Abstract # 145

Marked therapeutic efficacy of a novel poly(ethylene-glycol) conjugated SN38 conjugate in xenograft models of breast and colorectal cancers

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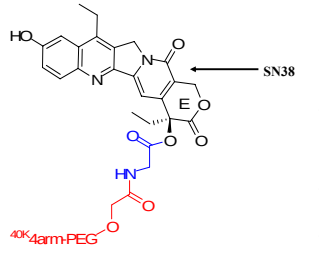
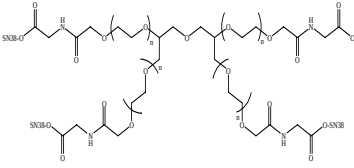
Introduction

SN38 (10-hydroxy-7-ethyl-camptothecin) is the active moiety of CPT-11 (Camptosar®). The clinical utility of SN38 has been severely limited due to its poor solubility. We have generated a novel water soluble conjugate, PEG-SN38 (EZN-2208), by linking SN38 with a multi-arm high molecular weight polyethylene-glycol (PEG). EZN-2208 conjugate is readily soluble and has *in vitro* potency equivalent to that of the free drug on a panel of tumor cell lines. Here we evaluate the pharmacokinetics and therapeutic efficacy of EZN-2208 in xenograft models of human breast, colorectal and pancreatic cancers.

Test compound (EZN-2208)





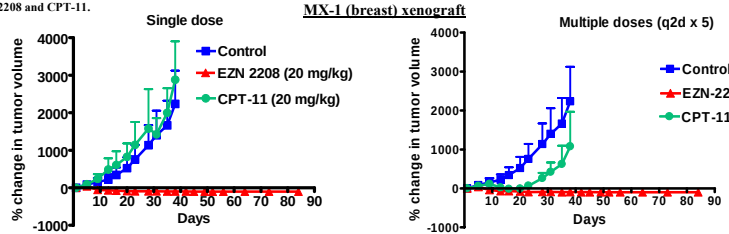
4arm-PEG

EZN-2208 is a novel water soluble prodrug of SN38, generated by conjugating SN38 to multi-arm PEG (40k 4-arm-PEG) via a glycine linker. EZN-2208 is readily soluble in saline (180 mg/ml). (For details on synthesis of compounds, refer poster # 154).

Therapeutic efficacy

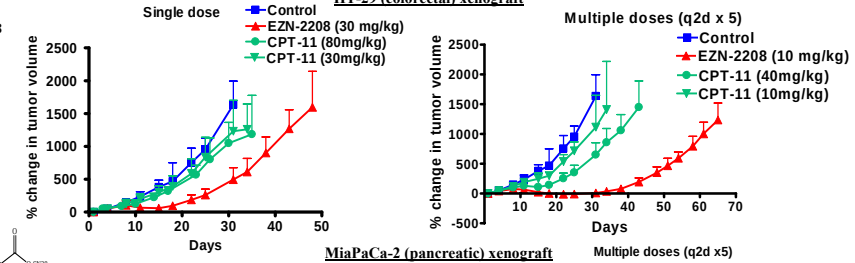
Therapeutic efficacy of PEG-SN38 was evaluated in nude mice (n=6-10/group) implanted with MX-1 breast tumor fragments, HT-29 colorectal cells or MiaPaCa-2 pancreatic cells subcutaneously. Treatment was initiated when tumors reached ~ 100 mm³. EZN-2208 formulated in saline was injected into mice intravenously either as a single dose or in multiple dose regimen (q2d x 5). In HT-29 and MiaPaCa-2 models, mice received EZN-2208 at its maximum tolerated dose (MTD). As controls, mice received CPT-11 at its MTD and equivalent dose level as EZN-2208. In MX-1 model, mice were dosed with lower doses than MTDs both for EZN-2208 and CPT-11.

MX-1 (breast) xenograft

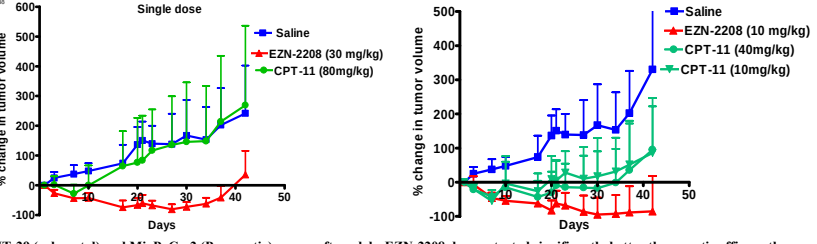


In MX-1 xenografts, treatment with either a single dose of 20 mg/kg or multiple doses of 5 mg/kg (q2d x 5) EZN-2208, led to 100% tumor growth inhibition and complete cures of all the animals. At equivalent dose levels, treatment with CPT-11 caused a 26 and 44% tumor growth inhibition when given as a single dose or multiple injections, respectively.

HT-29 (colorectal) xenograft



MiaPaCa-2 (pancreatic) xenograft



In HT-29 (colorectal) and MiaPaCa-2 (Pancreatic) xenograft models, EZN-2208 demonstrated significantly better therapeutic efficacy than CPT-11 at their respective MTDs as well as equivalent dose levels.

In vitro cytotoxicity (IC_{50} μM)

Indication	Cell Line	SN38	EZN-2208	CPT-11
Colorectal	Colo 205	0.08	0.03	20
	HT29	0.08	0.33	56
Pancreatic	PANC-1	0.84	0.62	52
	MIA PaCa-2	0.14	0.08	23
	BxPC-3	0.97	0.18	95
Ovarian	OVCAR-3	0.62	0.32	20
	SK-OV-3	0.17	0.94	52

EZN-2208 demonstrated potent *in vitro* cytotoxicity against a panel of cell lines as measured by MTS dye reduction assay. EZN-2208 or CPT-11 (dissolved in saline) and SN38 (dissolved in DMSO) were incubated with cells at 37ºC for 72h, after which MTS dye was added and plates were read at 490nm.

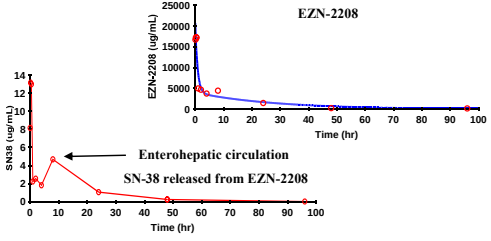
Maximum tolerated dose in nude mice

Compound	Dose level (mg/kg)	Survival/Total
EZN-2208 Single dose	25	5/5
	30	5/5
	35	4/5
EZN-2208 Multiple doses	10	5/5
	15	3/5
	20	0/5
CPT-11 Single dose	80	5/5
	100	6/10
	125	1/5
CPT-11 Multiple doses	40	5/5
	70	4/5
	100	1/5

Nude mice received a single injection of EZN-2208 (in saline). Mice were monitored for 14 days for mortality and signs of illness and sacrificed when body weight loss was >20% of the pretreatment body weight

Pharmacokinetics

Naïve (tumor free) Balb/C mice were injected with a single injection of 20 mg/kg EZN-2208. At various time points mice were sacrificed and plasma was analyzed for intact EZN-2208 and released SN38 by HPLC. Pharmacokinetic analysis was done using non compartmental analysis (WinNonlin)



Parameter	EZN-2208	SN38 Released from EZN-2208
AUC (h*μg/mL)	124,000	98.3
Terminal $t_{1/2}$ (hr)	19.3	14.2
C_{max} (μg/mL)	20,500	13.2
CL (mL/hr/kg)	5.3	202
Vss (mL/kg)	131	3094

Pegylation of SN38 provides long circulation half life and high exposure to native drug SN38. Enterohepatic circulation of EZN-2208 was observed.

Conclusions

1) EZN-2208 (PEG-SN38) is a novel water soluble prodrug of SN38 for direct parental applications
2) EZN-2208 displayed potent *in vitro* cytotoxicity against a panel of human cancer cell lines
3) EZN-2208 demonstrated excellent antitumor activity in xenograft models of human breast, colorectal and pancreatic cancer
4) Treatment with a single or multiple small doses of EZN-2208 led to complete cures of animals in MX-1 (breast) xenograft model
5) In HT-29 (colorectal) and MiaPaCa-2 (pancreatic) xenograft models, EZN-2208 demonstrated significantly better therapeutic efficacy than CPT-11 at their respective MTDs as well as equivalent dose levels
6) In naïve (tumor-free) mice, EZN-2208 provides a long circulation half life and exposure to the parent drug, SN38.
7) EZN-2208 has demonstrated excellent preclinical properties that merit its further evaluation in the clinic.

Refer Abstract # 154 for chemistry and *in vitro* properties of EZN-2208