Superior Therapeutic Profile of Poly-l-Glutamic Acid-Paclitaxel Copolymer Compared with Taxol in Xenogeneic Compartmental Models of Human Ovarian Carcinoma

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ABSTRACT

Previous preclinical studies with ectopic tumor models have demonstrated remarkable improvements in the therapeutic profile of paclitaxel, formulated as a copolymer with poly-l-glutamic acid, compared with paclitaxel in the clinical formulation, Taxol. In this study, we evaluated these formulations in two human ovarian carcinoma xenograft models, NMP-1 and HEY, in nude mice. i.p. implantation in female nude mice of either cell line gave rise to progressive disease within the peritoneum, in the parenchyma of visceral organs, and eventually at extraperitoneal sites; the resultant, increasing morbidity then required host sacrifice. i.p. administration of multiple-dose Taxol at its maximum tolerated dose 1 week after tumor implantation afforded minimal or no increased survival compared with controls in either model. Consistent with the predictions of drug copolymer behavior, paclitaxel, as the poly-l-glutamic acid-paclitaxel copolymer, displayed much less toxicity than Taxol in these hosts. When evaluated for antitumor efficacy in both the Taxol-resistant NMP-1 and HEY models, significant improvement in survival, and even some cures, were observed after a single i.p. treatment with this copolymer. The observed antitumor response correlated with histopathological analysis of peritoneal and extraperitoneal tumor burden in comparing control HEY mice sacrificed near the onset of morbidity with mice receiving paclitaxel copolymer. We conclude that both the i.p. NMP-1 and HEY models have significant value in establishing the efficacy of candidate agents, which might address Taxol-resistant human ovarian carcinoma. Furthermore, the poly-l-glutamic acid-paclitaxel copolymer has a superior therapeutic profile in these Taxol-resistant compartmental models.

INTRODUCTION

Ovarian cancer is the second most common and the most lethal gynecologic malignancy (1). Peritoneal implantation is a crucial and common aspect of this disease, because it predictably leads to a familiar clinical course attributable to progressive encasement of intra-abdominal organs followed by many morbid sequelae. Carcinomatous involvement of the peritoneum is present in ∼80% of patients with stage III–IV ovarian carcinoma (2). Recent clinical results support the value of an i.p. route of drug administration in this setting. Patients with minimal residual disease after cytoreductive surgery were randomized to i.v. CDDP (1) + cyclophosphamide or i.p. CDDP + i.v. cyclophosphamide arms; greater survival and reduced toxicities were seen in the latter group (3).

Treatment of ovarian carcinoma with platinum-containing regimens is widely considered standard therapy for stage III–IV disease (reviewed in Ref. 4). This treatment results in high initial response rates, but the vast majority of patients eventually represent with chemotherapy-resistant disease (4–6). This resistance is multifactorial and, because of treatment toxicities, cannot be readily circumvented by dose-intensification. Given the diverse mechanisms leading to CDDP resistance and treatment toxicities (7–15), new agents with distinct mechanisms of action and nonoverlapping toxicities are greatly needed.

One such agent, Taxol, the clinical formulation of paclitaxel, has already demonstrated activity in ovarian carcinomas (16). Paclitaxel increases tubulin polymerization, stabilizes microtubules, and prevents tubulin depolymerization, resulting in tubulin bundling (17–19). The mechanisms linking these effects to mitotic and G2/M arrest are complex and are concentration-dependent (20, 21). Taxol resistance has been linked to: (a) alterations in tubulin (22–25); (b) expression of the P-gp 170 drug-efflux pump (26, 27); (c) high Raf-1 kinase activity (28); and (d) overexpression of HER-2/neu (29–32). A number of recent observations suggest that dose-intensification with Taxol
to overcome resistance may be a situation of diminishing returns (reviewed in Ref. 33; Refs. 34, 35). This plateau effect has been attributed to saturation of the paclitaxel binding sites on β-tubulin (35) or to antagonistic effects of the Cremophor vehicle (36, 37). Furthermore, recent evidence suggests Taxol may interact negatively with 5-fluorouracil (38) and with CDDP (39), a caution against use of these drug combinations (40, 41). Thus, other approaches to Taxol-resistant disease strongly merit exploration.

Macromolecular drug delivery systems have been developed as one approach to overcome drug resistance and to improve the therapeutic index. Examples include polymeric conjugates of chemotherapeutic agents; these are internalized by endocytosis, resulting in their accumulation in perinuclear lysosomes, thereby rendering drug released from polymer both closer to nuclear targets and less accessible to membrane-linked efflux mechanisms than the free drug originally incorporated by diffusion (42). Drug copolymers may also have pharmacokinetic advantages over free drugs. The latter may readily extravasate to normal tissues, whereas the size of the former may restrict such distribution, potentially reducing toxicity. Despite this restriction, the leaky, irregular vasculature of solid tumors may still be readily traversed by these macromolecules, which, when combined with their greater retention in the tumor interstitium, results in superior tumor localization and less toxicity compared with free drug (43, 44).

Some copolymer formulations of paclitaxel have already been characterized; these include cyclodextrins; copolymers of ε, L-lactide, and polyethylene glycol (45–47); and PGA-TXL (48). PGA-TXL has already demonstrated key advantages over free paclitaxel (as Taxol): (a) greatly reduced toxicity; (b) greater localization to tumor implants; and (c) greater antitumor efficacy and even curative ability in several rodent models.

To date, PGA-TXL has been evaluated using systemic administration, in syngeneic murine and xenogeneic human tumor models, and in tumor models using both ectopic and orthotopic implantation (48–50). In the current studies, we have evaluated PGA-TXL in two human tumor xenograft models using i.p. implantation of tumor in nude mice. This route was used to facilitate characterization of the response of nascent disease invading i.p. from the ovarian capsule, or more realistically given the typically late detection of ovarian cancer, residual peritoneal tumor burden after surgical and chemotherapeutic debulking of advanced disease. In this report, we first demonstrate that these models, NMP-1 and HEY, are highly resistant to multiple-dose MTD Taxol, administered i.p. beginning 1 week after tumor implantation. Secondly, we demonstrate that a single i.p. administration of PGA-TXL administered at the same time as the first Taxol dose results in significant improvement in survival and even in some long-term cures in both models.

**MATERIALS AND METHODS**

**Cell Lines**

The human ovarian adenocarcinoma cell line OVCAR-3 was obtained from American Type Culture Collection (Rockville, MD). It was originally established from malignant ascites and is reported to be resistant to several drugs in vitro (51). After subsequent selection procedures, OVCAR-3 has been characterized as an i.p. tumor xenograft model in nude mice demonstrating progressive metastatic spread akin to the human disease (52). However, successful implantation i.p. required a high number of inoculated cells (≥10⁷).

A cisplatin (CDDP; Bristol-Myers Squibb)-resistant cell line, C-1, was derived from parental OVCAR-3 cells by in vitro incubation of OVCAR-3 cells with increasing concentrations of CDDP (53). Cells surviving several rounds of selection in CDDP-containing medium (1 μg/ml) were cloned by limiting dilution, expanded, and retested for CDDP sensitivity. C-1 cells demonstrated durable resistance to drug even after 3 months of passage in the absence of CDDP. NMP-1 cells were derived from ascites of nude mice into which C-1 cells had been implanted i.p. These NMP-1 cells retained in vitro CDDP-resistance levels identical to the original C-1 population.

The human ovarian carcinoma cell line HEY was originally established from a peritoneal deposit of a moderately differentiated papillary ovarian cystadenocarcinoma, and it has been reported to be moderately resistant to CDDP in a clonogenic assay (IC₉₀ ~ 1 μg/ml; Refs. 54, 55).

**PGA-TXL**

PGA-TXL was prepared by carbodiimide-mediated, ester coupling of hydroxyl groups of paclitaxel and γ-carboxyl groups of glutamic acid (as PGA), as described previously (48). Copolymers composed of 13% and 37% paclitaxel (w/w) were used in these studies. The molecular mass of the PGA backbone was Mₐ 30,000–40,000 daltons. PGA-TXL was dissolved in warmed (37°C) physiological saline before injection.

**In Vitro Cytotoxicity Assays**

NMP-1 and HEY cells were cultured overnight in 96-well plates in 100 μl of medium (DMEM/F12; Life Technologies, Inc.) supplemented with 5% FCS/well before treatment. Based on preliminary experiments, cell numbers were adjusted to 1 × 10⁶ cells/well in order to achieve subconfluent control cell monolayers at the end of the assay. The cytotoxic effects of Taxol (paclitaxel in Cremophor EL; Mead Johnson/Bristol-Myers Squibb) were established using a dose range of drug up to 4 μg/ml. Remaining viable cells were stained with neutral red after 96 hr, and the percentage of control survival as measured by optical density of incorporated dye was determined. The results from two to four experiments of each type are shown.

**In Vivo Efficacy Assays**

NMP-1. On Day 0, 1 × 10⁷ viable NMP-1 cells were injected into the peritoneal cavities of groups of 6–9-week-old female nude mice (Harlan). Five to 25 mice/experimental group were used as the basis for statistical analyses. i.p. therapy was initiated 1 week later (day 7). Histopathological examination of mice in parallel studies indicated that by this time frame, abdominal tumors were already present (data not shown). Taxol was administered on a q7d × 3 or q4d × 3 schedule, at ~20 mg/kg. Since it was important to evaluate PGA-TXL in a relevant preclinical setting, the clinical formulation of paclitaxel (Taxol) was used as a comparison rather than alternative formulations of paclitaxel with reduced or zero Cremophor content.
PGA-TXL was dissolved in PBS and administered at ≤200 mg/kg (paclitaxel equivalents) as a single i.p. injection on day 7, or at 180 mg/kg on a q7d × 3 schedule beginning on day 7, or as a single i.v. injection.

Since mice implanted i.p. with NMP-1 tumor cells develop marked, debilitating ascites as one of the earliest clinical signs of peritoneal tumor and substantially before other aspects of tumor progression, ascites fluid was repeatedly removed at intervals from mice, beginning after the fourth week. As the peritoneal tumor burden continued to increase as detected by direct visualization of tumor through the distended abdominal wall, relief from ascites removal became less effective. Cachexia, spine prominence, and other morbid symptoms became more severe, and these animals were humanely sacrificed by carbon dioxide asphyxiation.

HEY. On day 0, 3 × 10^5 viable HEY cells were injected into the peritoneal cavities of 6–9-week-old female nude mice. I.p. therapy was initiated on either day 2 or day 7. No macroscopic or histopathologic evidence of a tumor was observed in control mice that were sacrificed either 2 or 7 days after i.p. inoculation of HEY cells, reflecting the very low tumor burden at the times of treatment. Taxol was administered on a q7d × 3 schedule at ≤10 mg/kg, because toxicity had been observed with 20 mg/kg in the NMP-1 studies.

PGA-TXL was dissolved in PBS and administered at ≤180 mg/kg (paclitaxel equivalents), the most effective dose in the NMP-1 studies, as a single i.p. injection on day 2 or day 7, or at 180 mg/kg on a q7d × 3 schedule beginning on day 7.

For mice implanted with HEY tumors, the first sign of tumor growth was in the needle track in the muscle of the abdominal wall, and eventually palpable i.p. tumor was evident. As the latter progressed, cachexia became more significant. These morbid symptoms eventually required humane sacrifice. They did not display prominent abdominal distention from ascites as observed with the NMP-1 model.

On occasion, mice bearing either tumor succumbed between daily observations and before the opportunity to sacrifice them. In this case, the day of death was considered to be the day before the date they were discovered. The day of humane sacrifice/death was recorded for each mouse, and these values were compared among control and treatment groups by paired or unpaired Student’s t tests.

In addition to the survival end points described above, to provide objective, blinded data, mice were implanted with HEY tumor and randomized into three groups of four to six animals. These groups included: (a) controls to which saline was administered; (b) mice treated on day 2 with 180 mg/kg PGA-TXL; and (c) mice treated on day 7 with 180 mg/kg PGA-TXL. On day 31, before any deaths in the controls, mice were sacrificed and subjected to histologic and histopathologic analysis (see below) in a blinded fashion.

**Histopathology**

The mice were killed by exposure to CO₂. The skin was removed from the torso of each mouse, and 1 cc of 10% neutral phosphate-buffered formalin was injected into both the pleural and peritoneal cavities before the complete body of each animal being immersed in 10% neutral phosphate-buffered formalin for tissue fixation. The intracavitary infusion of formalin was added to improve fixation of abdominal and thoracic viscera. After the animals had fixed for ≥2 days, the bodies of the mice were immersed in 10% formic acid for 2–3 days to decalcify the osseous tissues. After fixation and decalcification, axial sections of the head, thorax, and two levels of the abdomen were routinely processed, and 4–6 μm paraffin sections, which were stained with H&E, were prepared for histopathologic examination. In addition to descriptions of the extent of tumor involvement in each tissue examined, the overall tumor burden in each animal was subjectively classified as no tumor present or modest (1+), moderate (2+), marked (3+), or severe (4+) levels of tumor burden within the abdominal wall and abdominal cavity. Tumor burden was classified based on the percentage of the area of the abdominal cavity and wall that the tumors composed, with modest = <25%, moderate = 25–50%, marked = 51–75%, and severe = >75% involvement.

**RESULTS**

**Taxol Response of NMP-1 and HEY in Vitro.** NMP-1 and HEY cells were treated with a concentration range of Taxol for 96 hr. Both cell lines demonstrated a loss of survival in response to Taxol, with NMP-1 cells being slightly more sensitive (Fig. 1). The concentration-response curves were shallow, with an effect evident at the lowest concentration but even the highest concentration being incapable of achieving 100% cell death in this time frame.

**NMP-1 Resistance to i.p. Multiple-Dose MTD Taxol in Vivo.** NMP-1 cells were implanted i.p. in nude mice on day 0. Beginning on day 7, q7d × 3 regimens were initiated using Taxol at 10 or 20 mg/kg/injection. The survival of treated animals and of control mice that received saline alone is shown in Fig. 2 and Table 1. Control animal survival was 43.4 ± 1.1 days (mean ± SE). Taxol administered at 10 mg/kg failed to improve survival (41.7 ± 0.5 days; P = 0.32). When a dose of 20 mg/kg was used, drug toxicity became evident, and host survival was reduced significantly on either a q7d × 3 (13.8 ± 4.1 days) or q4d × 3 (26.2 ± 3.3 days) regimen compared to controls (P < 0.0001). Therefore, this ovarian model, with treatment beginning 1 week after implantation of tumor, ap-
appeared to be highly Taxol-resistant using this multiple-dose MTD regimen.

**HEY Response to i.p. Multiple-Dose MTD Taxol in Vivo.** HEY cells were implanted i.p. on day 0. Beginning either on day 2 or on day 7, q7d × 3 regimens were initiated using Taxol at 5 and 10 mg/kg, administered i.p.; in light of the toxicity observed in the NMP-1 model with the 20 mg/kg Taxol regimen, this higher dose level was not evaluated in this model. The survival of treated animals and of control mice that received saline alone is shown in Fig. 3 and Table 2. Control animal survival was 36.3 ± 1.7 days. The effect of Taxol was both dose-dependent and tumor burden-dependent. When the q7d × 3 regimen was begun with the lower tumor burden present on day 2, 5 mg/kg Taxol increased survival nominally to 46.7 ± 6.1 days (P = 0.045; Table 2). However, a dose level of 10 mg/kg resulted in a substantial increase in mean survival to 56.0 ± 8.7 days (P < 0.007), reflecting sensitivity to the higher Taxol dose in animals with lower tumor burden. In contrast, when treatments were begun with the higher tumor present on day 7, even the 10 mg/kg dose level of Taxol could not improve survival (36.7 ± 0.3 days; P = 0.89). Comparing the NMP-1 and HEY models using initiation of intervention on day 7 for both, these models appear particularly resistant to multiple-dose MTD Taxol.

**NMP-1 Response to Single-Dose i.p. PGA-TXL in Vivo.** NMP-1 cells were implanted on day 0, and on day 7, a single i.p. injection of PGA-TXL was administered at 140, 160, 180, or 200 mg/kg (paclitaxel equivalents). Effects of these treatments on host survival are shown in Fig. 4 and Table 3. All four of the treatment arms demonstrated improved survival compared to controls (P ≤ 0.042). Whereas control survival in this experiment was 43.0 ± 1.3 days, two of five mice in the 180 mg/kg group were alive at >380 days at the termination of the experiment. Interestingly, the highest dose level used, 200 mg/kg, did not achieve the longest survival. These results, nevertheless,
control; P  

regimen, 7 days after implantation of NMP-1 cells. The day of death/sacrifice/death (q7d single i.v. injection, a single i.p. injection, or of multiple i.p. injections (q7d × 3) of PGA-TXL at 180 mg/kg, or three i.p. injections of PGA-TXL at 180 mg/kg each on a q7d × 3 regimen, 7 days after implantation of NMP-1 cells. The day of death/sacrifice is noted.

**Table 3** Responses of NMP-1 tumors to i.p. single-dose PGA-TXL

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Mean day of sacrifice/death</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>43.0 ± 1.3</td>
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<tr>
<td>PGA-TXL a</td>
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<tr>
<td>140 mg/kg</td>
<td>52.6 ± 3.0</td>
<td>0.0034</td>
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<tr>
<td>160 mg/kg</td>
<td>65.6 ± 9.8</td>
<td>0.0103</td>
</tr>
<tr>
<td>180 mg/kg</td>
<td>119.2 ± 43.8 a</td>
<td>0.0419</td>
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<tr>
<td>200 mg/kg</td>
<td>54.4 ± 4.9</td>
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* Compared with controls.

**Table 4** Responses of NMP-1 tumors to i.v. single-dose or i.p. single-dose or multiple-dose PGA-TXL

<table>
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<th>Group/treatment</th>
<th>Mean day of sacrifice/death</th>
<th>P</th>
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<td>Controls</td>
<td>42.6 ± 3.1</td>
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<td>PGA-TXL (180 mg/kg/dose) i.p., single dose a</td>
<td>50.8 ± 5.4</td>
<td>0.861 b</td>
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<tr>
<td>i.p., triple dosing q7d × 3, beginning day 7</td>
<td>58.6 ± 2.4</td>
<td>0.0004 b</td>
</tr>
</tbody>
</table>

* Administered on day 7.

**Fig. 5** Effects of single-dose i.p., or i.v. PGA-TXL, or multiple-dose i.p. PGA-TXL on survival of mice bearing 7-day i.p. implants of NMP-1 tumors. Groups of five or more female nude mice were given i.p. saline (Control), a single i.p. or i.v. injection of PGA-TXL at 180 mg/kg, or three i.p. injections of PGA-TXL at 180 mg/kg each on a q7d × 3 regimen, 7 days after implantation of NMP-1 cells. The day of death/sacrifice is noted.

**Fig. 6** Effects of single-dose i.p. PGA-TXL on survival of mice bearing 2-day or 7-day i.p. implants of HEY tumors. Groups of six female nude mice were given either i.p. saline (Control) or a single i.p. injection of PGA-TXL at 180 mg/kg, either 2 or 7 days after implantation of HEY cells. The day of death/sacrifice is noted.

Demonstrate striking efficacy of single-dose PGA-TXL in this Taxol-resistant model.

### NMP-1 Response to i.v. Single-, i.p. Single-, or Multiple-Dose PGA-TXL in Vivo

The effects on host survival of a single i.v. injection, a single i.p. injection, or of multiple i.p. injections (q7d × 3) of PGA-TXL (180 mg/kg/injection paclitaxel equivalents) administered after implantation of NMP-1 cells on day 0 are shown in Fig. 5 and Table 4. Single-dose administration on day 7 by the i.v. or i.p. routes resulted in similar improvements in survival (50.2 ± 2.4 and 50.8 ± 4.8 days; P = 0.86 for i.v. versus i.p.). In a subsequent experiment with five additional mice, some possible toxicity was evident using the i.v. route, because one of the mice expired within 11 days of drug administration. Combining the two experiments using i.v. administration, the nine surviving mice demonstrated improved survival compared to controls (52.8 ± 2.8 days; P < 0.038). The multiple-dose i.p. regimen additionally improved survival but only slightly to 58.6 ± 2.4 days (P < 0.0001 versus control; P = 0.073 versus single dose in this experiment).

There was more modest improvement in survival (treated versus control = 118 for single i.p. injection and 146 for triple i.p. injection) and no long-term survivors in this experiment using a 37% paclitaxel formulation of PGA-TXL compared with the more compelling improvements in survival and even cures observed with a single injection of the same dose (180 mg/kg) of the 13% paclitaxel formulation in the previous experiment (Table 3). This suggests that the extent of paclitaxel substitution may be an important variable in optimizing the efficacy of PGA-TXL.

### HEY Response to i.p. Single- or Multiple-Dose PGA-TXL in Vivo

HEY cells were implanted i.p. on day 0. On either day 2 or day 7, a single i.p. injection of 180 mg/kg (paclitaxel equivalents) PGA-TXL was administered to several groups of mice. The resultant effects of treatment on survival are shown in Fig. 6 and Table 5. There was clear benefit to survival whether PGA-TXL was administered with low tumor burden (day 2; survival = 226.7 ± 42.5 days with four of six mice alive at ≥222 days; P = 0.0002 versus control) or high tumor burden (day 7; survival = 76.8 ± 29.0 days with one of six mice alive at 222 days; P = 0.13 versus controls; P < 0.016 versus intervention on day 2). Earlier intervention clearly favored long-term survival. Curiously, multiple-dose administration (q7d × 3) beginning on day 7 did not improve survival (48.3 ± 5.7 days; P = 0.02 versus controls) compared with single-dose administration (P = 0.526).

To verify that the survival data reflected the consequences
of progressive tumor burden, mice were implanted i.p. with HEY tumor cells, and four to six animals per group were treated either with saline (controls) or a single i.p. injection of 180 mg/kg PGA-TXL on either day 2 or day 7 after tumor implantation. All of the mice were sacrificed on day 31 and prepared for blinded histopathological examination. The results are shown in Fig. 7.

All four of the control mice presented with single discrete 5–12 mm diameter tumors in the abdominal wall and s.c. adipose tissues (Fig. 7, A). These tumors were typically located along the ventral midline close to the area where the i.p. injections were performed. One of the control animals also had several other small 1–4 mm diameter tumors that were present in the muscle of the lateral abdominal wall adjacent to the larger midline tumor mass. At this time point, no evidence of tumor was present within the abdominal cavity or the abdominal wall of any of the four mice administered PGA-TXL on day 2 (Fig. 7, B). This observation correlates well with survival for mice on this protocol being ~227 days versus ~36 days for the controls (Table 5). Similarly, five of six mice administered PGA-TXL on day 7 did not have any histopathological evidence of tumor at day 31. Only one of these mice had a single, small (~2 mm) diameter tumor observed between the spleen and pancreas (Fig. 7, C). This also correlates with improved survival with this group (~77 days; Table 5) but with fewer cures achieved than with day 2 treatment.

**DISCUSSION**

Drug copolymers may have key advantages over free drugs, including reduced toxicity (56); high plasma Cmax values (57), and rapid extravasation, which contribute to toxicity of free drugs and should be largely abrogated with copolymer prodrugs, of which the diffusion rates are limited because of their size (58). However, the so-called EPR effect (59–61) should still allow copolymer accumulation and retention within the tumor interstitium followed by endocytic uptake by tumor and stromal cells, activation of the prodrug, drug access to intracellular targets, and cell death.

Some copolymer formulations of paclitaxel have already been characterized, including PGA-TXL (48–50). The latter has already demonstrated key advantages over free paclitaxel (as Taxol) as monotherapy or when combined with radiotherapy: (a) greatly reduced toxicity; (b) greater localization to tumor implants; and (c) greater antitumor efficacy. As seen in the current studies, the latter includes achieving some apparent cures in highly Taxol-resistant human ovarian carcinoma xenograft models. The strong in vivo resistance to Taxol was unexpected, given the sensitivity of both NMP-1 and HEY tumor cells to Taxol in vitro (Fig. 1). Based on a favorable preclinical profile, clinical trials of PGA-TXL are now underway.

There would appear to be two quite different mechanisms for PGA-TXL processing, which could be proposed. The first is that after fluid phase pinocytosis, subsequent activation involves two distinct steps: first, an endosomal protease of the appropriate specificity cleaves the amide bonds linking the PGA backbone, thereby liberating free glutamic acid and a glutamic acid ester linked via its γ-carboxyl group to one of the hydroxyl groups of paclitaxel. Endosomally degradable PGA but not PAA nor their D-isomers, as shown by the ability of all of the latter but not the first to protect against gentamycin-induced nephrotoxicity (62–64). Thus, only with PGA backbone will the amino acid/paclitaxel ester product be generated. Next, this ester is expected to be inherently chemically labile and to undergo spontaneous release of paclitaxel by an autocatalytic hydrolysis involving nucleophilic attack by the α-carboxylate group on the γ-ester linkage (Fig. 8). Therefore, only PGA-TXL will be internalized by endocytosis and then also successfully proteolyzed, ultimately rendering paclitaxel available intracellularly. A similar autocatalytic hydrolysis mechanism would not be expected to occur with PAA because of the lack of initial and requisite endosomal proteolysis of the backbone. This hypothesis is consistent with the observation that PAA-TXL does not share the potency of PGA-TXL (48).

An alternative to this two-step, proteolytic/autoesterolytic mechanism could involve exogenous ester-dependent release of the paclitaxel from the copolymer and does not depend on endosomal processing. The EPR effect alone would afford a superior response to that observed with Taxol. However, this is unlikely to be an efficient mechanism for paclitaxel release, since negatively charged molecules are poor substrates for carboxylate esterases (65). However, it should be noted that substituents such as hydroxylamine on PGA have been reported to increase the degradation by lysosomal proteases (reviewed in Ref. 66). On the other hand, conjugation of PGA to adriamycin resulted in an inactive produg (67) possibly because of the stability of the amide linkage between the backbone and the 3-amino group of the sugar. Whether substitution of PAA or PGA, for example with paclitaxel, would similarly increase catalysis by esterases is not established; if it is increased, this could contribute to the “leakiness” of the produg before tumor localization. Furthermore, a gradual and pH-dependent release of paclitaxel from PGA-TXL in PBS has been observed. A direct comparison of solvolysis rates for PGA-TXL with those for PAA-TXL would help to establish the relative importance of these produg processing mechanisms in vivo.

Although not extensively studied, there appeared to be some effect of varying the extent of paclitaxel substitution on antitumor efficacy of PGA-TXL in the NMP-1 model (compare Tables 3 and 4); in this case, lower substitution (13%) appeared to be more efficacious and gave more long-term survival benefit.

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4 C. Li, unpublished observations.
than with the 37% formulation. Perhaps as a result of differences in substitution and accessibility to proteases or esterases, these formulations differed in their processing by either the two-step or one-step mechanisms cited above. The extent of substitution should be considered a variable to be optimized in formulation based on mechanisms of prodrug processing.

In neither of the ovarian tumor models employed in our studies should the EPR effect be profound, given the low tumor burden of these models.
burden and minimal tumor angiogenesis expected, particularly in the day 2 or day 7 HEY model. Nevertheless, copolymer delivery of paclitaxel may contribute to its slower peritoneal clearance and ability to maintain effective drug doses over longer periods of time. This might particularly be the case when the much higher-tolerated doses of PGA-TXL compared with Taxol are considered.

Previous studies, both in experimental tumor models (68) and in the clinic (69), have suggested that i.p. administration of Taxol drugs may be beneficial for targeting ovarian carcinomas confined to the peritoneum. The present study supports the adequacy of this approach and has shown that the i.p. administration of this copolymer formulation of paclitaxel is much more effective and better tolerated than conventional Taxol in these two Taxol-resistant tumor models. In this light, it will also be important to establish the relative benefits of i.p. versus i.v. delivery on copolymer uptake by i.p.-implanted tumors.

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Superior Therapeutic Profile of Poly-l-Glutamic Acid-Paclitaxel Copolymer Compared with Taxol in Xenogeneic Compartmental Models of Human Ovarian Carcinoma

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