

# Synergistic anti-tumor activity observed for a fixed-ratio liposome formulation of Cytarabine:Daunorubicin against preclinical leukemia models

S. Johnstone<sup>1</sup>, P. Harvie<sup>1</sup>, C. Shew<sup>1</sup>, S. Kadhim, T. Harasym<sup>1</sup>, P. Tardi<sup>1</sup>, N. Harasym<sup>1</sup>, M. Bally<sup>2</sup> and L. Mayer<sup>1</sup>  
<sup>1</sup>Celator Technologies Inc. and <sup>2</sup>B.C. Cancer Agency, Vancouver, B.C., Canada

## INTRODUCTION

Cytarabine combined with an anthracycline such as Daunorubicin has been standard treatment for acute myelogenous leukemia (AML) for the past thirty years. Although this combination is effective in inducing tumor remission, the five year survival rate for AML is only 20%. Combination chemotherapy dosing regimens such as these typically have been established based on the tolerability of the individual agents. However, in vitro studies have revealed that the 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 parameters, resulting in exposure of tumor cells to drug ratios that may have inferior therapeutic activity. Celator's technology is based on improving antitumor efficacy by maintaining synergistic drug ratios in vivo.

We have developed CPX-351, a liposomal formulation designed to deliver a fixed molar ratio of Cytarabine and Daunorubicin. This development entailed (1) identifying the most desirable ratio of Cytarabine and Daunorubicin using an in vitro screening assay, (2) designing a liposome carrier which maintains this ratio in vivo by virtue of having similar release kinetics for each agent and (3) confirming optimal activity for this fixed-ratio formulation in animal tumor models.

## METHODS

**Optimal Drug:Drug Ratio Identification:** Cultured cells were exposed to individual agents and combinations of agents at fixed molar ratios. The fraction of surviving cells was determined using the MTT assay. CalcuSyn was used to determine the combined dose effect relationship via the median effect equation of Chou and Talalay (Adv. Enzyme Reg 22:27-55, 1984) and the combination indices (CI) were subsequently determined using the CI equation. Additive cytotoxicity produces a CI=1, synergy produces a CI <1 and antagonism produces a CI >1.

**CPX-351 Liposome Preparation:** Distearoylphosphatidylcholine:distearoylphosphatidylglycerol:cholesterol (7:2:1) liposomes were prepared by dispersing lipid films in 100mM copper gluconate 220 mM TEA pH 7.4 followed by extrusion through 100 nm filters. Cytarabine was passively encapsulated to achieve 0.5 mol Cytarabine/mol of lipid and Daunorubicin was actively loaded to achieve 0.1 mol Daunorubicin/mol of lipid (final Cytarabine:Daunorubicin molar ratio of 5:1).

**CPX-351 Plasma Elimination Kinetics:** Plasma elimination of Cytarabine and Daunorubicin co-encapsulated in a single liposome was monitored for 24 hours. 50 µmol/kg Cytarabine/10 µmol/kg of Daunorubicin was administered i.v. Blood was collected at 1, 2, 4, 8, 16 and 24 hours into EDTA tubes and plasma was separated by centrifugation (1800Xg 10 minutes). Cytarabine and Daunorubicin concentrations were determined by HPLC assay. Briefly, plasma samples were analyzed using reverse phase C18 column (mobile phase: ammonium acetate buffer, pH 4.8 and acetonitrile) and detected by UV (273.7 nm) for Cytarabine and by fluorescence (Ex/Em 480/ 560 nm) for Daunorubicin.

**CPX-351 Efficacy Studies:** BDF-1 female mice (6-10/group) were inoculated i.p. with 1X10<sup>6</sup> P388 or L1210 cells. Treatment was administered i.v. on a Q3DX3 schedule beginning day 1. Tumor progression as measured by body weight and survival was monitored for 60 days. Endpoints were physical or behavioral changes indicating advanced tumor-related illness. Determination of tumor cell doubling was based on survival times for mice inoculated with 10 to 10<sup>7</sup> cells. Statistical analysis to compare treatment groups used one-way ANOVA (Newman-Keuls method).

## RESULTS

In vitro cytotoxicity assays demonstrate that a Cytarabine:Daunorubicin molar ratio of 5:1 provides optimal drug:drug interaction.

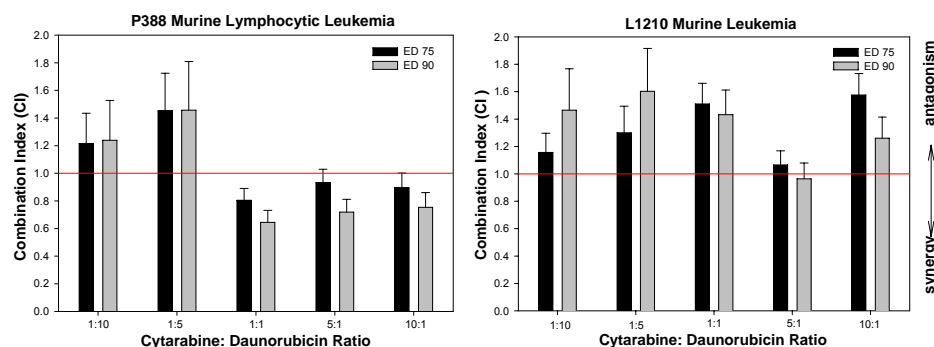


Figure 2. Ratio dependent synergy at high fraction affected dose, ED75 and ED90, for P388 and L1210 cells exposed to Cytarabine and Daunorubicin at various fixed molar ratios ± S.E.M.

Co-encapsulation of Cytarabine and Daunorubicin in DSPC:DSPG:Chol (7:2:1) liposomes (CPX-351) maintains the molar ratio of both agents in the desired range for up to 24 hours.

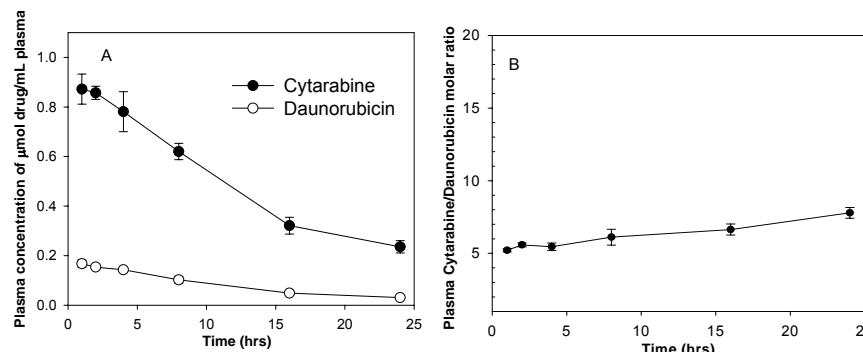


Figure 2. Plasma drug concentrations (A) and Cytarabine:Daunorubicin molar ratio (B) after i.v. injection of CPX-351 to BDF-1 mice (3 per time point).

Table 1. CPX-351 is significantly more efficacious in the P388 ascitic leukemia tumor model than free Cytarabine and Daunorubicin administered either individually or combined in saline at their MTD. Treatments were administered i.v. on days 1, 4 and 7 post i.p. tumor inoculation.

Treatment	Dose (µmol/kg)	Median Survival Time (Days)	% Increase Life Span
Saline	-	8	0
Free Daunorubicin (MTD)	21	23	188
Free Cytarabine (MTD)	4000	20	150
Free Cytarabine: Daunorubicin	50:10	19	138
Free Cytarabine: Daunorubicin (MTD)	2500:20	28	250
CPX-351 (MTD)	50:10	>60	>650

CPX-351 is more efficacious than liposomes containing single agents administered i.v. at their respective MTD.

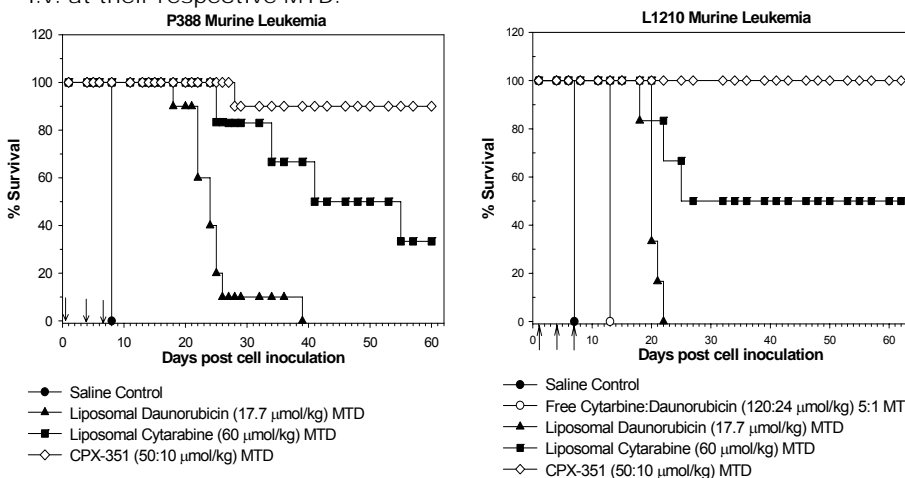


Figure 3. In vivo efficacy of CPX-351 vs. liposomes with single agents or free drug cocktails when administered at maximum tolerated dose to BDF-1 mice bearing P388 or L1210 tumors

Table 2. CPX-351 displays synergistic antitumor activity in vivo. Tumor cell kill analysis was used to determine whether CPX-351 efficacy in the P388 tumor model was additive or synergistic. Log cell kill values were determined from tumor doubling times (T<sub>d</sub>) and treatment-induced delays in median survival time according to the formula (T-C)/3.32XT<sub>d</sub>. CPX-351 exhibited a tumor log kill value (9.05) that was significantly greater than predicted based on the activity of the individual liposomal drugs (2.26 + 4.07 predicts value of 6.33).

Treatment	Dose (µmol/kg)	Mean Survival Time (days)	% Increase in Life Span	Log cell Kill
Liposomal Daunorubicin	4	13	62.5	2.26
Liposomal Cytarabine	20	17	112.5	4.07
CPX-351 Cytarabine: Daunorubicin	20:4	28	250	9.05

CPX-351 exhibits optimal efficacy against P388 tumors compared to Cytarabine and Daunorubicin formulated inside liposomes at other ratios

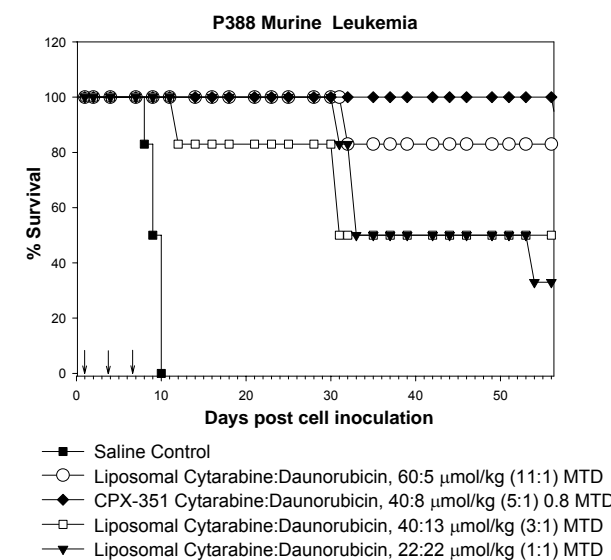


Figure 4. Efficacy of Cytarabine:Daunorubicin formulated inside DSPC:DSPG:Chol liposomes at drug:drug molar ratios ranging from 1:1 to 11:1. Mice were treated i.v. with the indicated dose on days 1, 4 and 7 post i.p. tumor inoculation.

## CONCLUSIONS

- In vitro cytotoxicity experiments indicate that Cytarabine and Daunorubicin combined at a 5:1 ratio is most synergistic and avoids antagonism.
- CPX-351 maintains the Cytarabine:Daunorubicin ratio in the synergistic range for extended times after i.v. administration.
- CPX-351 is more efficacious than free drugs and more efficacious than liposomes containing single agents.
- Maintaining synergistic ratios in vivo through liposome delivery dramatically increases the therapeutic efficacy of Cytarabine:Daunorubicin combination treatment.