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A phase I trial of intraperitoneal GEN-1, an IL-12 plasmid formulated with PEG-PEI-cholesterol lipopolymer, administered with pegylated liposomal doxorubicin in patients with recurrent or persistent epithelial ovarian, fallopian tube or primary peritoneal cancers: An NRG Oncology/Gynecologic Oncology Group study

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Conflicts of interest

Dr. Thaker reports grants from Merck, personal fees from Celsion, outside of the submitted work. Dr. Kathleen Moore reports personal fees from Astra Zeneca, personal fees from Tesaro, personal fees from immunogen, and personal fees from VBL Therapeutics, outside of the submitted work. Dr. Muller reports that she is involved in other ovarian cancer clinical trials with other chemotherapy and immunotherapy agents through NCI and other industry sponsors. Dr. R. Schilder reports personal fees received from Celsion. Dr. Fracasso reports that she became an employee of Bristol-Myers Squibb Company (BMS) as of May 1, 2014, and as such, she has stock with the company. Prior to her employment with BMS, she was a Professor of Medicine and Obstetrics and Gynecology at the University of Virginia where she is now affiliated as a Visiting Professor of Medicine and Obstetrics and Gynecology. This clinical study was done while was a Professor at the University of Virginia and no activities in this work have any relationship to her work at BMS. All other authors report that there are no conflicts of interest to disclose.

Abstract

Objective—The study's purpose was to assess safety and efficacy of escalating doses of weekly GEN-1 with pegylated liposomal doxorubicin (PLD) in patients with recurrent or persistent epithelial ovarian, fallopian tube or primary peritoneal cancers (EOC).

Methods—Patients had persistent or recurrent platinum-resistant EOC. The trial was a standard 3 + 3 phase I dose escalation design with patients receiving intravenous PLD 40 mg/m² (dose level 1 and 2) or 50 mg/m² (dose level 3) every 28 days and intraperitoneal GEN-1 at 24 mg/m² (dose level 1) or 36 mg/m² (dose level 2 and 3) on days 1, 8, 15, and 22 of a 28 day cycle. Cycles were repeated every 28 days until disease progression. Patients were monitored for toxicity, clinical efficacy, and evidence of systemic and intraperitoneal immunologic effect.

Results—Sixteen evaluable patients received a median of 4 cycles (range 1–8). No dose limiting toxicities were found. The adverse side effects were 4 grade 3 anemia, 2 grade 3 abdominal pain, 7 grade 3 neutropenia, and 2 grade 4 neutropenia. A clinical benefit of 57.1% (PR = 21.4%; SD = 35.7%) was found in the 14 patients with measurable disease. The highest number of partial responses (28.6%) and stable disease (57.1%) were found at dose level 3. The maximum tolerated dose was not reached. Increases in IL-12, IFN- γ , and TNF- α levels were found in peritoneal fluid following GEN-1 treatment.

Conclusions—GEN-1 in combination with PLD has encouraging clinical benefit and biological activity in recurrent or persistent EOC and warrants further investigation with escalating doses of GEN-1.

Keywords

GEN-1; IL-12 plasmid; Pegylated liposomal doxorubicin; Primary peritoneal cancer

1. Introduction

Advanced epithelial ovarian cancer (EOC) remains the most lethal gynecologic malignancy. Despite advances in surgical cytoreduction and chemotherapy, the five-year survival remains approximately 45.6% [1]. Due to EOC being an immunogenic tumor, there has been significant interest in developing immunotherapy strategies using cytokines, monoclonal antibodies, checkpoint inhibitors, immune modulators, therapeutic vaccines, adoptive T cell transfer and oncolytic viruses.

Interleukin-12 (IL-12) is a pluripotent cytokine with the ability to promote natural killer cell proliferation and activation, interferon gamma (IFN- γ) secretion, T-helper differentiation and the development of cytotoxic CD8+ T-cell responses, leading to tumor suppression [2]. Additionally, IL-12 also has anti-angiogenic properties in tumors that result in tumor death [3]. Unlike interferon alpha (IFN- α) or IFN- γ , IL-12 activates both innate and adaptive immunity as well as inhibits neo-vascularization of tumors. A phase II trial of 34 EOC patients with residual low volume disease after upfront chemotherapy was conducted to evaluate the benefit of recombinant interleukin-12 (rhIL-12) intraperitoneally (IP) weekly and had limited responses with moderate toxicity [4]. Hence novel delivery systems of IL-12 have been developed in order to overcome the toxicity of rhIL-12.

EGEN-001 (GEN-1) is a novel immunotherapeutic agent comprising a human IL-12 expressing plasmid which encodes for functional IL-12 protein and is encompassed within a synthetic polyethyleneglycol-polyethyleneimine-cholesterol (PPC) DNA delivery system to facilitate plasmid delivery *in vivo* [5,6]. GEN-1 allows for IP injection in order to produce an expanded IL-12 immune response against tumor cells at the injection site and at distant metastatic sites; this is accomplished with minimal increases in systemic circulation unlike rhIL-12 administration [5]. Preclinical studies demonstrated efficacy of inducing murine IL-12 and IFN- γ , reduction in murine ascitic vascular endothelial growth factor (VEGF) levels, and improved survival in ovarian tumor bearing mice receiving GEN-1 alone and with chemotherapy [7]. These data led to a phase I monotherapy trial evaluating 13 platinum-resistant EOC patients who were treated with IP GEN-1 at 0.6 mg/m², 3 mg/m², 12 mg/m² and 24 mg/m² weekly every 4 weeks evaluating for safety and tolerability. The most frequent adverse events (AE) were fever (69.2%) and abdominal pain (53.8%). Significant increases in IFN- γ were observed in the patient's peritoneal fluid but not in the serum. Disease responses appeared in the higher dose levels of GEN-1 and the maximum tolerated dose (MTD) was not reached [8]. Another phase I trial was conducted in platinum-sensitive EOC patients treated with intravenous (IV) carboplatin and docetaxel with escalating doses of IP GEN-1: 12 mg/m², 18 mg/m², or 24 mg/m² $\times$ 4 doses or 24 mg/m² $\times$ 6–8 doses. Similar AEs and dose dependent rises in IFN- γ concentrations were seen. No dose-limiting toxicities (DLT) were seen and the MTD was not reached. The addition of GEN-1 to chemotherapy did not attenuate the efficacy or exacerbate the side effects [9].

Given the previous clinical trial findings as well as the MTD not being reached, we sought to evaluate the toxicity profile and anti-tumor activity of weekly IP GEN-1 in platinum-resistant EOC patients receiving pegylated liposomal doxorubicin (PLD). PLD was chosen as the chemotherapy due to its known 8–20% response rate as monotherapy in platinum-resistant EOC patients and would allow for four weekly treatments of GEN-1 per cycle [10–12]. Moreover, doxorubicin is known to be an inducer of immunogenic cell death, which can lead to cross-presentation of tumor antigens [13].

2. Methods

2.1. Eligibility criteria

Patients were required to have recurrent or persistent EOC and could have a CA-125 recurrence or radiographically measurable or non-measurable disease per RECIST 1.1 [14]. Patients were required to have received at least one prior platinum-based chemotherapy regimen and could have received up to two additional cytotoxic regimens with no >1 non platinum, non-taxane regimen. Patients who received only one prior platinum regimen must have had progressive disease during primary treatment, had persistent disease at the completion of primary treatment, or had a platinum-free interval of <12 months. Patients could not have had a previous treatment with PLD or other anthracyclines.

Patients had a Gynecologic Oncology Group (GOG) performance status of 0–1 and adequate organ function [absolute neutrophil count 1500/ μ l, platelets 100,000/ μ l, creatinine to 1.5 $\times$ institutional upper limit normal (ULN) or calculated creatinine clearance 50 ml/min, bilirubin 1.5 $\times$ ULN, SGOT/SGPT 3 $\times$ ULN, alkaline phosphatase 2.5 $\times$ ULN and

neuropathy (sensory or motor less than or equal to CTCAE grade 1)]. Additionally, left ventricular ejection fraction had to be to the institutional lower level of normal as determined by a multigated acquisition scan or echocardiogram.

Patients had to be 18 years and without secondary malignancies. Patients who had received previous radiation or chemotherapy for non-gynecologic cancers within the last three years, with serious uncontrolled medical conditions, or abdominal surgery except IP port placement within 30 days of enrollment were excluded from the study.

Institutional Review Board and Institutional Biosafety Committee approvals were obtained at each institution and all eligible patients signed an informed consent before study entry in compliance with institutional, state, and federal regulations.

2.2. Treatment plan

Eligible patients had an IP catheter placed according to GOG guidelines (<https://gogmember.gog.org/manuals/pdf/surgman.pdf>) greater than one week prior to therapy initiation. PLD was administered IV at 40 mg/m² for dose levels (DLs) 1 and 2 and 50 mg/m² for DL 3 every 28 days with concurrent GEN-1 IP at 24 mg/m² for DL 1 and 36 mg/m² for DLs 2 and 3. After completion of PLD, there was an interval of at least 30 min to 3 h prior to administering IP GEN-1 when the catheter was flushed for patency. GEN-1 was reconstituted to achieve a dose of 24 mg/m² or 36 mg/m² and infused *via* gravity through the IP catheter as quickly as possible within 2 h. The IP catheter was flushed after GEN-1 administration. The GEN-1 treatment was given on an outpatient basis weekly (four weeks = 1 cycle). Patients were not allowed to receive white blood cell growth factors unless approved by the study chair. Patients were allowed to use acetaminophen or ibuprofen as needed for fever or pain. Patients continued until disease progression, AEs prohibited further therapy, or elected to withdraw from the study.

2.3. Evaluation of toxicity and treatment modification

Patients underwent weekly CBC evaluations and toxicity assessments. Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0). Dose-limiting toxicities during the first cycle only included the following: dose delay > three weeks due to failure to recover counts, febrile neutropenia, grade 4 neutropenia > 7 days, grade 4 thrombocytopenia, grade 3 thrombocytopenia with clinically significant bleeding, study-related grade 3 or 4 non-hematological toxicity (excluding grade 3 anorexia, fatigue, nausea or vomiting or diarrhea (unless >48 h despite maximal medical management), dehydration as a result of nausea and vomiting, constipation, PLD hypersensitivity reactions, metabolic abnormalities (that recover to grade 2 or less within 48 h with or without medical management), abdominal pain 72 h despite medical management, peritonitis, or cytokine release syndrome), and any drug-related death. Attribution to treatment or other causes was assigned to AEs by investigators. Subsequent cycles of combination therapy were not given until ANC 1500/μl and platelet count 100,000/μl, and all non-hematologic toxicity had recovered to grade 1.

PLD dose modifications for hematologic toxicity (ANC or platelets), hand-foot syndrome, stomatitis and impaired hepatic function were in the protocol. GEN-1 was delayed 2 weeks

if warranted due to drug related AEs. However, delays in GEN-1 dosing did not delay or shift timing of PLD. In the event of grade 3 or 4 toxicities were attributed to GEN-1, subsequent doses were delayed until recovery to grade 1. Patients who required dose delays of >2 weeks or required >2 such delays at a given dose had GEN-1 reduced by one DL and there was no dose reescalation. Patients who required >2 GEN-1 dose reductions due to drug-related toxicity were discontinued from the study. Patients who received the lowest dose of GEN-1 (18 mg/m² = DL-1) and required further dose reduction had therapy discontinued. Specific dose modifications of GEN-1 for nausea, vomiting and diarrhea or abdominal pain were in the protocol.

2.4. Assessment for clinical efficacy

Radiographic imaging (CT scan or MRI) was obtained within 28 days of initial treatment and then every other cycle for assessment of response in all patients regardless whether they had measurable or non-measurable disease. Response was evaluated by comparing pre- and post-radiographic imaging of target and non-target lesions using RECIST 1.1 guidelines [14]. Patients who received at least 1 cycle of therapy and had their disease re-evaluated were considered evaluable for response. Patients with global clinical deterioration requiring discontinuation of treatment without objective evidence of disease progression at the time were reported as symptomatic deterioration. Elevated CA-125 levels required normalization for a patient with resolution of measurable disease to be considered to have had a complete response. The best overall response was the best response recorded from treatment initiation until disease progression/recurrence.

2.5. Translational research

Blood and peritoneal fluid (either by lavage or ascites) samples were collected just before the first GEN-1 treatment and on days 2 and 4 and then before the second GEN-1 treatment and on days 9, 11, and 15. A total of seven blood and peritoneal fluid samples from each patient was planned. The samples were immediately stored in a -80 °C freezer until analysis. IL-12, IFN- γ , TNF- α and VEGF concentrations in plasma and peritoneal fluid samples were determined using commercially available ELISA kits per manufacturer protocol at the Immune Analysis Facility in the Center for Immunotherapy at Roswell Park Cancer Institute. The human IL-12 and TNF- γ assay kits were purchased from R&D Systems (Minneapolis, MN) and human IFN- γ and VEGF kits were purchased from eBioScience (San Diego, CA). After duplicate assay completion, plates were read in a microplate reader and cytokine concentration in the sample was determined with the standard curve. The cytokine data was expressed over 100 mg total protein, which was quantified with a Pierce BCA protein assay kit (Rockford, IL). There were several peritoneal wash samples that were too dilute and hence low in total protein. Samples with total protein <1 mg/ml were not considered adequate to include in data analysis. The average pre-treatment plasma or peritoneal cytokine levels from all evaluable patients were calculated for IL-12, IFN- γ , TNF- α and VEGF compared with peak post-treatment levels for each patient to calculate the treatment related fold changes. Patients with at least one post-treatment sample were included in the data analysis.

2.6. Statistical design

This study utilized a 3 + 3 design to identify the MTD of GEN-1 with PLD [15]. Dose escalation for each cohort of three patients continued until a DLT was observed during the first cycle of therapy (28 days). If one patient out of three experienced a DLT, an additional three patients were enrolled at that DL. The MTD was estimated by the maximum dose level at which <2 patient (among 6) experienced a DLT. The translational data was tabulated as Mean $\pm$ SEM.

3. Results

3.1. Patient characteristics

Sixteen eligible patients enrolled from September 2012 to July 2014. Four patients were not evaluable for DLT. Two patients (one each in DL 1 and 2) did not complete a full cycle of PLD and GEN-1 due to clinical deterioration, and two patients (one each in DL 2 and 3) voluntarily withdrew from study prior to completion of the first cycle. Characteristics for treated patients are listed in Table 1. The median age was 64 years (range 51–76). All patients were Caucasian and had serous histology; the majority had a GOG performance status of 0 and measurable disease (14 of the 16). A total of 63 cycles of the combination were administered to patients with a median of 4 cycles (range 1–8).

3.2. Adverse events and DLT

Twelve patients were evaluable for DLT. Three patients were initially treated in DL 1 (PLD 40 mg/m² q28 days and weekly IP GEN-1 24 mg/m²). One was not evaluable for DLT due to symptomatic deterioration prior to receiving Day 15 of GEN-1 and was replaced. There were no DLTs in the three evaluable patients at DL 1. Three patients were enrolled in DL 2 (PLD 40 mg/m² q 28 days and weekly IP GEN-1 36 mg/m²), two of which were not evaluable for DLT due to rapid clinical progression in one and voluntarily withdrawal of another due to abdominal pain after receiving cycle 1 of PLD and Day 1 of GEN-1 only. These two patients were replaced and there were no DLTs in three evaluable patients at this dose level. Three patients were then enrolled in DL 3 (PLD 50 mg/m² q 28 days and weekly IP GEN-1 36 mg/m²). One patient in this cohort voluntarily withdrew due to grade 1 abdominal pain, nausea and vomiting, and malaise six days after receiving PLD and Day 1 of GEN-1 and was replaced. There were no DLTs in the 3 evaluable patients, and as per protocol, DL 3 cohort was expanded for an additional 3 patients. None of the 6 evaluable patients at DL 3 had DLTs. No DLTs were noted in any of the dose levels proposed in this study.

Grade 3 or higher adverse events (AEs) considered at least possibly related to study treatment are tabulated by dose level in Table 2. The most frequently experienced non-hematologic AEs across all grades (data not shown) were fatigue (81%), constipation (63%), nausea (63%), abdominal pain (63%), oral mucositis (56%), anorexia (56%), diarrhea (44%), vomiting (38%), headache (38%), fever (44%), chills (31%), and peripheral sensory neuropathy (31%). The majority of the clinical AEs were grade 1/2 in nature, transient and medically manageable. In DL 3, two patients had grade 1 and another had grade 2 palmar-plantar erythrodysesthesia; two patients experienced grade 3 anemia and grade 3 oral

mucositis. The most frequently experienced hematologic AEs across all grades (data not shown) were leukopenia (88%), anemia (88%), neutropenia (81%) and thrombocytopenia (50%). Six patients (38%) had grade 3 leukopenia. Seven patients (44%) had grade 3 neutropenia and two patients (13%) had grade 4 neutropenia. Five of the seven patients with severe neutropenia were treated in DL 3 with 50 mg/m² PLD. Three patients required pegfilgrastim. One patient on DL 3 received 6 cycles of therapy with pegfilgrastim support starting at cycle 2 and could not receive cycle 7 due to neutropenia >3 weeks. There were no dose reductions in either PLD or GEN-1. There was one patient who had her IP port removed due to access issues and induration around the port after cycle 3 Day 15; the port could not be replaced causing the patient to come off of study.

3.3. Clinical responses

Two patients of the cohort were unevaluable for response since they did not have measurable disease leaving fourteen patients evaluable for response (Table 3). Both measurable patients in DL 1 had progressive disease (PD). In DL 2, one patient had a partial response (PR); another had stable disease (SD); one had progression while 2 patients were indeterminate due to the fact they did not have post-baseline imaging. In DL 3, two patients had a PR and four patients had SD while one patient was indeterminate. CA-125 also rose or declined according to disease status and was an accurate marker for patients receiving IP GEN-1. For DL 3, the clinical benefit rate was 86% (Table 3). Both progression-free (PFS) and overall survival (OS) are depicted in Fig. 1. Seven patients had a PFS of ≥ 6 months, and none of these patients had gone off of study and onto other therapies before six months. The duration of response for the 3 responders was 6.7, 10.0, and 10.3 months respectively, and the last patient had to be censored since she did not progress on follow-up. The median PFS was 4.7 (1.8, 8.0) months, and the median (95% CI) OS was 11.1 (6.6, >26) months.

3.4. Immunologic effects

Blood and peritoneal fluid/wash were collected at various time points from 13 patients. In two of the patients post-treatment peritoneal samples could not be collected or were too dilute (<1 mg total protein/ml fluid) for a meaningful analysis. Therefore, the translational results are based on 11 patients. From 11 patients, a total of 77 blood and peritoneal fluid samples were to be collected. Out of those 77 peritoneal fluid samples, 22 samples could not be collected due to technical difficulties or patient discomfort and 17 samples were too dilute for a meaningful analysis; therefore, 39 (51%) samples were evaluable for translational work. Five out of 11 patients had enough pre-treatment samples for IL-12 and IFN- γ assays and four out of 11 patients had enough samples for TNF- α and VEGF assays. All planned plasma samples (n = 77) were collected and analyzed for cytokine levels. The average pre-treatment and peak post-treatment values of IL-12, IFN- γ , TNF- α and VEGF in the evaluable peritoneal fluid or plasma samples are provided in Fig. 2A. The peak post-treatment value for IL-12, IFN- γ or TNF- α in the peritoneal fluid of every evaluable patient was higher than the overall pre-treatment study average and also in every paired pre-treatment and post-treatment comparison where available (five out of 11 pairs each for IL-12 and IFN- γ and 4 out of eleven pairs for TNF- α and VEGF).

The increases in IL-12, IFN- γ and TNF- α levels in peritoneal fluid samples from 10 of 11 evaluable patients were much higher than those in the paired corresponding plasma samples. The IL-12 levels before treatment were undetectable but increased to a peak average value of 65 ± 46.3 pg per 100 mg protein (range: 0.0–91.8, $n = 11$) after GEN-1 treatment. The IFN- γ and TNF- α levels before GEN-1 treatment were 4.84 ± 4.0 pg (range: 0.0–20.9, $n = 5$) and 8.24 ± 2.1 pg per 100 mg protein (3.8–13.7, $n = 5$), respectively. After GEN-1 treatment the IFN- γ and TNF- α levels increased by 46-fold and 13-fold respectively. There was a slight decrease (22%) in average VEGF values following GEN-1 treatment. However, the changes in cytokine levels in plasma were much smaller than in peritoneal fluid following GEN-1 treatment confirming the local effects of this novel therapy (Fig. 2B). The IL-12 levels in pre- and post-treatment plasma samples were undetected and were <1 pg per 100 mg protein respectively. The average fold increases in plasma IFN- γ , TNF- α , and VEGF were 1.68, 1.54, and 1.65, respectively.

The treatment related increases in peritoneal IL-12 lasted for several days after a single GEN-1 treatment. The levels reached peak values within 4 days and stayed above baseline 7 days after treatment. The time course data is averaged from 3 patients where all intended collection time points were evaluable (Supplemental Figure 1). The data plot of peritoneal IL-12 levels by tumor responses show highest average IL-12 levels in the PD group as compared to PR or PR + SD group (Fig. 3A). However, the individual values were highly variable as 0.00 pg, 4.32 pg, and 521 pg (PD); 4.26 pg, 4.92 pg and 48.78 pg (PR); and 0.37 pg, 10.58 pg, 23.1, and 91.83 pg (SD). The treatment-related trends in plasma IL-12 levels exhibited similar pattern although the absolute levels in plasma were much lower (Fig. 3B). The subject with highest IL-12 level in peritoneal fluid (521 pg) also had the highest IL-12 level in plasma (2.5 pg/100 mg of protein), which shows that the treatment-related changes in blood are consistent with those in the peritoneal cavity but at a much smaller scale. The treatment-related increases in IFN- γ and TNF- α in peritoneal fluid were highest in PR group and followed a similar trend in plasma but at a smaller scale (Fig. 3C). The treatment-related fold changes in VEGF levels were found to be small and in opposite trend to that of the cytokine data. For example, in PD group the post-treatment peritoneal and plasma VEGF levels increased by 1.7 fold and 2.6 fold, respectively (Fig. 3C) but decreased by ~50% in PR group. A modest increase in plasma VEGF post GEN-1 therapy is similar in magnitude of the increase seen with the use of anti-VEGF antibodies or tyrosine kinase inhibitors in cancer patients [16,17]. The post-treatment VEGF changes in our study are based on small number of patients, but are consistent with anti-angiogenic properties of IL-12 [3]. Although the mechanisms underlying increased VEGF levels following anti-angiogenic therapy are unclear, several mechanisms have been proposed and include the following: increased VEGF production by tumor, relocalization from tissue to circulation, or host-blockade of VEGF clearance [17–19].

4. Discussion

There have been two studies evaluating GEN-1 in the platinum-resistant EOC patient population. The first trial was a phase I monotherapy study of IP GEN-1 ranging from 0.6 mg/m² to 24 mg/m² weekly for a month [8]. The MTD was not reached in this study. Four patients (31%) had stable disease and nine (69%) had progressive disease five weeks after

the last dose. The translational studies revealed that there were detectable levels of IL-12-expressing plasmid DNA in the peritoneal fluid after administration of GEN-1. Additionally, significant increases in IFN- γ levels, critical in the induction of IL-12, were found in the peritoneal fluid of these patients.

A GOG phase II monotherapy trial of GEN-1 24 mg/m² weekly for four weeks had twenty evaluable patients, and the most frequent AEs related to GEN-1 were grade 1/2 fatigue, fever, chills, abdominal pain, nausea, vomiting, and myelosuppression [20]. Three of the twenty patients voluntarily withdrew from study due to treatment-related toxicities of grade 1 abdominal pain after cycle 1, grade 1 neuropathy and dyspnea after cycle 3, and grade 1 anemia and leukopenia during cycle 2. No patients had a partial or complete response. Thirty-five percent of the evaluable patients had stable disease. The authors concluded that there was limited activity as a monotherapy in platinum resistant EOC patients, but acknowledged that since 30% of patients received 1 cycle, the short duration of treatment may have limited the ability to assess tumor activity of GEN-1.

PLD was chosen for this trial due to the beneficial effects of it in combination with IL-12 or other immunomodulatory cytokines in several tumor models [21–24]. Zhu and colleagues reported that doxorubicin enhanced the accumulation of IL-12 induced IFN- γ in both squamous and adenocarcinomas *in vitro* and *in vivo* [21]. They also found that only when IL-12 was localized in tumors not systemically in the host was signal transducer and activator of transcription 1 (STAT1) deficiency-associated with maximum therapeutic efficacy obtained by IL-12 gene therapy [21,25]. Coukos and associates showed that ovarian cancer cells ID8 surviving PLD therapy upregulated surface molecules that are critical for immune recognition, attached to MHC class I and Fas receptor through an unknown mechanism, and exhibited increased sensitivity to killing by cytotoxic lymphocytes and to apoptosis mediated by Fas *in vitro* [23]. Hence, in addition to direct tumor killing and the immunization effect derived by immunogenic cell death, PLD exerts an important immunomodulatory effect upon the tumor fraction surviving drug exposure and this effect does not appear to be apoptotic-driven like other anthracyclines. Doxorubicin can also modulate macrophages and natural killer cells of the peritoneal cavity and impairs immune-suppressing cells [26–30]. In randomized phase III clinical trials of single agent PLD in platinum resistant EOC patients the clinical benefit rate was 40–45.3%, but in our trial this rate was 57.1% indicating the potentially synergistic benefit of adding GEN-1 to PLD [31,32].

The translational data from this study provides evidence for immunological activity of IP GEN-1 in EOC patients. The production of human IL-12 following GEN-1 treatment indicates that the IL-12 expressing plasmid is taken up by cells of the peritoneal cavity or ascites [7]. The IL-12 increase was predominantly in peritoneal cavity while only minuscule amounts (<1 pg/100 mg protein) were detected in plasma. The stimulation of IL-12 levels in evaluable patients lasted for several days after a single injection which is consistent with the IL-12 transfection time course observed in an EOC mouse model [7]. Persistent local delivery of IL-12 at tumor site with little distribution to systemic circulation is novel and has distinct pharmacokinetic advantages over bolus rhIL-12 therapy. IL-12 is considered more efficacious when delivered in persistent low levels at tumor site [33]. Significant increases in

IFN- γ and TNF- α levels following GEN-1 treatment demonstrates activation of immune pathways that are downstream to IL-12 production and are known to mediate IL-12 response to cancer. As observed with IL-12, the increases in IFN- γ and TNF- α levels were mainly in the peritoneal cavity with little changes in the blood. In comparison to IL-12, IFN- γ and TNF- α , the changes in VEGF levels were much smaller and in the opposite direction.

Since about half of peritoneal fluid samples could not be collected due to poor ascites volume, patient discomfort, or were too dilute for a meaningful analysis, the translational results are from a small sample size. The comparisons of study averages, for example between pre-treatment and post-treatment values, have lesser variability due to relatively larger number of patients ($n = 11$) whereas averages from subgroups analyses, such as for dose comparison have higher variability as result of smaller number of patients ($n = 3$). Nonetheless, interesting trends were observed from limited analysis and warrants future studies. For example, the average fold-increases in peritoneal and plasma levels of IFN- γ , a central mediator of IL-12 actions, was relatively higher in patients with tumor responses than in patients with progressive disease, which is consistent with the notion that increases in IFN- γ levels is important for anticancer activity. In one of the patients the increase in post-treatment IL-12 levels in peritoneal fluid and plasma was the highest among all the patients studied but no increases in IFN- γ in the evaluable samples could be detected. This may be indicative of a potential impairment in the downstream signaling mechanism of IL-12 in that patient [34–36]. Due to a limited number of paired pre- and post-treatment samples and inconsistency in the peritoneal fluid density from patient to patient the cytokine data should be interpreted with caution. Future evaluation of translational parameters would be useful in improving our understanding of the GEN-1 mechanism of action and the underlying immunological changes that may be relevant to designing better treatment approaches for EOC. In an ongoing neoadjuvant study of GEN-1 in combination with the first line chemotherapy (NCT02480374), primary tumor samples collected at laparoscopy and interval debulking are being collected for a comprehensive immunological evaluation [37].

In conclusion, we found that monthly PLD at 50 mg/m² and weekly GEN-1 at 36 mg/m², which is the highest dose of GEN-1 to date tested in a patient population, was overall tolerable with five of the seven patients receiving four or more courses of therapy. More importantly, the clinical benefit of 86% in this cohort is an encouraging result that warrants further development of PLD with escalating doses of GEN-1 in this difficult to treat patient population. The translational data from this study demonstrated that GEN-1 treatment is associated with the activation of the immune system that may be related to its clinical efficacy. The translational data also shows intriguing trends between cytokine changes and tumor responses but due to small sample size additional studies are warranted. An ongoing phase I trial is utilizing escalating doses of IP GEN-1 36 mg/m² to 79 mg/m² concurrently with IV weekly paclitaxel and every three week carboplatin in neoadjuvant EOC patients (NCT02480374). Since this will be the first trial of GEN-1 in newly diagnosed EOC patients, untreated and post GEN-1 treated tumors will be available for a comprehensive translational evaluation [37].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Recurrent EOC patients were treated with GEN-1 and pegylated liposomal doxorubicin.
- Three had partial responses and five had stable disease of the 16 patients.
- IL-12 expression and downstream immunological effect were found.
- Maximum tolerated dose of GEN-1 was not achieved.

Fig. 1. Kaplan-Meier plots of (A) progression-free (PFS) and (B) overall survival (OS). The median PFS was 4.7 (1.8, 8.0) months and the median OS was 11.1 (6.6, >26) months.

Fig. 2.

A. Cytokine levels in peritoneal fluid or peritoneal washes before and after GEN-1 treatment. The pre-treatment values (Mean $\pm$ SEM) are from samples collected before the first weekly GEN-1 treatment (Day-1) and on Day 2, Day-4, and Day-8 after the first and second GEN-1 treatments. B: cytokine levels in plasma before and after GEN-1 treatment. The pre-treatment values (Mean $\pm$ SEM) are from samples collected before the first weekly GEN-1 treatment (Day-1) and on Day 2, Day-4, and Day-8 after the first and second GEN-1 treatments.

Fig. 3.

Peak IL-12 levels in peritoneal fluid (A) or plasma (B) samples after GEN-1 treatment plotted by tumor response. (C) Cytokine levels of IFN- γ , TNF- α , and VEGF in peritoneal fluid and plasma samples after GEN-1 treatment are plotted by tumor response. The values are Mean $\pm$ SEM, with n = 11 for all evaluable study patients (All), n = 3 each for patients with progressive disease (PD) and partial response (PR) and n = 7 for patients with partial response + stable disease (PR + SD). IL-12 levels were not detectable in pre-treatment samples. (C) Cytokine levels of IFN-g, TNF-a, and VEGF in peritoneal fluid and plasma are.

Table 1

Baseline characteristics – all eligible and treated patients.

Total	N = 16 (%)
Age group (years)	
50–59	6 (38)
60–69	7 (44)
70–79	3 (19)
Ethnicity	
Hispanic or Latino	1 (6)
Non-Hispanic	14 (88)
Unknown/not specified	1 (6)
Race	
Caucasian	16 (100)
Performance status	
0	9 (56)
1	7 (44)
Histology	
Serous adenocarcinoma	16 (100)
Measurable disease	
No	2 (13)
Yes	14 (88)
Platinum status	
Platinum resistant	15 (94)
Platinum sensitive	1 (6)
Number of prior chemotherapy regimens	
1	8 (50)
2	4 (25)
3	4 (25)

Table 2

Grade 3 or higher adverse events at least “possibly” related to GEN-1 treatment.

System organ class/term	Dose level/adverse event grade							
	EGEN-001 24 mg/m ² IP weekly + PLD 40 mg/m ² IV Day 1 (N = 4)		EGEN-001 36 mg/m ² IP weekly + PLD 40 mg/m ² IV Day 1 (N = 5)		EGEN-001 36 mg/m ² IP weekly + PLD 40 mg/m ² IV Day 1 (N = 7)		EGEN-001 36 mg/m ² IP weekly + PLD 50 mg/m ² IV Day 1 (N = 7)	
	3	4	3	4	3	4	3	4
Blood/lymphatics	1	0	1	0	2	0	0	0
Anemia	1	0	1	0	2	0	0	0
Gastrointestinal	0	0	1	0	3	0	0	0
Abdominal pain	0	0	1	0	1	0	0	0
Mucositis oral	0	0	0	0	2	0	0	0
Infections/infestations	0	0	0	0	1	0	0	0
Urinary tract infection	0	0	0	0	1	0	0	0
Investigations	2	0	1	0	4	1	1	1
Neutrophil count decreased	2	0	1	0	4	1	1	1
Platelet count decreased	0	0	1	0	0	0	0	0
White blood cell decreased	1	0	1	0	4	0	0	0
Metabolism/nutrition	1	0	0	0	1	0	0	0
Hyperglycemia	1	0	0	0	0	0	0	0
Hypokalemia	0	0	0	0	1	0	1	0

Table 3

Objective tumor response – in measurable disease patients.

Dose level		GEN-1 24 mg/m ² IP weekly + PLD 40 mg/m ² IV	GEN-1 36 mg/m ² IP weekly + PLD 40 mg/m ² IV	GEN-1 36 mg/m ² IP weekly + PLD 50 mg/m ² IV
All	GEN-1 24 mg/m ² IP weekly + PLD 40 mg/m ² IV	GEN-1 36 mg/m ² IP weekly + PLD 40 mg/m ² IV	GEN-1 36 mg/m ² IP weekly + PLD 40 mg/m ² IV	GEN-1 36 mg/m ² IP weekly + PLD 50 mg/m ² IV
n (%)	Day 1 n(%)	Day 1 n(%)	Day 1 n(%)	Day 1 n(%)
Total	14	2	5	7
<i>Objective tumor response</i>				
Partial response	3 (21)	0 (0)	1 (20)	2 (29)
Stable	5 (36)	0 (0)	1 (20)	4 (57)
Progressive disease	3 (21)	2 (100)	1 (20)	0 (0)
Indeterminate	3 (21)	0 (0)	2 (40)	1 (14)