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**Title and Subtitle**
New Agents for Taxol-Resistant Ovarian Carcinoma

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**Abstract**
Taxol resistance is an important issue in relapsing ovarian cancer. Two approaches to address this resistance include the use of drug copolymers and drug targeting. In this proposal, paclitaxel covalently coupled to backbones of poly(L-glutamic) acid (non-targeted) or hyaluronic acid (CD44 targeted) as prodrugs will be evaluated in CD44 over-expressing human ovarian carcinoma i.p. (orthotopic) xenografts. We have now documented both the greatly reduced toxicity as well as the anti-tumor efficacy of a lead formulation of HA-TXL compared to Taxol in human ovarian tumor (NMP-1)-bearing nude mice. Additional studies will attempt to develop a superior HA-TXL formulation and compare its toxicity and efficacy to PGA-TXL, which is already in clinical trials.

**Subject Terms**
PGA-TXL, HA-TXL, Taxol Resistance

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INTRODUCTION

Although Taxol has proven to be a most worthy addition to the chemotherapeutic regimens which can be offered to Stage III/IV ovarian cancer patients following surgical debulking, as with other drugs, evidence for resistance to taxanes has emerged. New agents and strategies are urgently needed to address Taxol-resistant ovarian cancer. Among the approaches to overcome drug resistance and to enhance the therapeutic index are the use of drug copolymers and drug targeting.

Drug copolymers are high molecular weight conjugates that can be actively transported to the endosome, where they are then cleaved to release free drug at this organelle. For DNA-targeting drugs, this may afford superior nuclear access compared to import via diffusion as occurs with free drug. Further, it restricts the gradient of export of conjugate-released drug via membrane-localized drug efflux mechanisms, e.g., P-gp170, that are clearly operant on free drug including Taxol. This approach has already proven to be successful for doxorubicin in vitro.

In vivo, other considerations may be more relevant, including distribution to tumor vs. normal tissue. High molecular weight drug copolymers may restrict diffusion-controlled uptake by normal tissues that occurs with free drug; but at the same time enhance extravasation across the abnormal tumor endothelium, thereby enhancing tumor localization compared to free drug.

In these studies, two paclitaxel copolymers will be evaluated. The first copolymer to be employed, poly(L-glutamic acid)-paclitaxel (PGA-TXL), has shown both reduced toxicity and greater tumor localization compared to Taxol in animal models, thereby fulfilling two expectations of copolymer behavior. PGA-TXL should be considered non-targeted, as there is no known receptor for its uptake, and most likely is endocytosed by pinocytosis. PGA-TXL is already in advanced clinical testing as XYOTAX™. The other paclitaxel copolymer is designed to target CD44 expressed on the tumor cell surface. It is based on a hyaluronic acid (HA) backbone that will also serve as a ligand for receptor-mediated uptake by CD44. In this proposal, we will establish the toxicity, pharmacokinetics and anti-tumor efficacy of these paclitaxel copolymers in human ovarian adenocarcinoma xenograft models in nude mice. Comparison of the efficacy of these copolymers with each other and to Taxol will provide evidence by which to judge the merits of tumor targeting of novel copolymers of established drugs.
BODY

Task 1 Synthesis and characterization of hyaluronic acid-paclitaxel (HA-TXL) conjugates with ester or acid-labile linkages

We have returned to essentially our original plans: to couple HA to the NHS ester of paclitaxel via a hydrazide linkage, following the general procedures of Prestwich and coworkers (1). Prestwich has synthesized an HA-TXL formulation with a modestly-sized HA backbone, ~11kDa, with the reported intent to exploit renal clearance of this relatively small conjugate. However, we have focused on a much larger HA backbone as the pilot formulation, ~42kDa. Since a major interest of ours as articulated in this proposal is the application of HA-TXL in i.p. therapy of ovarian carcinoma peritoneal xenografts, we propose that a larger conjugate should have a longer clearance time from the peritoneal cavity. Moreover, other evidence suggests that HA polymers of ~20 disaccharides or larger (up to ~40 or more) have increased binding avidity, most likely due to multiple binding interactions with more than one CD44 molecule. We propose that these favorable CD44-binding and pharmacokinetic characteristics should lead to greater anti-tumor efficacy.

Our reproduction of Prestwich’s synthesis, as well as the synthetic scheme in his issued patents, has been partially successful, as we in fact have demonstrated paclitaxel modification of HA. Further (see below), we have initial evidence for reduced toxicity and much greater antitumor efficacy of this formulation compared to Taxol. However, the yields have not been up to expectations, and other issues (e.g., pre-existing intellectual property) may make further pre-clinical development of this particular formulation difficult. Therefore, we will next explore alternative linker chemistry to attempt to enhance yield and the simplicity of synthesis, as well as to create a patent position, a key to interesting potential industrial sponsors to support further pre-IND development.

Task 2 Mechanistic Studies: Effects on Cell Cycle Distribution/Apoptosis and RAF-1 Kinase Activation

Most of these experiments have already been conducted as reported in previous annual reports..

Task 3 Pharmacokinetics: Cellular and IP Administration

These studies have not yet been undertaken, in large part because of the delays in the synthesis of the lead formulation of HA-TXL.

Task 4 Efficacy Studies: Her-2/neu- and CD44- high and low expression models

In a pilot study, mice were implanted with NMP-1 human ovarian carcinoma cells i.p. according to our published protocol (2). On Day 7, the presence of implanted peritoneal tumor was confirmed by MRI using techniques we have developed under Dr. Roger Price’s direction here at MD Anderson in the Small Animal Cancer Imaging Research Facility (3; and manuscripts in preparation). On Day 8, mice received either buffer control or HA-TXL as
synthesized by the Prestwich method (1); the HA-TXL dose levels initially evaluated were 100 and 200 mg/kg, based on previous experience with PGA-TXL, suggesting that this might be near the MTD for such paclitaxel copolymer prodrugs (2). These dose levels were in fact readily tolerated, with no detectable weight loss or any other clinical symptoms. This compares favorably with PGA-TXL, and is far above the MTD of free Taxol; levels of 20 mg/kg in our hands are above the Taxol MTD (2). The control mice all subsequently succumbed due to tumor burden before Day 40, where as the 100 mg/kg dose level of HA-TXL caused ~70% increase in lifespan compared to controls, and the 200 mg/kg dose level is still under observation.

Although only a pilot study, we are indeed greatly encouraged by these results, which are also the first demonstration of proof-of-principle for an HA-paclitaxel formulation. These studies will next be expanded pending validation of the improved synthetic scheme. (see Task 1).

KEY RESEARCH ACCOMPLISHMENTS

- Documented the greatly reduced toxicity of a lead formulation of HA-TXL compared to Taxol in human ovarian tumor-bearing nude mice
- Documented the anti-tumor efficacy of a lead formulation of HA-TXL compared to Taxol in Taxol-resistant, human ovarian tumor-bearing nude mice

REPORTABLE OUTCOMES

Pending additional data with improved HA-TXL formulation and comparison to PGA-TXL.

CONCLUSIONS

We have demonstrated both the greatly reduced toxicity as well as the anti-tumor efficacy of a lead formulation of HA-TXL compared to Taxol in human ovarian tumor (NMP-1)-bearing nude mice.

REFERENCES


APPENDICES

N/A