Chemoimmunotherapy Using Pegylated Liposomal
Doxorubicin and Interleukin-18 in Recurrent Ovarian Cancer:
A Phase I Dose-Escalation Study

Fiona Simpkins1, Aurea Flores1, Christina Chu2,3, Jonathan S. Berek4, Joseph Lucci III5, Sharon Murray5, John Bauman6, Herbert Struemper6, Fiona Germaschewski5, Zdenka Jonak5, Olivia Gardner5, John Toso5, and George Coukos2,3

Center, Stanford University School of Medicine, Stanford, California; and
Cancer Research Center, 3Division of Gynecology Oncology, University
of Pennsylvania, Philadelphia, Pennsylvania; 4Stanford Women’s Cancer
Research Online (http://cancerimmunolres.aacrjournals.org/).

Abstract
Recombinant interleukin (IL)-18 (SB-485232) is an immunostimulatory cytokine, with shown antitumor activity in combination with pegylated liposomal doxorubicin (PLD) in preclinical models. This phase I study evaluated the safety, tolerability, and biologic activity of SB-485232 administered in combination with PLD in subjects with recurrent ovarian cancer. The protocol comprised four cycles of PLD (40 mg/m2) on day 1 every 28 days, in combination with SB-485232 at increasing doses (1, 3, 10, 30, and 100 μg/kg) on days 2 and 9 of each cycle, to be administered over five subject cohorts, followed by discretionary PLD monotherapy. Sixteen subjects were enrolled. One subject withdrew due to PLD hypersensitivity. Most subjects (82%) were platinum-resistant or refractory, and had received a median of three or more prior chemotherapy regimens. SB-485232 up to 100 μg/kg with PLD had an acceptable safety profile. Common drug-related adverse events were grade 1 or 2 (no grade 4 or 5 adverse events). Concomitant PLD administration did not attenuate the biologic activity of IL-18, with maximal SB-485232 biologic activity already observed at 3 μg/kg. Ten of 16 enrolled subjects (63%) completed treatment, whereas five (31%) subjects progressed on treatment. A 6% partial objective response rate and a 38% stable disease rate were observed. We provide pilot data suggesting that SB-485232 at the 3 μg/kg dose level in combination with PLD is safe and biologically active. This combination warrants further study in a phase II trial. Cancer Immunol Res; 1(3): 168–78. © 2013 AACR.

Introduction
Epithelial ovarian and primary peritoneal cancers carry the highest fatality to case ratio among all gynecologic malignancies and are the fifth most common cause of cancer-related mortality in women in the United States (1). Unfortunately, approximately 75% of cases are diagnosed at advanced stages (stage III or IV), and up to 70% of these patients will experience recurrence following initial therapy (2). Multiple, single-agent cytotoxic chemotherapeutics have shown a clinical benefit as second-line treatment of platinum-resistant ovarian cancer (2). Pegylated liposomal doxorubicin (PLD) was shown to have a 14% to 20% response rate as monotherapy in platinum resistant ovarian cancer (3, 4) and has received U.S. Food and Drug Administration (FDA) approval for this indication. There is evidence that host antitumor cell-mediated immune mechanisms play a role in controlling malignant progression of ovarian carcinoma (5–8). In subjects with advanced epithelial ovarian cancer, the absence of intraepithelial tumor-infiltrating T lymphocytes (TIL) in fresh primary tumors was associated with shorter progression-free survival (PFS) and overall survival (OS). The 5-year OS rate was 38% among subjects whose tumors contained intraepithelial TIL and 4.5% among subjects whose tumors contained no intraepithelial TIL (9). A recent meta-analysis of 10 studies comprising more than 1,800 patients has confirmed the significant positive association between the presence of intraepithelial TILs and prolonged survival in ovarian cancer (10). Although the ovarian cancer microenvironment is quite immunosuppressive, in many tumors the presence of intraepithelial TILs was associated with evidence of TIL activation, including increased tumor expression of IFN-γ (9). This suggests that activation of immune effector cells could produce clinical benefit in this patient population.

One therapeutic strategy that holds promise is to combine immunostimulatory drugs with standard of care cytotoxic chemotherapy. Ideally, a positive interaction is created if the cytotoxic agent sensitizes tumor cells to immune-mediated

Authors' Affiliations: 1Division of Gynecology Oncology, University of Miami Leonard M. Miller School of Medicine, Miami, Florida; 2Ovarian Cancer Research Center, 3Division of Gynecology Oncology, University of Pennsylvania, Philadelphia, Pennsylvania; 4Stanford Women’s Cancer Center, Stanford University School of Medicine, Stanford, California; and 5GlaxoSmithKline, Inc., Research Triangle Park, North Carolina

Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

Corresponding Authors: Fiona Simpkins, Division of Gynecology Oncology, University of Miami Leonard M. Miller School of Medicine, 1475 NW 12th Avenue, Suite 3500, Miami, FL 33136. Phone: 305-243-2239; Fax: 305-243-9038; E-mail: fsimpkins@med.miami.edu; and John Toso, John.F.Toso@gsk.com

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killing such that immune effector cells can target and eliminate
tumor cells that would otherwise survive the chemotherapy
insult. The interaction could be even more positive if the
cytotoxic drug exerted independent positive immunomodula-
tory effects. However, the cytotoxic agent of choice should not
deplete or inactivate effector immune cells activated by the
immunostimulatory agent (11). In vitro and clinical studies
have shown synergistic activity of IFN-γ with platinum com-
pounds (12–14). In a randomized phase III study of subjects
with previously untreated stage IC through stage IIIC epithelial
ovarian cancer, who received first-line therapy consisting of
cisplatin and cyclophosphamide with or without IFN-γ, the 3-
year PFS was significantly improved in those women who were
also receiving IFN-γ (15). However, another phase III trial
testing IFN-γ in combination with carboplatin and paclitaxel
failed to show any benefit with the addition of IFN-γ. In this
study, IFN-γ was administered weekly instead of every other
week, which was associated with increased toxicity and a
higher rate of patients unable to complete six cycles of che-
moimmunotherapy (16). Furthermore, adverse biologic inter-
actions might have also accounted for the lack of benefit in
the combination with paclitaxel, as the accompanying steroids
can suppress effector T-cell function and induce regulatory
T cell (Treg) activation (17–19). The choice of chemotherapy
and immunostimulatory drugs is therefore critical in these
combinations.

Doxorubicin has interesting immunomodulatory properties.
Although many chemotherapy drugs induce immunologically
silent apoptosis, doxorubicin kills tumor cells by immunogenic
apoptosis, that is, apoptosis that elicits an antitumor immune
response (20). This is mediated by calreticulin exposure on the
surface of dying cells, which facilitates tumor cell phagocytosis
by dendritic cells and tumor antigen presentation (21). Doxo-
rubicin-killed tumor cells recruit intratumoral CD11c+
CD11b+Ly6Chi myeloid cells, which efficiently engulf tumor
antigens and present them to T lymphocytes, thus inducing in
situ vaccination (22). As a result, doxorubicin can enhance the
efficacy of tumor vaccines in mouse models (23, 24), and has
been shown to synergize with immunostimulatory cytokines,
such as interleukin (IL)-2, IL-12, or TNF-α (25–27). PLD is a
unique formulation of doxorubicin, in which a water-soluble
polyethylene glycol layer surrounds a doxorubicin-containing
liposome. This formulation minimizes hematopoietic side
effects and could be optimal for chemotherapy/immunotherapy
combinations. In a mouse model of ovarian cancer, we reported
that tumor cells surviving the direct toxicity of PLD, upregu-
lated surface expression of MHC class I molecules and the
death receptor Fas and became susceptible to immune attack,
enhancing recognition and killing by activated T and natural
killer (NK) cells (28).

IL-18 is an immunostimulatory cytokine known to induce the
production of T-helper cell (Th1)-type cytokines and chemokines
such as IFN-γ and CXCL10. IL-18 enhances cellular immunity by activating key immune effector cells such as
NK cells and T lymphocytes, and increases the infiltration of
these cell types in tumors in preclinical models (29–31). IL-18
also promotes the differentiation of CD4+ T lymphocytes into
Th1 cells and induces the generation of memory cytotoxic
CD8+ T lymphocytes. In addition, IL-18 upregulates Fas ligand
(FasL) expression on NK and T cells, which may enhance
antitumor activity (27–29, 32–34). Using a mouse model of
ovarian cancer, we previously showed that IL-18 in combina-
tion with PLD resulted in synergistic antitumor activity.
Although IL-18 or PLD monotherapy had a moderate antitu-
mor effect, in combination, they significantly restricted tumor
growth, augmented OS rate, and generated long-term protect-
tive immunity (28). Therefore, we hypothesized that the anti-
tumor activity of PLD can be enhanced by IL-18 in patients
with ovarian cancer. IL-18 has been evaluated as monother-
apy in phase I/II studies in patients with cancer with
advanced solid tumors and lymphomas, and was found to be
biologically active and well-tolerated without reaching a
maximum-tolerated dose (MTD) even at 1,000 μg/kg (35, 36).
The biologic effects of IL-18 included transient lymphopenia,
increased activation of NK and CD8+ T cells, and increased
Th1 cytokines (IFN-γ) in blood. Similar pharmacokinetic and
pharmacodynamic effects are seen with daily dosing for 5
consecutive days compared with weekly dosing (37). These
studies defined the biologically active dose range and sche-
dule for IL-18 and provided the rationale for combining IL-18
with PLD (37).

To combine IL-18 with PLD, we made several dose and
schedule decisions. PLD is FDA approved and recommended
as a single agent for recurrent ovarian cancer at a dose of 50
mg/m2 i.v. every 4 weeks for a minimum of four cycles as per
the manufacturer’s recommendation. Several prospective nonran-
domized trials have shown 40 mg/m2 every 28 days to be
equally effective as 50 mg/m2, but with lower toxicities (skin
toxicity and mucositis; ref. 38). Thus, to minimize toxicity, PLD
was given at a dose of 40 mg/m2, at the recommended
frequency of every 28 days, for a minimum of four cycles.
Because of the concern for toxicity, four cycles of PLD plus IL-
18 were initially studied. Because the primary objective of this
study was to access the safety and tolerability of IL-18 in
combination with PLD, we felt that the standard four cycles
of PLD gave sufficient time to assess feasibility and safety of
the combination with IL-18. Patients were able to continue PLD
after four cycles, if there was a clinical benefit (i.e., no pro-
gression), as determined by the treating physician. IL-18 dose
range of 1 to 100 μg/kg was chosen for the present study. In the
prior phase I and II trials, biologic activity and clinical efficacy
were documented at the lowest dose of 10 μg/kg, with a PR rate
of 5% and a disease stabilization rate of 29% (39). Thus, we
chose to test lower doses (1 and 3 μg/kg) in this study. The
schedule of IL-18 on days 2 and 9 was chosen, given the similar
pharmacokinetic and pharmacodynamic effects shown with
dosing over 5 consecutive days versus dosing once a week. Also,
a weekly dosing regimen allowed for IL-18–binding protein (IL-
18BP) to decrease, thereby preventing attenuation of the
cytokine response (37).

We reached the primary objective of this study, which was to
access the safety and tolerability of IL-18 in combination with
PLD. In this study, an MTD was not reached and toxicity was
minimal across the dose range studied. A secondary objective
was to evaluate biologic activity; we gathered pilot pharma-
codynamic data that 3 μg/kg is the most biologically effective
dose. Given the small sample size, this dose must be further evaluated in a future phase II trial.

Materials and Methods

Subject selection

Females at least 18 years of age with a histologically confirmed recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma, who were candidates to receive PLD, were eligible to enter this study. A predicted life expectancy of at least 4 months and Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 were required.

Study design

This was an open-label, nonrandomized, dose-escalation, safety, and tolerability phase I clinical study (GlaxoSmithKline Clinical Study II108621; ClinicalTrials.gov NCT00659178), which was conducted at three centers: University of Miami (Miami, FL), University of Pennsylvania (Philadelphia, PA), and Stanford University (Palo Alto, CA). The protocol was approved by the Institutional Review Board of each institution. Written informed consent was obtained from each subject before enrollment. Study drug SB-485232, a recombinant form of human IL-18 (rhIL-18) supplied by GlaxoSmithKline, in combination with a standard regimen of PLD, was given to subjects with recurrent epithelial ovarian cancer. Subjects received up to four cycles of combination therapy. One cycle of experimental treatment lasted 28 days, consisting of one dose of PLD (40 mg/m² i.v.) on day 1 plus two doses of SB-485232, on days 2 and 9. The starting dose of SB-485232 was 1 µg/kg, which was escalated in subsequent cohorts (3 patients/ cohort) to 3, 10, 30, and 100 µg/kg. SB-485232 was administered intravenously over 2 hours, at least 24 hours after the start of PLD infusion, and in the absence of any acute PLD infusion-related toxicities. Subjects, who completed all four cycles of experimental treatment, had a follow-up visit at least 2 weeks after the final dose of SB-485232, and were then followed at 3-month intervals for progression or survival for up to 1 year. All subjects, who experienced disease-stabilization or PR after completing four cycles of experimental treatment, were allowed to continue PLD monotherapy during any follow-up period, as per standard of care. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria Version 3.0. Dose-limiting toxicity (DLT) was defined as any grade 3 or 4 toxicity observed during cycle 1 and assessed to be related to the study drug, excluding grade 4 lymphopenia and hyperglycemia, and grade 3 fever, nausea, vomiting, diarrhea, constipation, anorexia, asthenia, hand-foot syndrome, stomatitis, anemia, thrombocytopenia, hyperglycemia, leukopenia, and neutropenia.

Pharmacokinetics

Blood samples were collected for quantification of SB-485232 concentrations before initiation, 1 hour after initiation, immediately before termination of infusion (2 hours), and at 4, 6, 8, 48, and 168 hours after initiation of SB-485232 infusion on days 2 and 4 of cycles 1 and 4. SB-485232 maximum plasma concentration ($C_{max}$), minimum plasma concentration ($C_{min}$), area under the plasma concentration–time curve from time zero to time $t$ (AUC$_{0–t}$), terminal plasma elimination rate-

constant ($\lambda_e$), clearance, and volume of distribution at steady state ($V_{ss}$) were estimated. The apparent terminal elimination half-life ($t_{1/2}$) was calculated as ln(2)/$\lambda_e$ and clearance (CL) was calculated as dose/AUC$_{0–\infty}$.

Pharmacodynamics and biomarkers

Blood samples were collected for quantification of plasma cytokines and chemokines including granulocyte macrophage colony-stimulating factor (GM-CSF), CXCL10 (IP-10), CXCL9 (MIG), CCL2 (MCP-1), IFN-$\gamma$, TNF-$\alpha$, IL-1, IL-2, IL-6, IL-8, IL-10, and IL-12, before initiation of PLD infusion on day 1 of cycles 1 to 4, and before and 4 hours after initiation of each SB-485232 infusion. Also, blood samples were collected for quantification of plasma IL-18BP before initiation of PLD infusion on day 1 of cycle 1, and before initiation of each SB-485232 infusion. Blood samples were collected for flow cytometry analysis before initiation of PLD infusion (cycles 1 and 4), before initiation of SB-485232 infusion, and 4, 48, and 168 hours after initiation of SB-485232 infusion on day 2 of cycles 1 and 4.

Evaluation of response

Within 28 days of the first dose of study drug, baseline disease was documented by radiologic imaging [i.e., computed tomography (CT) scan or MRI]. Radiologic assessments (using the same methodology as was used at baseline) were conducted within 7 days of follow-up visit 1 (at least 2 weeks after completion of study drug combination) and approximately every 3 months for 1 year during the follow-up II period or more frequently as clinically indicated. Target lesion response (complete response, PR, stable disease, and progressive disease) was determined in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) criteria v 1.0. In some patients, CA-125 was used to make therapeutic decisions along with other clinical symptoms or CT if indicated.

Statistical analysis

Analysis of safety and efficacy data was descriptive in nature, with counts and percentages determined for categorical data and mean, median, SD, minimum, and maximum for continuous data.

Results

Patient characteristics

A total of 16 subjects were enrolled (Supplementary Table S1). Most subjects were heavily pretreated, with 81% of patients having received three or more prior regimens. Fifteen subjects received at least one cycle of combination therapy. Eight of 16 subjects received prior PLD and none had progressed on PLD. Ten subjects (63%) completed combination therapy through cycle 4 and follow-up visit 1 (2 weeks after the last dose of study medication), and of these, 3 subjects completed the study as planned (i.e., were followed for 1 year after treatment period in study). The remaining 5 subjects did not complete study treatment because of disease progression. Two subjects were withdrawn from the study due to adverse events. One subject withdrew due to PLD hypersensitivity without receiving SB-485232, and another subject (100 µg/kg SB-485232) withdrew.
due to ascites, nausea, and dyspnea that were due to disease progression.

Toxicity

SB-485232 up to 100 μg/kg in combination with PLD had an acceptable toxicity profile (Table 1). The most common drug-related adverse events were grade 1 or 2 chills (81%), nausea (75%), anemia (63%), fatigue (56%), hyperglycemia (50%), or pyrexia (50%), which did not seem to be dependent on the dose of SB-485232 (Table 2). Hematologic toxicity was as follows: for neutropenia, 13% grade 1, 13% grade 2, and 19% grade 3. For anemia, we found 25% grade 1, 25% grade 2, and 19% grade 3. For thrombocytopenia, we found 6% grade 1, 25% grade 2, and 19% grade 3 adverse events were PLD hypersensitivity and hyperglycemia. Because IL-18 is known to induce hyperglycemia in subjects with impaired glucose tolerance, grade 3 hyperglycemia (reversible within 24 hours of treatment) was not classified as a DLT in this study.

No fatal adverse events were reported. Four subjects experienced 10 nonfatal, serious adverse events. Of these, three were considered to be related to the study drug: grade 3 anemia (at 3 μg/kg SB-485232), grade 3 drug hypersensitivity to PLD (no SB458232 was administered), and grade 2 cytokine release syndrome (at 100 μg/kg SB-485232). The subject with reported cytokine release syndrome developed signs and symptoms on day 2 of cycle 2 during the first hour of SB-485232 infusion. The infusion was stopped after the subject experienced rashes, pallor, tachypnea, hypotension, and nausea and was symptomatically managed with corticosteroids, antihistamines, oxygen, and antiemetics. The subject responded to the symptomatic treatment and was rated as a DLT. The three cases of grade 3 anemia were considered related to the study drug but due to the known association of anemia with PLD treatment, they were not classified as DLTs in this study. The other drug-related grade 3 adverse events were PLD hypersensitivity and hyperglycemia. Because IL-18 is known to induce hyperglycemia in subjects with impaired glucose tolerance, grade 3 hyperglycemia (reversible within 24 hours of treatment) was not classified as a DLT in this study.

No fatal adverse events were reported. Four subjects experienced 10 nonfatal, serious adverse events. Of these, three were considered to be related to the study drug: grade 3 anemia (at 3 μg/kg SB-485232), grade 3 drug hypersensitivity to PLD (no SB458232 was administered), and grade 2 cytokine release syndrome (at 100 μg/kg SB-485232). The subject with reported cytokine release syndrome developed signs and symptoms on day 2 of cycle 2 during the first hour of SB-485232 infusion. The infusion was stopped after the subject experienced rashes, pallor, tachypnea, hypotension, and nausea and was symptomatically managed with corticosteroids, antihistamines, oxygen, and antiemetics. The subject responded to the symptomatic treatment and was admitted to the hospital with a temperature of 98°F, heart rate of 100 beats/min, respiratory rate 18 breaths/min, and blood pressure of 102/62 mmHg; the subject's vital signs normalized over the next several hours. This subject was admitted 1 week later for grade 2 ascites, grade 2 dyspnea, and grade 1 nausea, which was associated with disease progression and was withdrawn from the study.

Table 1. Most frequently reported adverse events (at least 4 subjects) regardless of causality

<table>
<thead>
<tr>
<th>Preferred term</th>
<th>1 μg/kg N = 3</th>
<th>3 μg/kg N = 4</th>
<th>10 μg/kg N = 3</th>
<th>30 μg/kg N = 3</th>
<th>100 μg/kg N = 3</th>
<th>Total N = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chills</td>
<td>2 (67)</td>
<td>3 (75)</td>
<td>3 (75)</td>
<td>2 (67)</td>
<td>3 (100)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (100)</td>
<td>2 (50)</td>
<td>2 (67)</td>
<td>3 (100)</td>
<td>2 (67)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1 (33)</td>
<td>3 (75)</td>
<td>2 (67)</td>
<td>3 (100)</td>
<td>2 (67)</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (33)</td>
<td>2 (50)</td>
<td>1 (33)</td>
<td>3 (100)</td>
<td>2 (67)</td>
<td>9  (56)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1 (33)</td>
<td>1 (25)</td>
<td>2 (67)</td>
<td>2 (67)</td>
<td>2 (67)</td>
<td>8  (50)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>2 (50)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>7  (44)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>1 (33)</td>
<td>2 (50)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>7  (44)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1 (33)</td>
<td>2 (50)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>0</td>
<td>6  (36)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>5  (31)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>0</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>5  (31)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (33)</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>0</td>
<td>5  (31)</td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>1 (25)</td>
<td>2 (67)</td>
<td>0</td>
<td>1 (33)</td>
<td>4  (25)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (33)</td>
<td>0</td>
<td>1 (33)</td>
<td>0</td>
<td>2 (67)</td>
<td>4  (25)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td>4  (25)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>2 (67)</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>0</td>
<td>0</td>
<td>4  (25)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (33)</td>
<td>1 (25)</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td>0</td>
<td>4  (25)</td>
</tr>
</tbody>
</table>

Abbreviation: AE, adverse event.
Cardiac toxicity was evaluated by 12-lead electrocardiography (ECG) before, during, and after treatment, and by pre- and posttreatment Multigated Acquisition (MUGA) scans. None of the subjects had clinically relevant abnormal findings during the study with respect to mean or median ECG values. Of the 15 subjects with postbaseline MUGA scans, 13 had normal results. One subject (subject 2004; 10 mg/kg SB-485232) had a mildly enlarged right atrium, but exhibited normal myocardial function and unchanged left ventricular ejection fraction (LVEF; 62% pre and 65% post), whereas one subject (subject 2005; 30 mg/kg SB-485232) had hyperdynamic contractility (LVEF; 56% pre and 70% post), which was thought to be due to differences in hemodynamic status or recent strenuous activity. In addition, subject 5008 (30 mg/kg SB-485232) had a grade 1 adverse event of left ventricular dysfunction at follow-up, approximately 2 weeks after the last dose of study drug. The subject’s LVEF was 69% at screening, after treatment, LVEF was 50%. Her follow-up MUGA was normal with an ejection fraction of 77%.

Four deaths occurred during this study, 3 subjects died because of disease progression (at the dose of 1, 10, or 100 μg/kg SB-485232, respectively), whereas another subject (at the dose of 30 μg/kg SB-485232) had evidence of stable disease after completing the last cycle of study treatment (day 115), but died during the follow-up period (day 196) in hospice care and without documentation of disease progression.

The toxicity profile was similar to that observed in patients receiving SB-485232 as monotherapy (37) except for an increase in the incidence of hematologic toxicities such as neutropenia and anemia, which are typically observed with PLD (40). A MTD of SB-485232 was not identified. Antibodies to SB-485232 were not detected in any patient treated on this study.

### Pharmacokinetics of rIL-18

Mean $t_{1/2}$ values ranged from 43.5 to 72.7 hours during cycle 1 and from 52.7 to 79.9 hours during cycle 4. The increases in $C_{\text{max}}$ and AUC values seemed to be less than dose-proportional (Fig. 1). This nonlinear dose–exposure relationship was also observed in other SB-485232 studies and is likely due to saturated binding of IL-18 to IL-18BP, and the fact that the pharmacokinetic assay measures the total IL-18 concentration (41). The mean $C_{\text{max}}$ and AUC values were generally similar in cycles 1 and 4, indicating no accumulation after multiple doses.

### Table 2. Adverse events with maximum grade 3

<table>
<thead>
<tr>
<th>SB-485232 doses + DOXIL 40 mg/m²</th>
<th>1 μg/kg N = 3</th>
<th>3 μg/kg N = 4</th>
<th>10 μg/kg N = 3</th>
<th>30 μg/kg N = 3</th>
<th>100 μg/kg N = 3</th>
<th>Total N = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred term</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Any AE</td>
<td>1 (33)</td>
<td>3 (75)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
<td>2 (50)</td>
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<td>0</td>
<td>1 (33)</td>
<td>3 (19)</td>
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<tr>
<td>Abdominal pain</td>
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<td>0</td>
<td>1 (33)</td>
<td>0</td>
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<td>1 (6)</td>
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<td>Asthenia</td>
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<td>0</td>
<td>1 (33)</td>
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<td>1 (6)</td>
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<tr>
<td>Dehydration</td>
<td>1 (33)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Drug hypersensitivity (to PLD)</td>
<td>0</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Edema peripheral</td>
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<td>0</td>
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<td>Fatigue</td>
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<td>0</td>
<td>1 (33)</td>
<td>0</td>
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<td>1 (6)</td>
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<td>Hyperglycemia</td>
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<td>0</td>
<td>0</td>
<td>1 (6)</td>
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<td>Hyperkalemia</td>
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<td>1 (25)</td>
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<td>0</td>
<td>0</td>
<td>1 (6)</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>1 (33)</td>
<td>0</td>
<td>1 (6)</td>
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<tr>
<td>Musculoskeletal pain</td>
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<td>0</td>
<td>1 (33)</td>
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<td>1 (6)</td>
</tr>
<tr>
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<td>0</td>
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<td>1 (6)</td>
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<td>0</td>
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<td>1 (6)</td>
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<tr>
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<td>0</td>
<td>1 (33)</td>
<td>0</td>
<td>0</td>
<td>1 (6)</td>
</tr>
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Abbreviation: AE, adverse event.

Figure 1. Profile of median SB-485232 plasma concentrations by dose group.
of SB-485232 (3 to 100 μg/kg) in combination with 40 mg/m² PLD.

Biologic effects of rhIL-18

Leukocyte markers showed a rapid (within hours of rhIL-18 administration) and reversible response. Lymphocyte counts (total lymphocytes, CD4⁺ and CD8⁺ T cells, and NK cells) had a marked response pattern: a sharp drop of plasma cell counts at 4 hours, followed by a rebound to baseline levels by 48 hours postdose (see Fig. 2 for CD8⁺ T cells). Importantly, no increase or attenuation of responses was observed between cycles, indicating no attenuation of the IL-18 effect by repeat PLD administrations. The most prominent biologic effects were seen with NK cells, both in cell counts and changes in activation status. CD56dim CD16⁺ NK cells showed a dose-dependent increase in the percentage of activated cells expressing CD69 at the 4- and 48-hour time points (Fig. 3A). Interestingly, the maximal biologic effect with activated CD56dim CD16⁺ NK cells was observed at a dose as low as 3 μg/kg in this study. The levels of activated CD56dim CD16⁺ NK cells induced after dosing were similar after cycles 1 and 4, showing that the biologic response was maintained after 4 cycles of dosing and was not attenuated by repeat PLD dosing (Fig. 3B). The fractions of activated CD16⁺ CD56dim or CD16⁺ CD56bright NK cells expressing both Fasl and IL-18Ra were also increased for most subjects at the 4-hour time point for all dose levels (data not shown). No obvious effects were observed after SB-485232 treatment on CD4⁺ CD25⁺ FoxP3⁺ Tregs, monocytes, or neutrophils (data not shown).

Almost all the measured soluble cytokine and chemokine biomarkers showed strong responses 4 hours after SB-485232 dosing, with a several-fold increase from predose levels. In individuals dosed with 3 to 100 μg/kg, levels of IFN-γ were consistently increased from undetectable levels to peak levels up to 60 pg/mL at the 4-hour time point, and reverted back to undetectable levels 1 week postdose. Serum levels of IFN-γ, CCL2, CXCL9, and CXCL10 were elevated several fold from baseline (Fig. 4). Interestingly, different dose responses were seen for the different cytokines and chemokines. IFN-γ increased to reach a maximum at the 10 μg/kg dose. IFN-inducible chemokines CXCL9 and CXCL10 reached serum peak levels already at the 3 μg/kg dose, whereas CCL2 reached a maximum at the 10 μg/kg dose (Fig. 4). The levels of TNF-α and GM-CSF were not significantly changed at the 1 and 3 μg/kg doses.
but reached peak levels at 10 μg/kg dose. The levels of IL-6, IL-8, and IL-10 were also unchanged at the 3 μg/kg dose, and trended toward a plateau at the 10 μg/kg dose (Fig. 4). Importantly, cytokine or chemokine responses to SB-485232 were not attenuated over the four cycles of dosing, indicating no immune suppression by repeat PLD dosing. Mean IL-18BP levels generally showed little change between cycles or across time within a cycle (data not shown).

Tumor response
Of the 16 enrolled subjects, 10 (63%) completed the four treatment cycles and were evaluated on follow-up visit 1, and 5 (31%) subjects progressed while receiving treatment drugs. By RECIST criteria, this drug combination resulted in a PR rate of 6% [1 of 16; 95% confidence interval (CI), 0%–18.1%] and the stable disease rate was 38% (6 of 16). The objective response was similar to that of PLD used as monotherapy in the same population, based on reported studies (42). A waterfall plot with the maximum percentage reduction in tumor burden was created for 9 subjects, who completed cycle 4 and had evaluable tumor by CT (Fig. 5A). On the basis of this analysis, 8 of 16 subjects had either stable overall tumor burden or a reduction of the overall tumor burden. Three heavily pretreated subjects had stable disease or a PR and did not progress for significant periods of time during the follow-up period; of these 3 subjects, only subject 5004 received additional therapy (six cycles of PLD) during the follow-up period. Subject 5004 (3 μg/kg; six lines of prior therapy) had the greatest reduction in tumor volume (53%) but was considered to have progressive disease because of the identification of a new lesion. The majority of patients exhibited stable or declining CA-125 by end of cycle 4 (Fig. 5B).

Discussion
Rationale for developing chemoimmunotherapy combinations for ovarian cancer is quite strong, based on the significant impact of antitumor immune response on survival in these patients. In this approach, chemotherapy could sensitize tumor cells to immune attack, thereby resulting in increased efficacy. However, identifying the right combination of cytotoxic and immunomodulatory drugs along with optimal dose and schedule is critical.

IL-18 is an immunostimulatory cytokine that seems ideal for combination with cytotoxics, as it is known to activate key effector cells such as NK cells and T lymphocytes, and it is a potent inducer of TH1 cytokines and chemokines. IL-18 (SB-485232) was previously evaluated as monotherapy in patients with advanced solid tumors and was found to be safe and well-tolerated up to a dose of 1,000 μg/kg and has shown immunomodulatory activity (35, 36). The biologic activity of IL-18 in vivo in patients with cancer included lymphocyte activation and the induction of IFN-γ and IFN-inducible chemokines including CXCL9 and CXCL10 in peripheral blood.

Preclinical models can be used to select optimal combinations of cytotoxics with immunomodulatory drugs. We previously screened combinations of IL-18 with chemotherapy in a mouse model of ovarian cancer and found that among drugs commonly used for ovarian cancer, PLD induced the best results in terms of mobilizing antitumor immunity, establishing memory, and
improving survival of mice (28, 43). This could be explained by the fact that doxorubicin has independent immunomodulatory effects that can synergize with IL-18, including enhancing antigen presentation through immunogenic tumor cell death as well as enhancing immune recognition through upregulation of surface MHC class I and Fas in surviving tumor cells. In addition, the pharmacodynamic effects of PLD did not preclude immune activation during repeated drug administration in the mouse.

Current regimens for recurrent ovarian cancer include FDA-approved PLD alone or in combination with carboplatin that resulted in improved PFS and reduced toxicity over carboplatin/paclitaxel in platinum-sensitive patients in a phase III randomized study (44). In addition, PLD has been studied in combination with other chemotherapies, and current efforts focus on combining PLD with immunotherapeutic and targeted agents (45, 46). Thus, PLD seems suitable for combinations with immunostimulatory therapy and has been tested in the clinic.

The objective of this study was to determine the safety, tolerability, and biologic activity of IL-18 combined with PLD for recurrent ovarian cancer. The present phase I study shows that SB-485232 is well tolerated when used in combination with the standard acceptable dose of PLD in this heavily pretreated, recurrent ovarian cancer population. The majority of patients (82%) was platinum-resistant and had received more than three prior regimens. Importantly, there was no positive drug interaction in terms of toxicity, and the most common adverse events were grade 1 to 2 chills and nausea. The safety and tolerability profile of SB-485232/PLD was similar to SB-485232 when used as monotherapy (36), with the exception of anemia and neutropenia, which were attributed to PLD and were in fact similar to the incidence observed with PLD monotherapy (40). No DLTs were identified.

SB-485232 administration in combination with PLD rapidly and reversibly induced upregulation of markers of immune activation, such as TNF-α and GM-CSF (e.g., IFN-γ, CXCL9, CXCL10, CCL2), and expression of immune effector cell activation markers (e.g., CD69 and FasL on NK and CD8^+ T cells), similar to what was seen with SB-485232 monotherapy. This was most evident in the NK population and to a slightly lesser degree in CD8^+ and CD4^+ T cells, and this activation was quite rapid. At the 48-hour time point, CD69 expression was still increased for CD56^dim NK cells. CD69 is an important marker of NK cell activation. In previous murine studies using E14 T cell lymphoma, where combination

Figure 4. Mean cytokine and chemokine induction at 4 hours after receiving SB-485232 averaged over available cycles [absolute change for IFN-γ; fold change (% change/100) for CXCL9, CXCL10, CCL2, TNF-α, GM-CSF, IL-2, IL-6, and IL-8; % change for IL-10].
of IL-18 with doxorubicin showed synergistic antitumor activity. IL-18 significantly enhanced NK cell activation and upregulated CD69 (unpublished data). Furthermore, in a recent phase I study in patients with CD20-positive non-Hodgkin’s lymphoma, IL-18 (SB-485232) in combination with rituximab, a monoclonal antibody against CD20, induced an increase in the percentage of peripheral blood CD69\(^+\) NK cells 48 hours after the infusion of SB-485232 (47).

Given our small sample size, and the low number of patients with clinical benefit, we cannot conclude which biomarker is most predictive of the best dose level for SB-485232. However, at the 3 \(\mu\)g/kg dose, we observed an optimal Th1 cytokine and chemokine activation profile, including a significant increase in serum IFN-\(\gamma\) and maximal increase in serum CXCL9 and CXCL10, suggesting that effective Th1 immune activation can be obtained at this dose, which was very well tolerated. Importantly, the biologic effect with activated CD56\(^{dim}\) CD16\(^+\) NK cells seemed to plateau at the dose of 3 \(\mu\)g/kg. In addition, IL-6, IL-8, and IL-10 variably involved in promoting tumor growth through inflammation, angiogenesis, and immune suppression, respectively, were low at the 3 \(\mu\)g/kg dose and consistently increased at the 10 \(\mu\)g/kg dose, further supporting the 3 \(\mu\)g/kg dose level as the biologically optimal dose. The two subjects, who showed the most significant decrease in tumor burden, were both treated at 3 \(\mu\)g/kg; one subject had a PR and the other experienced progressive disease because of a new liver lesion by RECIST criteria, but showed a PR by immune-related response criteria (48).

An important finding of our study is that concomitant and repeat administration of PLD did not attenuate the biologic effects of IL-18. The combination led to the transient reduction of circulating CD4\(^+\) T, CD8\(^+\) T, and NK cells in ovarian cancer subjects, with a rapidly occurring nadir in circulating lymphocyte counts 4 hours postexposure to SB-485232. This was interpreted as likely due to cell activation and margination rather than PLD-induced depletion of activated T cells, as the same acute lymphopenia was observed after SB-485232 monotherapy (37). In fact, the number and degree of activation of immune cells and blood cytokine or chemokine response was not decreased with repeated PLD administration. In addition, the patterns of immune stimulation and dose-response to SB-485232 seen with the SB-485232/PLD combination were

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**Figure 5.** A, waterfall plot of maximum percentage reduction from baseline in tumor size by subject and best overall response. B, waterfall plot of maximum percentage reduction of CA-125.
similar to those seen previously with SB-485232 monotherapy (49) or when SB-485232 was combined with rituximab (47). This is the first demonstration that PLD chemotherapy does not attenuate immunostimulatory therapy in the human. Thus, PLD at the dose of 40 mg/m² seems to be suitable for combining with immunostimulatory drugs, and the optimal expansion phase dose of SB-485232 seems to be 3 μg/kg.

It has been shown previously that IL-18, depending on dose or schedule, could either suppress or promote tumor functions in mouse tumor models that involved NK cells (50). In one such model, IL-18 at low doses induced expansion of an immunosuppressive population of Kit⁺ NK cells expressing programmed cell death ligand 1 (PD-L1), which could be prevented by anti-PD-1 blockade (51). However, in the same model, a different schedule of IL-18 (which reached serum levels >1 ng/mL) resulted in immune activation and tumor suppression, with a proinflammatory TNFα cytokine and chemokine profile, which is more similar to the biologic effects observed in our patients. Furthermore, plasma levels of SB-485232 were more than 10 ng/mL for subjects in the lowest dose cohort receiving 1 μg/kg. Nevertheless, the observation that IL-18 upregulates PD-L1 in NK cells (48), suggests that PD-L1-neutralizing antibodies could enhance the therapeutic effect of IL-18 in patients with cancer.

In conclusion, the present study shows the safety, tolerability, and biologic efficacy of SB-485232 in combination with PLD. The evidence that sufficient biologic activity is observed at a low dose of SB-485232 (3 μg/kg), which is not attenuated (and based on mouse data could be enhanced) by concomitant PLD, is encouraging for the design of a future phase II trial to evaluate the efficacy of SB—485232 plus PLD combination in recurrent ovarian cancer.

Disclosure of Potential Conflicts of Interest
C. Chu is a consultant/advisory board member of Prima Biomed. S. Murray has ownership interest (including patents) in GlaxoSmithKline. J. Bauman and O. Gardner are employed as Clinical Development Manager in GlaxoSmithKline and have ownership interest (including patents) in the same. J. Tosò is employed as Director in GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Simpkins, A. Flores, C. Chu, J.S. Berek, J. Lucci III, J. Bauman, J. Tosò
Writing, review, and/or revision of the manuscript: F. Simpkins, A. Flores, C. Chu, J.S. Berek, J. Lucci III, S. Murray, J. Bauman, H. Streumper, F. Germaschewski, Z. Jonak, O. Gardner, J. Tosò, G. Coukos
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Bauman
Study supervision: J. Bauman, O. Gardner

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References
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Fiona Simpkins, Aurea Flores, Christina Chu, et al.


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