

## Cytarabine:Daunorubicin combined inside liposomes at a fixed synergistic ratio leads to potent therapeutic activity against a range of preclinical leukemia models

S. Johnstone, P. Harvie,, S. Kadhim, T. Harasym, P. Tardi, N. Harasym, and L. Mayer  
Celator Pharmaceuticals, Vancouver, B.C., Canada and Princeton, N.J. USA

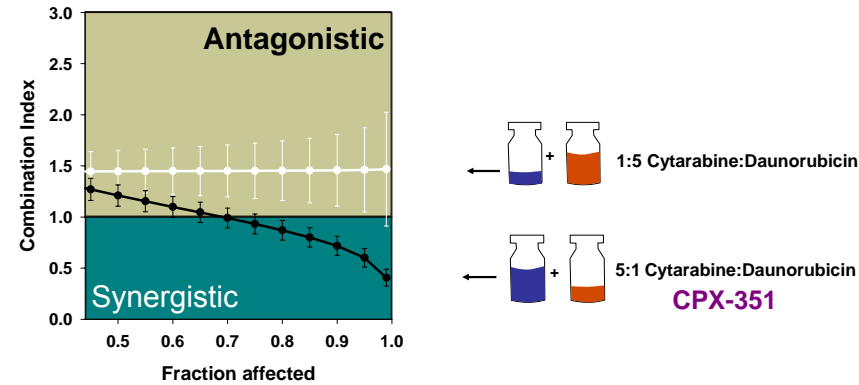
### INTRODUCTION

Cytarabine and daunorubicin combination chemotherapy is standard of care for AML. This combination is effective at inducing tumor remission. However, in less than 5 years more than 80% of the patients relapse. In vitro studies suggest that optimal drug:drug ratios can enhance efficacy and unfavourable drug:drug ratios can limit efficacy. Celator's technology is based on improving antitumor efficacy by maintaining synergistic drug ratios.

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 matched release kinetics for both agent and [3] confirming optimal activity for this fixed-ratio formulation in animal tumor models. We show here that CPX-351 is more efficacious than free drug cocktails as well as individual liposomal drugs in a range of preclinical leukemia models in vivo.

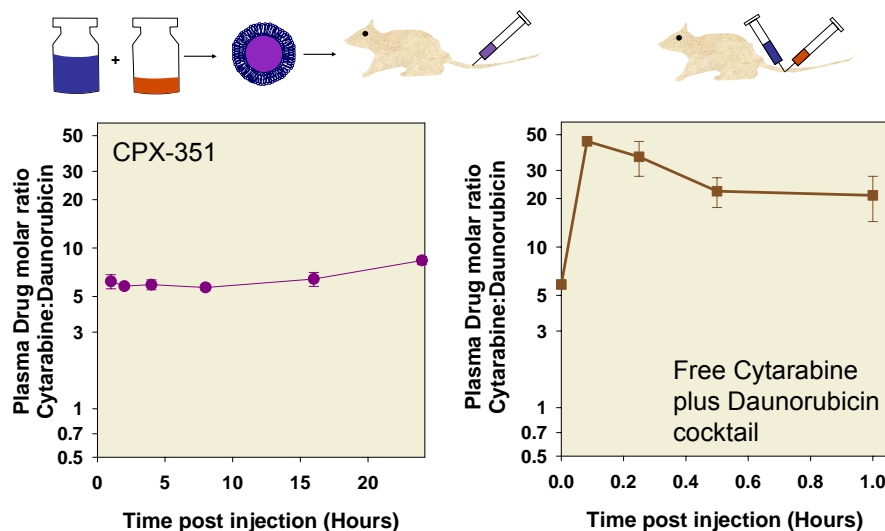
### DEVELOPMENT OF CPX-351

#### 1 Median effect method is used to select the optimum drug:drug molar ratio



Cultured cells were treated with cytarabine or daunorubicin and with cytarabine and daunorubicin in combination. The median effect equation was determined for each combination (Chou and Talay Adv. Enzyme Reg. 22:27-55).

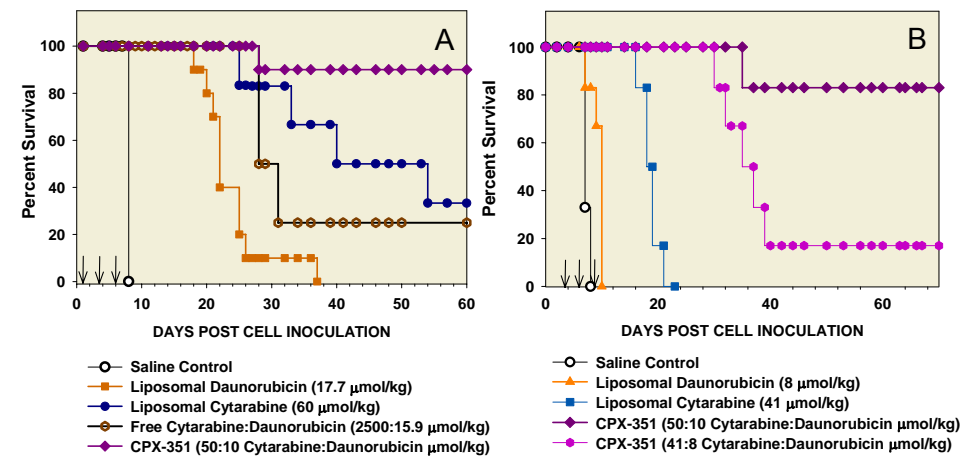
#### 2 Cytarabine and Daunorubicin were encapsulated in a liposome that maintains the optimal ratio in contrast to free drug cocktail



Formulated in CPX-351 the optimal ratio of 5:1 Cytarabine:Daunorubicin is maintained in the circulation for >20 hours. The differential clearance rate of the free agents alters the drug:drug ratio.

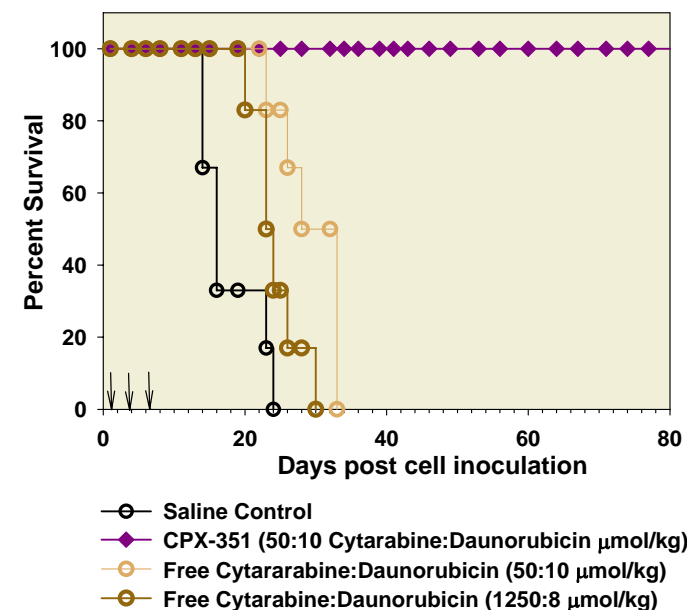
### RESULTS

#### 1 CPX-351 is more effective than free drug cocktails and individual liposomal drugs against P388 murine lymphocytic leukemia models utilizing both early or delayed treatment protocols



BDF-1 mice were inoculated i.p. with  $10^6$  P388 tumor cells. Treatment Q3dX3 began on day 1 (A) or was delayed until day 4 (B). In vivo synergy of cytarabine and daunorubicin in CPX-351 is demonstrated by the greater than additive effect on the log cell kill at matched doses.  
Liposomal Daunorubicin (8  $\mu\text{mol/kg}$ ) Log cell kill= 5.9  
Liposomal Cytarabine (41  $\mu\text{mol/kg}$ ) Log cell kill= 1.4  
CPX-351 (41:8 Cytarabine:Daunorubicin  $\mu\text{mol/kg}$ ) Log cell kill >10

#### 2 CPX-351 is markedly more efficacious than free drug cocktails against the murine myelomonocytic leukemia WEHI-3B model



#### 3 CPX-351 is more efficacious than free drug cocktail or other liposomal drugs in a wide range of murine and human leukemia tumor models

Tumor Model	Treatment	Cytarabine $\mu\text{mol/kg}$	Daunorubicin $\mu\text{mol/kg}$	Long Term Survivors/total	Percent increase life span
P388 Murine lymphocytic leukemia Early R <sub>x</sub>	CPX-351	50	10	9/10	>675
	Liposomal Cytarabine	60		2/6	488
	Liposomal Daunorubicin		17.7	0/10	175
	Free	2500	15.9	1/4	293
P388 murine lymphocytic leukemia Delayed R <sub>x</sub>	CPX-351	50	10	5/6	>814
	Liposomal Cytarabine	60		0/6	179
	Liposomal Daunorubicin		17.7	0/5	314
	CPX-351	50	10	6/6	>814
L1210 murine lymphocytic leukemia	Liposomal Cytarabine	60		3/6	521
	Liposomal Daunorubicin		17.7	0/6	171
WEHI-3B murine monomyelocytic leukemia	CPX-351	50	10	6/6	>413
	Free	2500	15.9	0/6	209
Human acute T-lymphoblastic leukemia xenograph	CPX-351	50	10	5/5	>152
	Free	1250	8	0/6	57
HL-60B Human acute promyelocytic leukemia xenograph	CPX-351	50	10	0/6	43
	Free	825	5.3	0/6	20

Leukemia models were treated with CPX-351, liposomal agents and free agents at maximum tolerated doses. Doses were administered on a Q3dX3 schedule.

### CONCLUSIONS

- A liposomal formulation was designed to capture and maintain the synergistic 5:1 molar ratio of cytarabine:daunorubicin after i.v. injection
- Comparison of CPX-351 and individual liposomes in vivo revealed that the combination synergizes in vivo at the 5:1 molar ratio
- CPX-351 is superior when compared to free drug cocktails of cytarabine:daunorubicin in a range of leukemia models
- The CPX-351 therapeutic efficacy profile suggests that CPX-351 is a promising candidate for evaluating in clinical trial setting