POSTER # 2645

Customized PEG Linkers Improve the Pharmaceutical Properties of Cytotoxic Small Molecules

Snehlata Tripathi*, Hong Zhao, Dechun Wu, Jing Xia, Yoany Lozanguiez, Syed Ali, Prakash Sai, Charles D. Conover, Lee M. Greenberger, Ivan D. Horak

Enzon Pharmaceuticals Inc., 20 Kingsbridge Road, Piscataway, NJ 08854

E-mail: snehlata.tripathi@enzon.com

Introduction

PEGylation describes a method of linking polyethylene glycol to a protein, oligonucleotide or small molecule. It is an established delivery technology for proteins that can decrease immunogenicity and prolong circulation half-life. PEGylation may also address delivery issues of small cytotoxic molecules by overcoming poor solubility, improving pharmacokinetic (PK) profiles and reducing toxicities. Unlike PEGylation of proteins, releasable PEGylation is essential for delivery of cytotoxics since the ability to regenerate the native small molecule is critical for their biological activity. We report here the use of releasable Customized Linker Technology® to enhance the therapeutic index of several cytotoxic agents including SN38, Daunorubicin & Cytarabine (Ara-C).

PEG-SN38 (EZN-2208)[1-3]

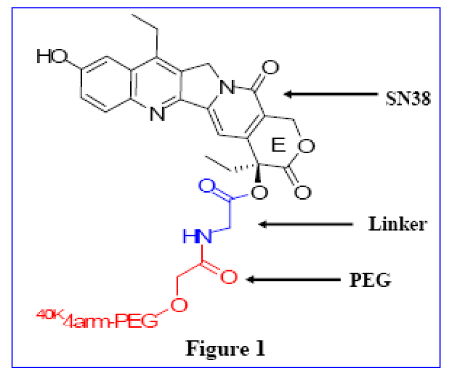


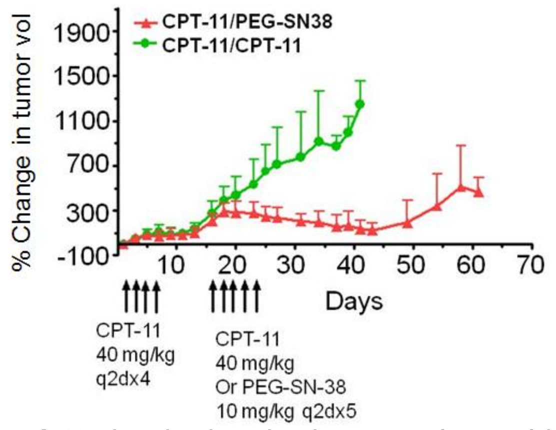
Figure 1

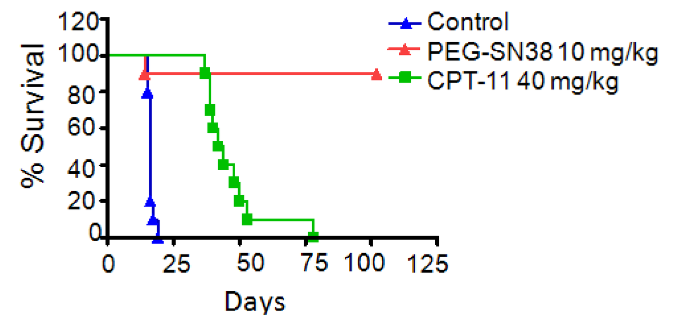
SN38 is the active metabolite of the CPT-11. It is 100 to 1000 times more potent than CPT-11. However, clinical utility of SN38 has been hampered by poor aqueous solubility. We have developed a novel PEG-SN38 conjugate by linking SN38 at the C20 position with a 4-arm PEG via glycine linker. This strategy serves a dual purpose. It stabilizes the E ring in closed and active form. In addition, it increases the solubility of SN38 about 1000-fold. PEG-SN38 has shown remarkable *in vivo* efficacy in preclinical models of solid tumors, hematological cancers, and even in CPT-11 refractory models.

Improved aqueous solubility of PEG-SN38 vs. SN38

	SN38	PEG-SN38
Solubility (mg/ml)	0.0072	6.7 (eq. of SN38)

Superior *in vivo* efficacy: PEG-SN38 vs. CPT-11

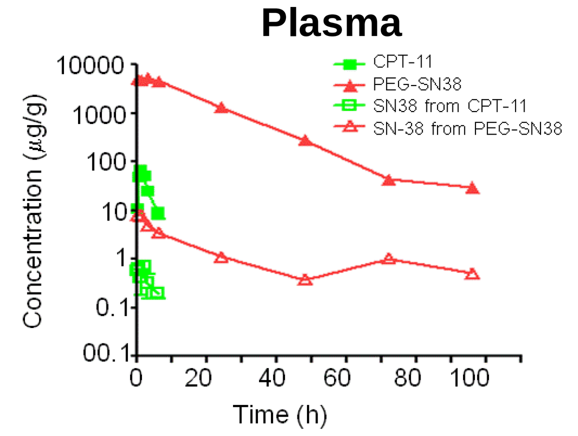


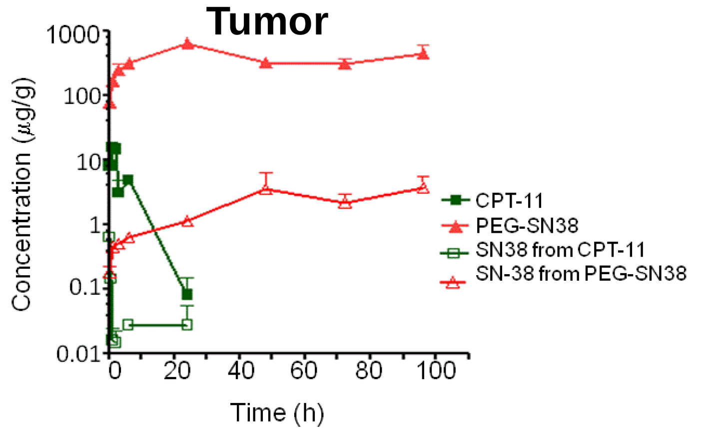


Method: Nude mice bearing human colorectal (HT-29) xenografts; Dose: 10 mg/kg q2dx5
Result: PEG-SN38 showed effective antitumor inhibition in CPT-11 resistant model.

Method: CB17 SCID mice; inoculated with Raji cells; Dose: multiple q2d x 5
Result: Multiple doses of PEG-SN38 cured 90% of the animals.

Improved Exposure to SN38 (both in Plasma & in Tumor)



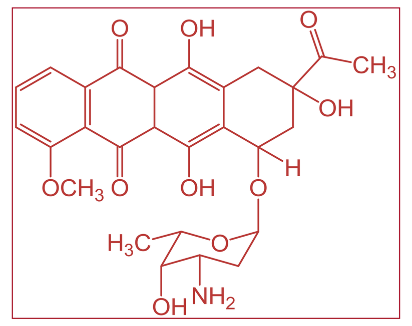


Conclusion: PEG-SN38 outperformed CPT-11 in the pre-clinical settings, showing efficacy in solid tumor and hematological human tumor models, including superior effects in CPT-11 resistant models.

Clinical Status:

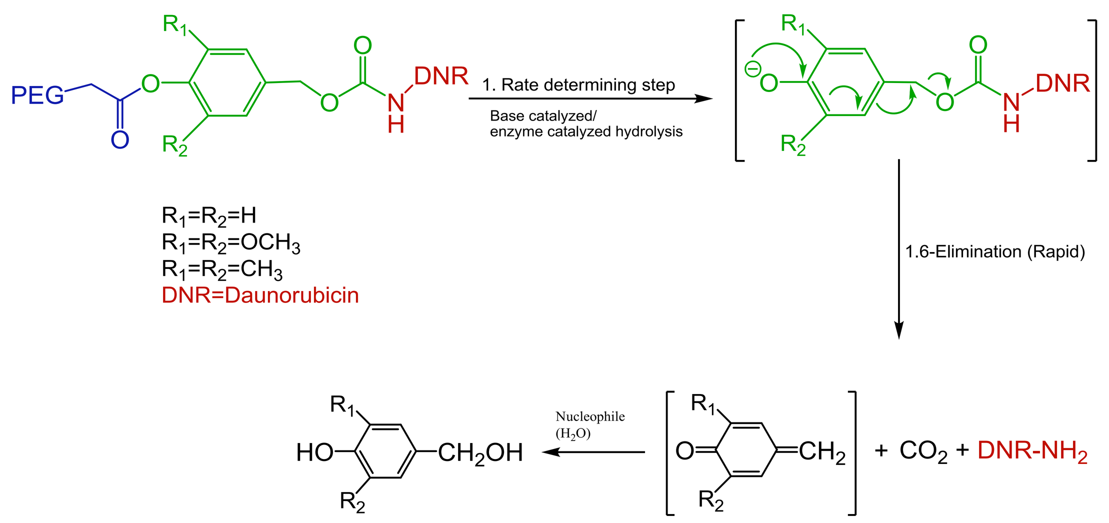
- PEG-SN38 was well tolerated in Phase I studies in heavily pretreated patients with advanced malignancies. PK data demonstrated high AUC and prolonged exposure to SN38.
- Currently, PEG-SN38 conjugate is being evaluated in Phase II trials for metastatic colorectal carcinoma and breast carcinoma as well as a Phase I trial in pediatric cancer.

PEG-Daunorubicin

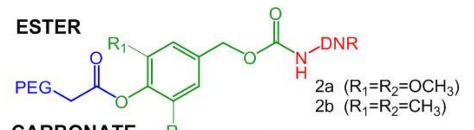
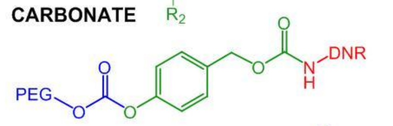
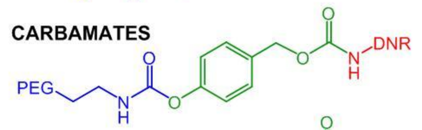
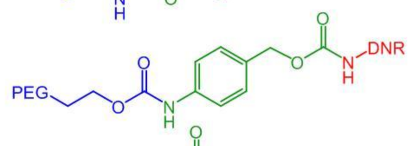
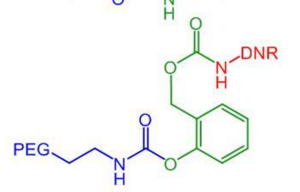
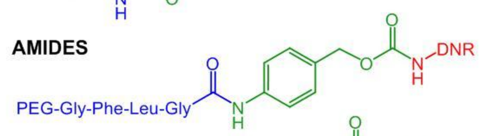


Daunorubicin (DNR) is an anthracycline antibiotics that is an intercalating agent used to treat leukemias. Our goal was to improve the efficacy of DNR without enhancing toxicity. Formulation efforts by utilizing non-aromatic $-NH_2$ group on the sugar ring are found to be challenging. We have developed novel amine based releasable PEG linkers to improve the delivery of DNR[4]. In particular, we have designed a tripartate prodrug approach based on Benzyl Elimination (BE) system, consisting of a trigger and a linker. Hydrolysis of the trigger is followed by the rapid 1,4 or 1,6- benzyl elimination releasing the native molecule. The trigger chemistry strategically combined with introduction of steric hindrance allowed us to generate a series of PEG-BE-drug conjugates with variable releasing profiles in plasma. By successfully applying this novel releasable linker technology, we have synthesized a number of conjugates with enhanced efficacy in animal models.

Mechanism of Benzyl Elimination



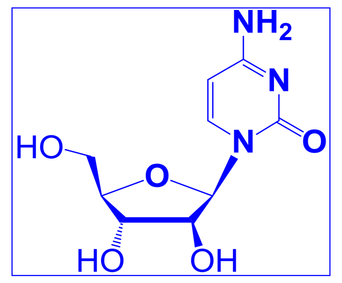
In vitro & *in vivo* results of PEG-Daunorubicin conjugates

Compound	IC_{50} (nM) P388/0	t1/2 (h) Rat plasma	M109 % T/C by week 2	SKOV3 % T/C by Week 5
Daunorubicin (DNR)	3	----	117.0	35.2
ESTER  2a ($R_1=R_2=OCH_3$)	27	1.0	48.2	-
2b ($R_1=R_2=CH_3$)	55	1.9	67.9	-
CARBONATE 	179	2.9	74.4	-
CARBAMATES 	15	4	64.6	7.6
	415	>24	129.0	51.7
	35	3	68.7	-
AMIDES 	160	13	82.6	-
	825	>24	204.3	-

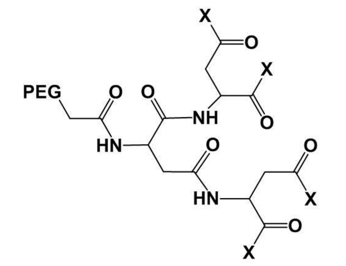
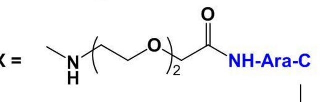
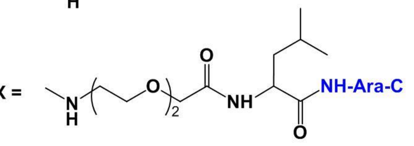
Method: Tumor volume (at the start of treatment)= 70 mm^3 (approx.) Dose: 3 mg/kg/dose i.v. (1, 5 & 9 day schedule)

Conclusion: Releasable PEG-BE linkers were successfully applied to $-NH_2$ containing DNR. PEG-DNR conjugates with different stability in plasma were synthesized. Most of the PEG- conjugates demonstrated better *in vivo* efficacy against solid tumors as compared to native DNR.

PEG-Ara-C



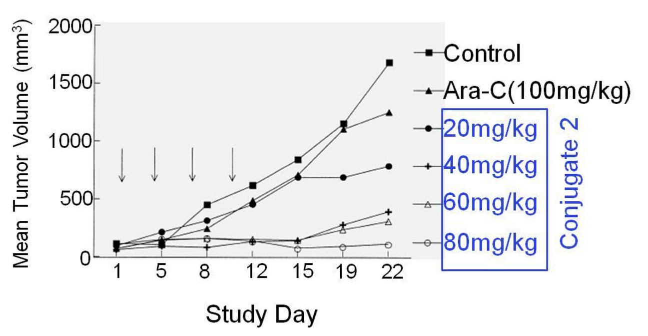
Ara-C (cytosine arabinoside) is used mainly for hematological malignancies. It lacks activity against solid tumors. The therapeutic limitation has been attributed to its short plasma half-life due to rapid conversion to inactive form. We have applied our Customized Linker Technology® to synthesize a series of PEG-conjugates of Ara-C with varied pharmacokinetic properties[4]. Certain conjugates showed superior *in vivo* antitumor activity in solid tumor models. Furthermore, the loading of the Ara-C was incrementally increased by using branched PEG.

Compound	t1/2 (h) Rat plasma	IC_{50} (nM) P388/0
Ara-C (1)	ND	10
		
Conjugate 2 X = 	4.3	39
Conjugate 3 X = 	11.3	40

Dose (i.v.): (1) 100 mg/kg; (2&3) 20 mg/kg on day 1,4,7 and 10.

Antitumor efficacy of PEG-Ara-C In Xenograft models

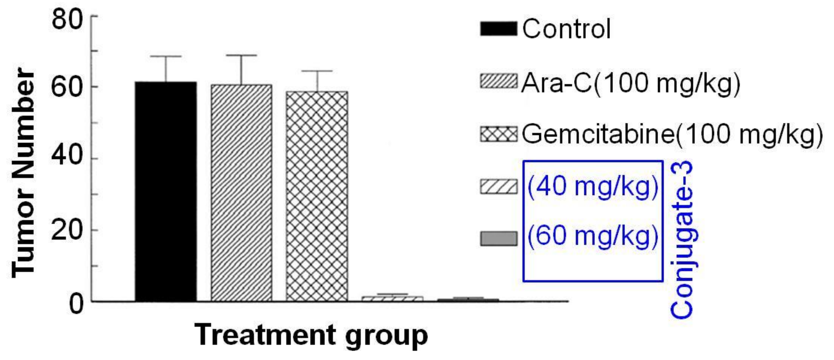
Antitumor efficacy of Conjugate-2 in LX-1 model



Method: Dose: Day 1,4,7 & 10 ; Tumor vol. (at start of treatment) =75 mm^3 (day 1)

Result: All doses of Conjugate-2 greater than 20 mg/kg were able to significantly inhibit tumor growth.

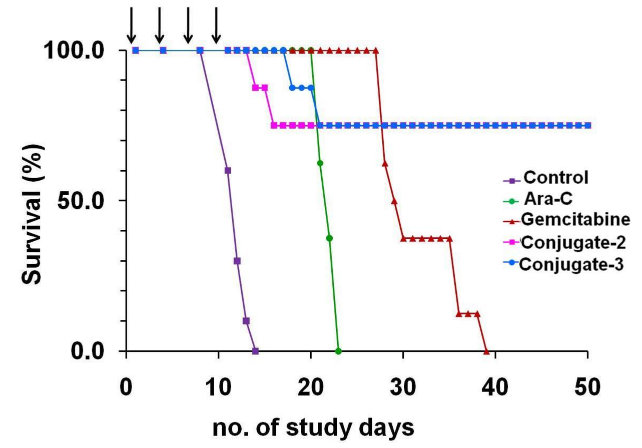
Efficacy of conjugate-3 in orthotopic PANC-1 xenograft



Method: SCID mice implanted with PANC-1
Dose: q3d x 5 i.v.
Study period: 40 days

Result: Conjugate-3 showed tumor inhibition in a model that failed to respond to Ara-C & Gemcitabine.

Effect of conjugates on the survival of CD2F1 mice



Method: P388/0 cells implanted i.p. (day 0) and dosed twice a week for two weeks. (Ara-C & Gemcitabine: 100/mg/kg); (Conjugate-2 & 3; 60 mg/kg)
Result: In ascite model, both Conjugate-2 & Conjugate-3 were able to increase life span and cured >70% animals. The effects were superior to Ara-C & Gemcitabine.

Conclusion: PEGylation of Ara-C using releasable Customized Linker Technology® helps in improving the pharmacokinetic property thus making the drug efficacious against solid tumors. Synthesis of tetrameric and octameric PEG-Ara-C prodrugs was achieved by using aspartic acid (Asp) and AspAsp dendrons (branched PEG).

Conclusions

A series of releasable customized PEG linkers have been developed to improve the delivery of cytotoxic molecules. PEG-conjugates were synthesized with improved solubility and in general, increased the exposure time of the parent molecule.

- PEG-SN38 showed markedly improved solubility leading to significantly enhanced therapeutic efficacy in various xenograft models (including tumors refractory to CPT-11). These encouraging results led to the clinical evaluation of PEG-SN38 (currently in Phase II program).
- The releasable PEG-BE linker system, used in PEG-Daunorubicin conjugate, is an excellent example of releasable PEG-linker Technology whereby small molecules can be released in controlled fashion.
- In case of PEG-Ara-C, optimal pharmacokinetic properties are achieved by applying releasable Customized Linker Technology®. Certain PEG-Ara-C conjugates showed remarkable activity against solid tumors as well as ascites model consistent with improved bioavailability of native Ara-C.

References

1) Zhao H, Rubio B, Sapra P, *et. al.*, **2008**, Novel Prodrugs of SN38 using multi-arm Polyethylene glycol (PEG) linkers. *Bioconjugate Chem.*, 19: 849-859.
2) Sapra P, Zhao H, Mehlig M, *et. al.*, ***2008***, Novel delivery of SN38 markedly inhibits tumor growth in xenografts. *Clin. Cancer Res.*, 14: 1888-1896.
3) Sapra P, Kraft P, Mehlig M, *et. al.*, ***2009***, Marked therapeutic efficacy of a novel polyethylene glycol-SN38 conjugate, EZN-2208, in xenograft models of B-cell non-Hodgkin's lymphoma. *Haematologica*, 94: 1456-1459.
4) Greenwald, RB, and Zhao, H. **2007**, In Book Chapter "Poly (ethylene glycol) Prodrugs: Altered Pharmacokinetics and Pharmacodynamics" of Poly (ethylene glycol) prodrugs: altered pharmacokinetics and pharmacodynamics. *Prodrugs: Challenges and Rewards. Part 1*, Series: Biotechnology: Pharmaceutical Aspects, Stella, VJ, Borchardt, RT, Hageman, MJ, Oliyai, R, Maag, H, Tilley, JW (Eds.) pp 283-338, ISBN: 978-0-387-49782-2.