ABSTRACT SUMMARY
Nano-scale liposomes and solid core nanoparticles can be used to enhance the activity of anticancer drug combinations that benefit from simultaneous exposure. Liposomes appear best suited for combinations of water soluble agents whereas prodrug nanoparticles may be necessary to coordinate the in vivo exposure of drug combinations exhibiting disparate physico-chemical properties. Coordinating drug release characteristics from nano-delivery systems can dramatically improve the efficacy of anticancer drug combinations.

INTRODUCTION
Inherent in any delivery system-based drug combination formulation is the presence of a fixed drug ratio. As a result, selection of agents to be combined must consider their drug ratio-dependent synergy/antagonism interactions as well as the physico-chemical properties that may impact the ability to control their rates of release from nano-scale drug carriers in vivo (1).

The formulation and in vivo delivery of drug combinations with such systems presents unique technical challenges including co-encapsulation, drug-drug interactions and coordinated pharmacokinetics of combined agents. Typical liposomes containing >30% cholesterol are often unsuitable for in vivo delivery of anticancer drug combinations due to the fact that different drug classes (e.g. nucleoside analogues and topoisomerase I/II inhibitors) exhibit very different permeabilities to such membranes. Titration of cholesterol content in PG-containing gel-phase liposomes affects membrane permeability for such combinations differentially and can coordinate drug release.

For combinations containing more lipophilic agents such as taxanes, we developed a library of hydrophobic prodrugs where the release of individual agents from solid core nanoparticles could be manipulated by varying the hydrophobicity of the prodrug anchor as well as linker lability. In this manner, the PK of chemically disparate drugs such as paclitaxel and gemcitabine can be coordinated to ensure simultaneous exposure of the two agents.

Here we examine the relationships between drug carrier design features and the ability to maintain synergistic drug ratios in vivo. Further, the marked efficacy improvements achievable with such formulations are presented.

EXPERIMENTAL METHODS
Unilamellar 100nm diameter liposomes were prepared by extrusion through polycarbonate filters. Drugs were loaded into pre-formed liposomes by passive diffusion at elevated temperatures for nucleoside analogues such as cytarabine (Cyt) or active encapsulation in response to transmembrane copper gradients (e.g. daunorubicin, Daun). Drugs were quantified by HPLC and liposomal lipid was monitored using 3H-cholesterylhexadecylether as a non-exchangeable lipid marker.

Prodrugs of paclitaxel (Pac) and gemcitabine (Gem) were synthesized by covalently attaching hydrophobic alcohols via bis-carboxylate linkers. Prodrugs were co-dissolved with PS-PEG block copolymer surface stabilizers and POPC co-lipid in EtOH/THF and then rapidly mixed with water in a continuous flow impinging jet mixer to spontaneously form homogeneous nanoparticles with a diameter of approximately 20 nm².

PK properties of liposome and nanoparticle drug combination formulations were determined by quantifying plasma levels of drugs by HPLC post IV injection to mice. Efficacy comparisons of nano-carrier formulated drug combinations with free drug cocktails and individual carrier formulated agents were made in human xenograft solid tumor and leukemia preclinical tumor models using IV treatment regimens.

RESULTS AND DISCUSSION
Co-encapsulating Cyt:Daun at a 5:1 molar ratio shown to be synergistic in vitro (1,3) was accomplished with heating by including copper gluconate-TEA, pH 7.4 inside the liposomes. As shown in Figure 1, within 24h after IV injection to mice, >80% of Daun
and <20% Cyt leaked from DSPC:Chol (55:45) liposomes in the plasma. In contrast, both drugs were released at similar rates from DSPC:DSPG:Chol (7:2:1) liposomes with approximately 50% of the drugs retained in the liposomes at 24h.

Although Pac and Gem have remarkably different water:oil partitioning properties, creating hydrophobic prodrugs of both enabled the two drugs to be co-formulated in solid core nanoparticles. Greater anchor hydrophobicity (dimyristoyl glycerol) was required for Progem retention in nanoparticles and when combined with Propac 7 (C22:0 anchor), the formulated Propac:Progem ratio was maintained in the plasma after IV injection (Figure 3).

CONCLUSION
Nano-scale drug delivery vehicles can be designed to coordinate the PK of a wide range of anticancer drug combinations. This enables drug combination synergy to be fully exploited in vivo, thereby increasing the likelihood of favorable therapeutic outcomes.

REFERENCES

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