Synthesis of targeted docetaxel-polymer conjugate and its anti-tumor efficacy

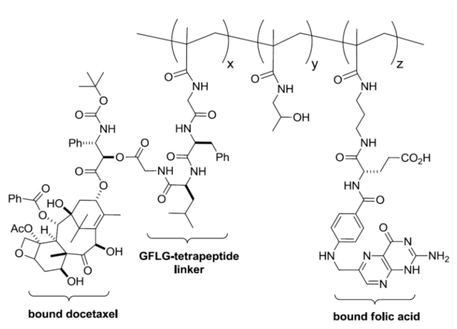
Young Bok Lee[1], Deog Joong Kim[1], Miyoung Yang[1], Chang-Ho Ahn[1], Anjan Nan[2], Robert J. Lee[3], Hong Li[3].
[1]Rexahn Pharmaceuticals, Inc., Rockville, MD; [2]University of Maryland, Baltimore, MD;
[3]Ohio State University, Columbus, OH

ABSTRACT # 4485

Over the last decade, it has been shown that nanopolymer therapeutics can be engineered to target cancer cells specifically and can deliver the cancer agent in a time-release fashion, thereby leaving normal healthy cells unaffected by toxic drugs such as docetaxel (Doc). Docetaxel has been used to treat several types of cancers, but it has provided pharmaceutical challenges due to its poor solubility and toxicities associated with the co-solvents. Given that nanotechnology can enhance the specificity, efficacy and safety of cancer agents and with the need of a new alternative formulation for docetaxel delivery, we developed a poly N-(2-hydroxypropyl) methacrylamide (HPMA)-Docetaxel conjugate with Folate (FA) targeting ligand and evaluated the effect of the product in both in vitro human cancer cell lines and in vivo mice xenograft tumor models. Analysis of the product displayed the incorporation of 7.9% of docetaxel and 2.0% of folate with 38.6kDa MW and 1.92 of polydispersity, and an improvement in water solubility. The product inhibited the proliferation of a variety of human cancer cells in nM ranges in in vitro study. The maximal tolerated dose (MTD) of the product in mice was more than 150 mg/kg (as a docetaxel amount), which is much higher than that of free docetaxel (~25 mg/kg). In mice bearing tumor xenografts, treatment with the polymer-conjugate strongly inhibited the growth of various human tumors, extended survival, and enhanced tumor regression significantly without effects on body weight compared to control animals. The results clearly demonstrate that our new polyHPMA-docetaxel-folate conjugate compound is a promising candidate for anti-tumor chemotherapeutics with reduced toxicity and prolonged survival.

RESULTS

polyHPMA-GFLG-docetaxel-folate (RX-11-JM)

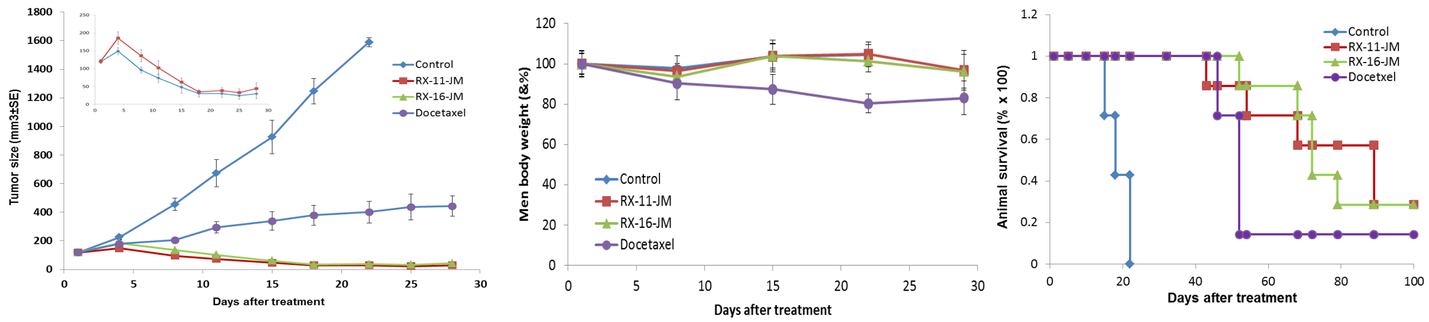


Characterization

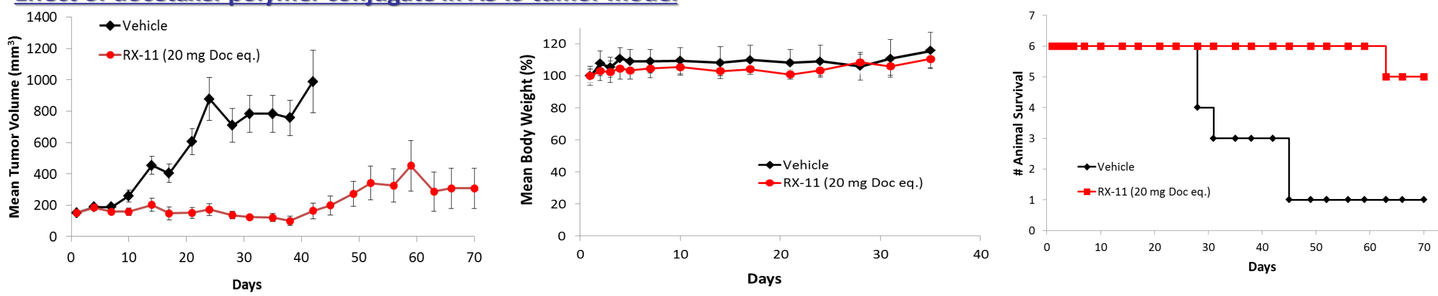
	RX-11-JM	RX-16-JM
Mw	38.6	114.4
Mn	20.0	51.4
PDI	1.92	2.22
Docetaxel content	7.9 wt%	6.7 wt%
Folate content	2.0 wt%	NA
Solubility in water	>400 mg/ml (>30 mg/ml as a Doc)	>180 mg/ml (>12 mg/ml as a Doc)

* Docetaxel solubility in water: 5~16 μg/ml

Effect of docetaxel-polymer conjugate in KB xenograft tumor model: (10mg/kg, as docetaxel equivalent)



Effect of docetaxel-polymer conjugate in A549 tumor model



In-vitro cytotoxicity against human cancer cell lines

Drugs	IC50 (nM) of drug equivalent			
	UMRC2	MDA-MB-231	PANC-1	HCT116
Docetaxel	26	0.77	1.2	0.55
RX-11	68	4.0	4.3	2.8
RX-16	33	1.4	6.6	1.0

Pharmacokinetics

	Docetaxel		RX-11-JM (as Dct eq)	
	Male	Female	Male	Female
	IV	IV	IV	IV
Dose(mg/kg)	15	15	15	15
$AUC_{0\text{-}inf}$ (hr.ng/ml)	5339	4880	1086317	1328072
C_{max} (ng/ml)	16120	18620	175600	265400
$T_{1/2}$ (hr)	19.5	19.4	8.82	10.9
Conjugate (%) in total			87.5	90.5

MATERIALS & METHODS

Chemistry: Intermediates, MA-GFLG-docetaxel, HPMA monomer and APMA-folate, were synthesized using general synthetic methods. HPMA copolymer-docetaxel conjugates were synthesized from the co-monomers, by free radical precipitation copolymerization of the co-monomers HPMA, MA-GFLG-Drug and with (RX-11 & RX-11-JM) or without (RX-16 & RX-16-JM) APMA-folate in acetone/DMSO at 50°C for 24 h using AIBN as the initiator.

***In vitro cell studies*:** Cancer cells were plated in 96-well plates. After 24 hours, the cells were treated with various concentrations of compounds for 96 hours. Cell growth inhibition was measured by sulforhodamine B (SRB) assay and IC50s were obtained.

***PK studies*:** Pharmacokinetic study of docetaxel and RX-11-JM was performed in CD1 Mice following intravenous administration.

Efficacy study: Xenograft studies in nude mice were performed with implanted human cancer cells. Docetaxel and polymer-docetaxel conjugates were administrated intravenously with qwk x 4 (KB model) or qwk x 3 (A549 model) schedule. Tumor volume, total body weight and the number of survived animals were measured at the indicated time points.

CONCLUSION/DISCUSSION

1. **Docetaxel-polyHPMA conjugates are highly water soluble, more than 1000 times increased as a docetaxel amount when compared to free docetaxel.**
2. **Docetaxel-polyHPMA conjugate showed the complete tumor growth inhibition in xenograft model without significant changes in body weight (BW) compared to free docetaxel.**
3. **Docetaxel-polyHPMA conjugate showed the prolonged survival compared to free docetaxel.**
4. **Majority of the docetaxel in the conjugate existed in system circulation as conjugate form in mice.**
5. **This study clearly demonstrated that polyHPMA-docetaxel-folate conjugate is a promising candidate for anti-tumor chemotherapeutics with reduced toxicity and prolonged survival.**

For further information about polyHPMA-docetaxel conjugates and Rexahn Pharmaceuticals please contact:
Dr. DJ Kim:
kimdj@rexahn.com, 240-268-5300 X306
or Dr. Vikas Sharma:
sharmav@rexahn.com, 240-268-5300 X314