whereas synergy is seen at a 1:1 ratio.

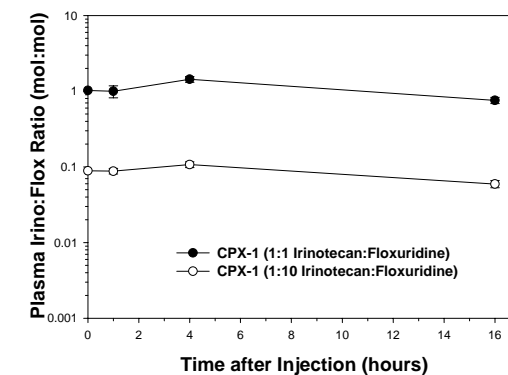


Figure 3: Liposome formulations can be designed to maintain a wide range of formulated drug ratios after IV administration.

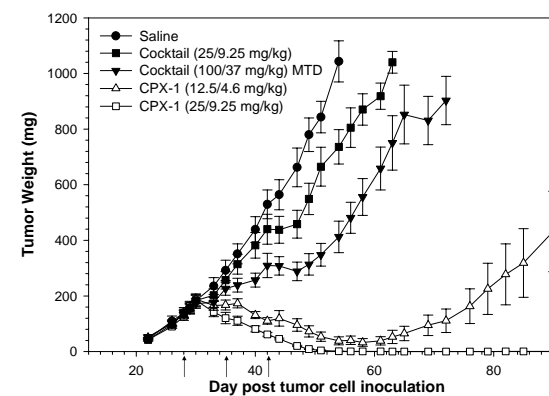


Figure 4: CPX-1 causes tumor regression and is significantly more active than the MTD of the free drug cocktail, which unlike CPX-1, is unable to control the drug:drug ratio after injection. Arrows indicate day of treatment.

Table 1: Log cell kill comparisons for synergistic and antagonistic ratios. The log cell kill for CPX-1 is greater than predicted from adding the activity of the individual liposomal drug components, consistent with *in vivo* synergy. The antagonistic ratio is no more active than liposomal floxuridine alone, consistent with *in vivo* antagonism.

Treatment	Dose (mg/kg)	Irino:Flox Molar Ratio	Tumor growth delay (days)	% Tumor growth delay [(T-C) x 100]	Log cell kill*
Free Irino	100	NA	15	35%	0.65
Free Flox	250	NA	10	23%	0.43
L-Irino	25	NA	34	79%	1.46
L-Flox	9.25	NA	7	16%	0.30
L-Flox	18.5	NA	10	23%	0.43
Antagonistic	5:18.5	1:10	10	23%	0.43
CPX-1	5:1.85	1:1	9	21%	0.39
CPX-1	25:9.25	1:1	42	98%	1.98**

* Log cell kill = $[T - C] / (3.32 \cdot [T_d])$ where T - C is the treatment induced delay for tumors to reach 500 mg (in days) and T_d is the tumor doubling time in days. Days for control tumors to reach 500 mg = 43 days.
** Statistically different ($p < 0.05$) from all other groups using Student-Newman-Keuls analysis.

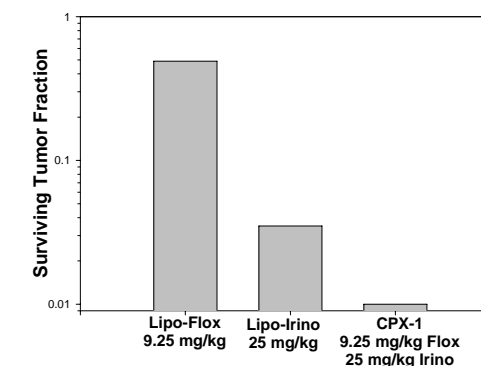


Figure 5: The number of Capan-1 human pancreatic tumor cells remaining after treatment with CPX-1 is significantly lower than that for individual liposomal drugs ($p < 0.05$, numbers derived from data in Table 1).

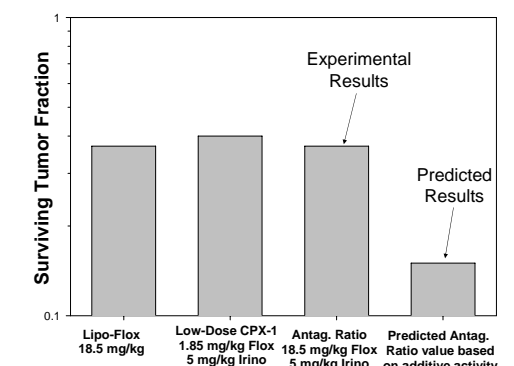


Figure 6: The number of Capan-1 human pancreatic tumor cells remaining after treatment with an antagonistic drug ratio in liposomes is significantly greater than predicted for additive antitumor activity, consistent with *in vivo* antagonism (numbers derived from data in Table 1).

Conclusions

- Cytotoxicity analysis of Irinotecan and Floxuridine combinations revealed that *in vitro* synergy in Capan-1 pancreatic cancer cells is dependent on the drug:drug ratio.
- Administration of Irinotecan and Floxuridine at a fixed synergistic ratio of 1:1 in a liposome delivery vehicle dramatically enhanced antitumor activity compared to free drug cocktails in the Capan-1 human pancreatic xenograft model.
- Log cell kill values from *in vivo* efficacy studies indicated synergistic antitumor activity at a 1:1 Irinotecan:Floxuridine ratio and antagonism at a 1:10 ratio. These data demonstrate that efficacy of Irinotecan:Floxuridine depends on maintaining synergistic drug ratios *in vivo*.