

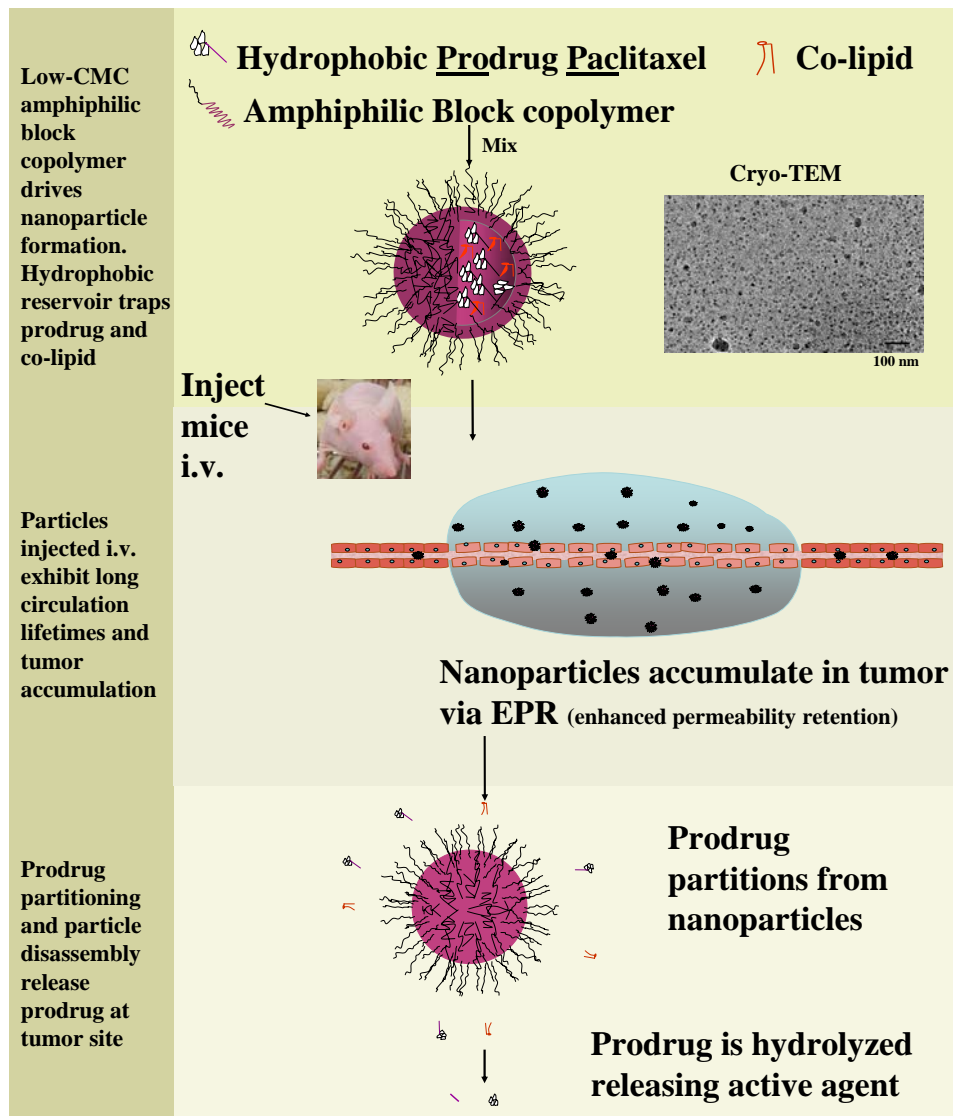
# Development of highly efficacious hydrophobic paclitaxel prodrugs delivered in nanoparticles for fixed-ratio drug combination applications

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## INTRODUCTION

In vitro evidence has revealed that many drug combinations can act either synergistically or antagonistically, depending on the exposed drug ratio. Since the pharmacokinetic behaviour of the individual drugs cannot be controlled when administered in a conventional aqueous based cocktail, drug delivery systems must be utilized to maintain optimal drug ratios. We report here the development of nanoparticle delivery systems for hydrophobic drugs where plasma drug levels can be controlled in a manner that can be readily adapted to deliver drug combinations. We have generated a series of paclitaxel prodrugs through changes in the hydrophobicity of the anchor with the objective of enhancing and controlling drug circulation lifetime of the prodrug when incorporated into nanoparticles. We describe the identification of prodrug and nanoparticle features that optimize therapeutic activity in preclinical tumour models. In addition, paclitaxel could be co-formulated with hydrophobic prodrug analogues of water-soluble agents such as gemcitabine in a manner that maintained the formulated drug ratio after injection. Formulating hydrophobic prodrugs in nanoparticles provides a novel approach to co-deliver anticancer drug combinations with widely differing physicochemical properties and maintain optimal drug:drug ratios in vivo.

## SCHEME FOR PROPAC NANOPARTICLES



Low-CMC amphiphilic block copolymer drives nanoparticle formation. Hydrophobic reservoir traps prodrug and co-lipid

Hydrophobic Prodrug Paclitaxel  
Amphiphilic Block copolymer  
Co-lipid

Inject mice i.v.

Particles injected i.v. exhibit long circulation lifetimes and tumor accumulation

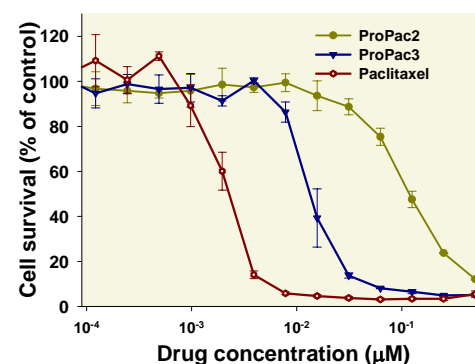
Nanoparticles accumulate in tumor via EPR (enhanced permeability retention)

Prodrug partitions from nanoparticles

Prodrug is hydrolyzed releasing active agent

Nanoparticles are comprised of amphiphilic polystyrene-block-polyethyleneglycol (3K:2.5K), co-lipid (1-palmitoyl 2-oleoyl phosphatidylcholine) and prodrug. These nanoparticles are stable for several months at 4°C. Cytotoxic activity in vitro and in vivo indicate that prodrug is released from nanoparticles then hydrolyzed to active agent as indicated in the above scheme.

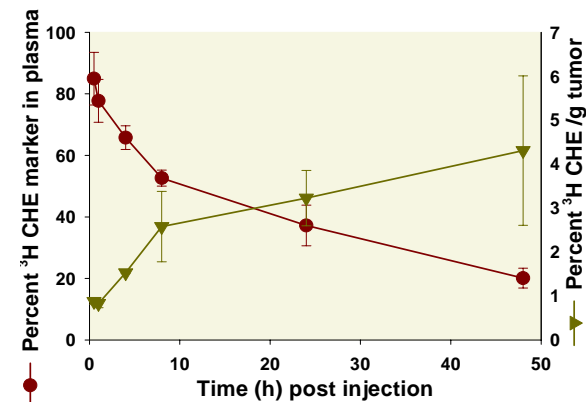
## 1 INCREASE IN HYDROLYSIS RATE INCREASES CYTOTOXIC POTENCY OF PACLITAXEL CONJUGATES



Name	Conjugate
ProPac2	Oleyl succinate (18:1)
ProPac3	Oleyl diglycolate (18:1)
ProPac5	Stearyl diglycolate (18:0)
ProPac6	Eicosanyl diglycolate (20:0)
ProPac7	Docosanyl diglycolate (22:0)
ProPac8	Cholesteryl diglycolate
ProPac10	Dimyristoylglycerol diglycolate (14:0)
ProGem12	Distearoylglycerol diglycolate (18:0)

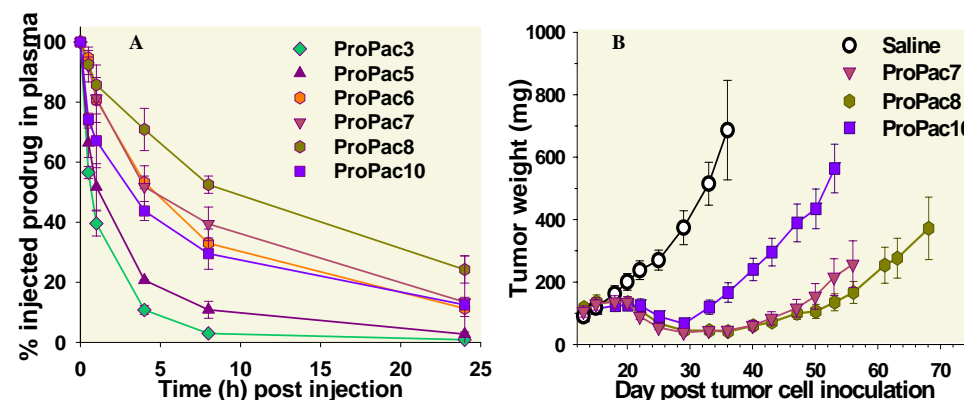
Paclitaxel prodrugs are less active than the parent compound, thus recovery of cytotoxic activity is an indicator of prodrug hydrolysis. Cultured A2780 human ovarian carcinoma cells were exposed to either ProPac2 or ProPac3 nanoparticles or Taxol<sup>®</sup> for 72 hours. Surviving cell fraction was determined by MTT assay. In vitro activity approaching that of paclitaxel is observed for readily hydrolyzed linkages such as ProPac3 (oleyl diglycolate paclitaxel), while reduced activity is observed with the more stable succinate linkage of ProPac2.

## 2 LONG CIRCULATING NANOPARTICLES ACCUMULATE AT THE TUMOR SITE



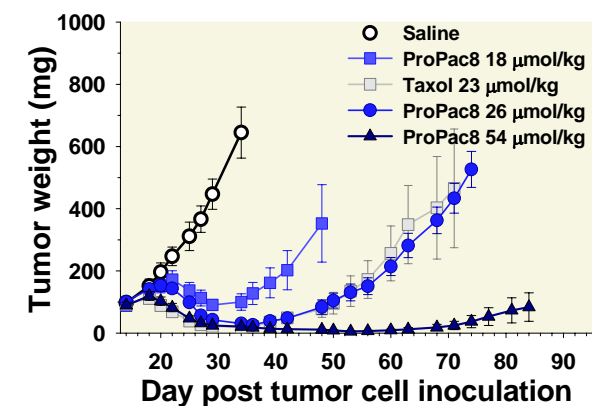
Long circulating nanoparticles accumulate in solid tumors. The biodistribution and tumor accumulation of nanoparticles can be demonstrated using a well-retained hydrophobic marker. Nanoparticles were prepared with tritiated cholesteryl-hexadecylether [<sup>3</sup>H]CHE. Mice bearing 300 mg human colon carcinoma HT29 tumors were injected with ProPac-containing [<sup>3</sup>H]CHE-labelled nanoparticles. At indicated times plasma and tumors were collected and analysed for [<sup>3</sup>H]CHE marker. Nanoparticle accumulation in tumors is most rapid in the first 8 hours.

## 3 EFFICACY OF PACLITAXEL NANOPARTICLES INCREASES WITH INCREASED CIRCULATION LIFETIME



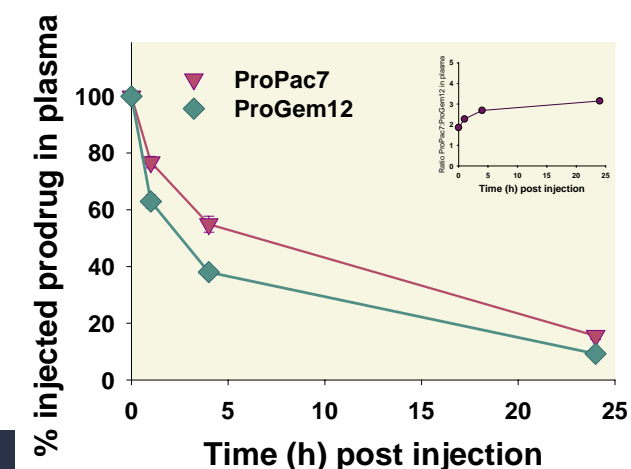
Nanoparticles are long circulating (see panel 2), consequently the plasma concentration over 24 hours correlates with the retention of the drug conjugate by the nanoparticle. **Panel A.** Plasma elimination of nanoparticle formulations of propac conjugates were monitored for 24 hours. Increasing the hydrophobicity of the conjugate correlated with increased plasma retention. **Panel B.** Mice bearing HT29 tumors were treated with matched molar doses of paclitaxel conjugates Q2DX5 beginning day 17. ProPac3 and ProPac5 were released most rapidly from nanoparticles (A) and consequently these nanoparticles had negligible anti-tumor activity (data not shown). ProPac8, the most well-retained paclitaxel conjugate, was the most active against tumors (B).

## 4 OPTIMIZED NANOPARTICLES ARE SIGNIFICANTLY MORE EFFICACIOUS THAN TAXOL<sup>®</sup>



Efficacy of optimized nanoparticles was compared to Taxol<sup>®</sup> at maximum tolerated dose. Treatment began on day 14, when mean HT29 tumor size was 80-120 mg. Mice were treated at the indicated drug dose using a Q2DX5 schedule. Data represent the mean measurement of six animals ±sd.

## 5 NANOPARTICLES COORDINATE DRUG RELEASE OF TWO HYDROPHOBIC DRUG CONJUGATES FORMULATED WITHIN THE SAME PARTICLE



Nanoparticles were prepared containing a combination of ProPac7 and a gemcitabine prodrug, ProGem12, at a fixed molar ratio of 2:1. Plasma elimination of the two prodrugs was monitored for 24 hours. Drug release was coordinated when two drugs were co-formulated within the same nanoparticle as demonstrated by maintenance of the ProPac/ProGem at the formulated ratio (inset).

## SUMMARY

- Paclitaxel prodrug conjugates (ProPacs) were generated to improve retention of paclitaxel in mixed nanoparticle formulations while maintaining the ability to regenerate the parent drug for activity.
- Increasing the hydrophobicity of the ProPac increased its retention in a nanoparticle delivery system. Drug retention in the nanoparticle correlated with increased circulation time and improved efficacy.
- Optimized ProPac formulated in nanoparticles showed dose-response therapeutic activity in the HT-29 solid tumor model and was more efficacious than Taxol at their respective MTDs.
- Molar ratios of two-drug combinations can be maintained in vivo by producing hydrophobic prodrugs of each agent and formulating the drug combination in nanoparticle delivery systems.