

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

Fiona Simpkins¹, Aurea Flores¹, Christina Chu^{2,3}, Jonathan S. Berek⁴, Joseph Lucci III¹, Sharon Murray⁵, John Bauman⁵, Herbert Struemper⁵, Fiona Germaschewski⁵, Zdenka Jonak⁵, Olivia Gardner⁵, John Toso⁵, and George Coukos^{2,3}

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: 305-243-4938; E-mail: fsimpkins@med.miami.edu; and John Toso, John.F.Toso@gsk.com

doi: 10.1158/2326-6066.CIR-13-0098

©2013 American Association for Cancer Research.

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 immunomodulatory 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 compounds (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 chemoimmunotherapy (16). Furthermore, adverse biologic interactions 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). Doxorubicin-killed tumor cells recruit intratumoral CD11c⁺ CD11b⁺ Ly6C^{hi} 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 chemoimmunotherapy combinations. In a mouse model of ovarian cancer, we reported that tumor cells surviving the direct toxicity of PLD, upregulated 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 (T_H1)-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 T_H1 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 combination with PLD resulted in synergistic antitumor activity. Although IL-18 or PLD monotherapy had a moderate antitumor effect, in combination, they significantly restricted tumor growth, augmented OS rate, and generated long-term protective immunity (28). Therefore, we hypothesized that the antitumor activity of PLD can be enhanced by IL-18 in patients with ovarian cancer. IL-18 has been evaluated as monotherapy 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 μ g/kg (35, 36). The biologic effects of IL-18 included transient lymphopenia, increased activation of NK and CD8⁺ T cells, and increased T_H1 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 schedule 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 nonrandomized 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 assess 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 progression), as determined by the treating physician. IL-18 dose range of 1 to 100 μ g/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 μ g/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 μ g/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 assess 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 pharmacodynamic data that 3 μ g/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 ILI108621; 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 (λ_z) clearance, and volume of distribution at steady state (V_{ss}) were estimated. The apparent terminal elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$ and clearance (CL) was calculated as $dose/AUC_{(0-\tau)}$.

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- γ , TNF- α , 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

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

Preferred term	SB-485232 doses + DOXIL 40 mg/m ²					Total N = 16
	1 µg/kg N = 3	3 µg/kg N = 4	10 µg/kg N = 3	30 µg/kg N = 3	100 µg/kg N = 3	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any AE	3 (100)	4 (100)	3 (100)	3 (100)	3 (100)	16 (100)
Chills	2 (67)	3 (75)	3 (100)	2 (67)	3 (100)	13 (81)
Nausea	3 (100)	2 (50)	2 (67)	3 (100)	2 (67)	12 (75)
Anemia	1 (33)	3 (75)	2 (67)	2 (67)	2 (67)	10 (63)
Fatigue	1 (33)	2 (50)	1 (33)	3 (100)	2 (67)	9 (56)
Hyperglycemia	1 (33)	3 (75)	1 (33)	1 (33)	2 (67)	8 (50)
Pyrexia	1 (33)	1 (25)	2 (67)	2 (67)	2 (67)	8 (50)
Constipation	0	2 (50)	2 (67)	1 (33)	2 (67)	7 (44)
Decreased appetite	1 (33)	2 (50)	1 (33)	2 (67)	1 (33)	7 (44)
Abdominal pain	1 (33)	2 (50)	1 (33)	2 (67)	0	6 (38)
Hypertension	0	1 (25)	1 (33)	2 (67)	1 (33)	5 (31)
Leukopenia	0	1 (25)	1 (33)	2 (67)	1 (33)	5 (31)
Vomiting	1 (33)	1 (25)	1 (33)	2 (67)	0	5 (31)
Back pain	0	1 (25)	2 (67)	0	1 (33)	4 (25)
Headache	1 (33)	0	1 (33)	0	2 (67)	4 (25)
Neutropenia	0	1 (25)	1 (33)	1 (33)	1 (33)	4 (25)
Pruritus	2 (67)	1 (25)	1 (33)	0	0	4 (25)
Rash	1 (33)	1 (25)	1 (33)	1 (33)	0	4 (25)

Abbreviation: AE, adverse event.

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 following: for neutropenia, 13% grade 1, 13% grade 2, and no grade 3 or 4. For leukopenia, there was 31% grade 1, and 6% grade 2. For anemia, we found 25% grade 1, 25% grade 2, and 19% grade 3. For thrombocytopenia, we found 6% grade 1 and 0% grade 2, 3, or 4. No patients developed hand-foot syndrome, but 25% of patients developed a rash. Nineteen percent of patients developed mucosal inflammation. Chills, nausea, fatigue, hyperglycemia, and pyrexia were previously reported as short-term adverse events after SB-485232 monotherapy infusion (32, 37). SB-485232-induced hyperglycemia was previously seen in patients with impaired glucose tolerance, and is typically reversible within 24 hours.

Eight of 16 subjects (50%) had grade 3 adverse events, which included 3 subjects with anemia (19%) and 1 subject each (6%) with abdominal pain, asthenia, dehydration, PLD hypersensitivity, edema, fatigue, hyperglycemia, hyperkalemia, jaundice, pain, nausea, vomiting, or pyelonephritis (Table 2). The majority of adverse events were found to be related to disease progression or not related to the study drug, and was not

considered DLTs. 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 SB485232 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 rigors, 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 2. Adverse events with maximum grade 3

Preferred term	SB-485232 doses + DOXIL 40 mg/m ²					
	1 µg/kg N = 3	3 µg/kg N = 4	10 µg/kg N = 3	30 µg/kg N = 3	100 µg/kg N = 3	Total N = 16
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any AE	1 (33)	3 (75)	1 (33)	2 (67)	1 (33)	8 (50)
Anemia	0	2 (50)	0	0	1 (33)	3 (19)
Abdominal pain	0	0	1 (33)	0	0	1 (6)
Asthenia	0	0	1 (33)	0	0	1 (6)
Dehydration	1 (33)	0	0	0	0	1 (6)
Drug hypersensitivity (to PLD)	0	1 (25)	0	0	0	1 (6)
Edema peripheral	0	0	0	1 (33)	0	1 (6)
Fatigue	0	0	0	1 (33)	0	1 (6)
Hyperglycemia	0	1 (25)	0	0	0	1 (6)
Hyperkalemia	0	1 (25)	0	0	0	1 (6)
Jaundice	1 (33)	0	0	0	0	1 (6)
Musculoskeletal pain	0	0	0	1 (33)	0	1 (6)
Nausea	0	0	1 (33)	0	0	1 (6)
Pyelonephritis	0	0	0	1 (33)	0	1 (6)
Vomiting	0	0	1 (33)	0	0	1 (6)

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 µg/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 µg/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 µg/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 of 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 rhIL-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_{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_{max} and AUC values were generally similar in cycles 1 and 4, indicating no accumulation after multiple doses

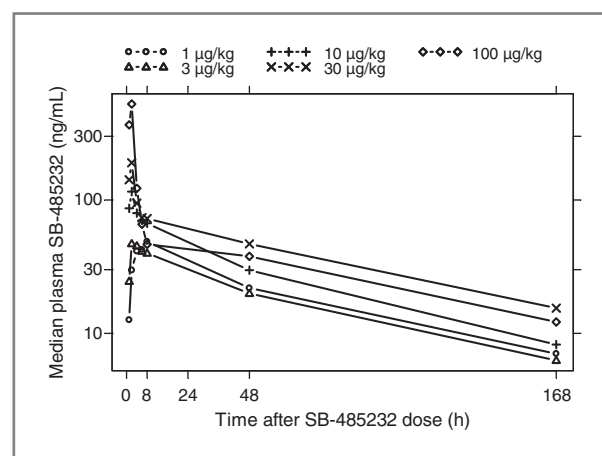


Figure 1. Profile of median SB-485232 plasma concentrations by dose group.

of SB-485232 (3 to 100 $\mu\text{g}/\text{kg}$) in combination with 40 mg/m^2 PLD.

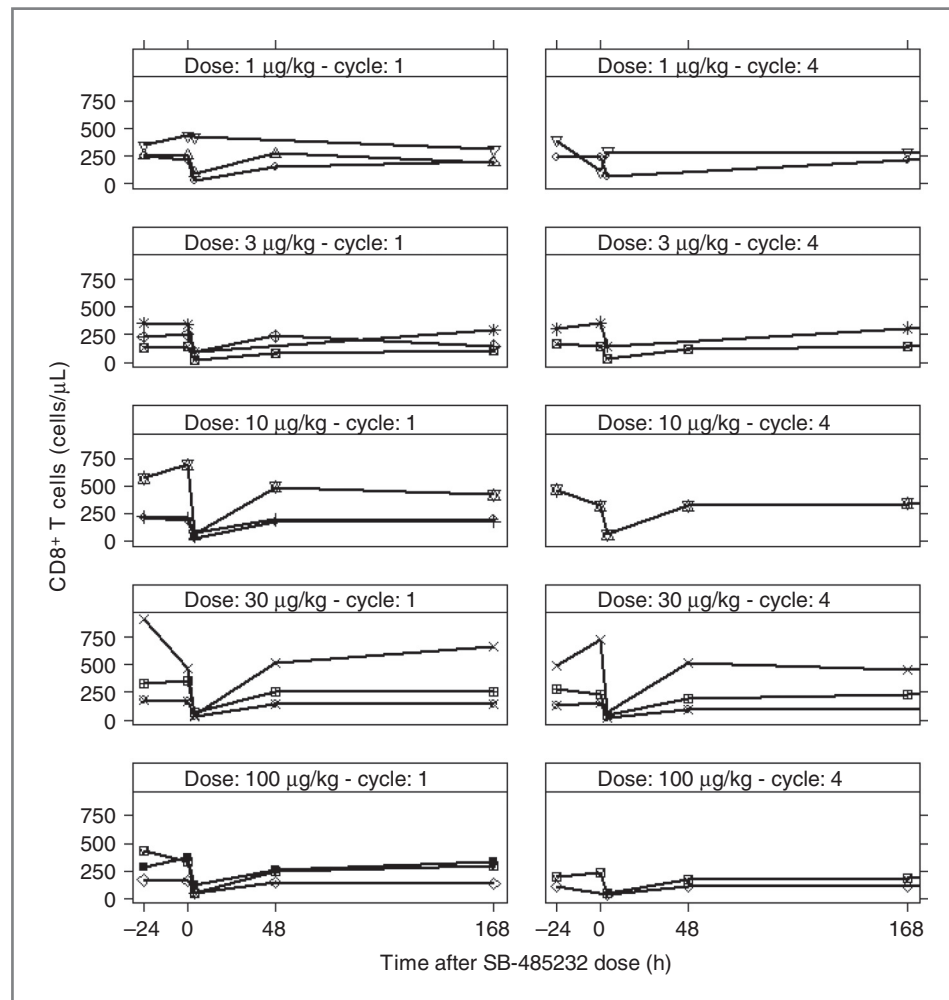
Biologic effects of rhIL-18

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. $\text{CD56}^{\text{dim}} \text{CD16}^+$ 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 $\text{CD56}^{\text{dim}} \text{CD16}^+$ NK cells was observed at a dose as low as 3 $\mu\text{g}/\text{kg}$ in this study. The levels of activated $\text{CD56}^{\text{dim}} \text{CD16}^+$ 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 $\text{CD16}^+ \text{CD56}^{\text{dim}}$ or $\text{CD16}^+ \text{CD56}^{\text{bright}}$ 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 $\text{CD4}^+ \text{CD25}^+ \text{FoxP3}^+$ 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 $\mu\text{g}/\text{kg}$, levels of $\text{IFN-}\gamma$ 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 $\text{IFN-}\gamma$, CCL2, CXCL9, and CXCL10 were elevated several fold from baseline (Fig. 4). Interestingly, different dose responses were seen for the different cytokines and chemokines. $\text{IFN-}\gamma$ increased to reach a maximum at the 10 $\mu\text{g}/\text{kg}$ dose. $\text{IFN-}\gamma$ -inducible chemokines CXCL9 and CXCL10 reached serum peak levels already at the 3 $\mu\text{g}/\text{kg}$ dose, whereas CCL2 reached a maximum at the 10 $\mu\text{g}/\text{kg}$ dose (Fig. 4). The levels of $\text{TNF-}\alpha$ and GM-CSF were not significantly changed at the 1 and 3 $\mu\text{g}/\text{kg}$ doses,

Figure 2. Absolute CD8^+ T-cell counts by subject for cycles 1 and 4 at predose, 4, 48, and 168 hours after receiving SB-485232.



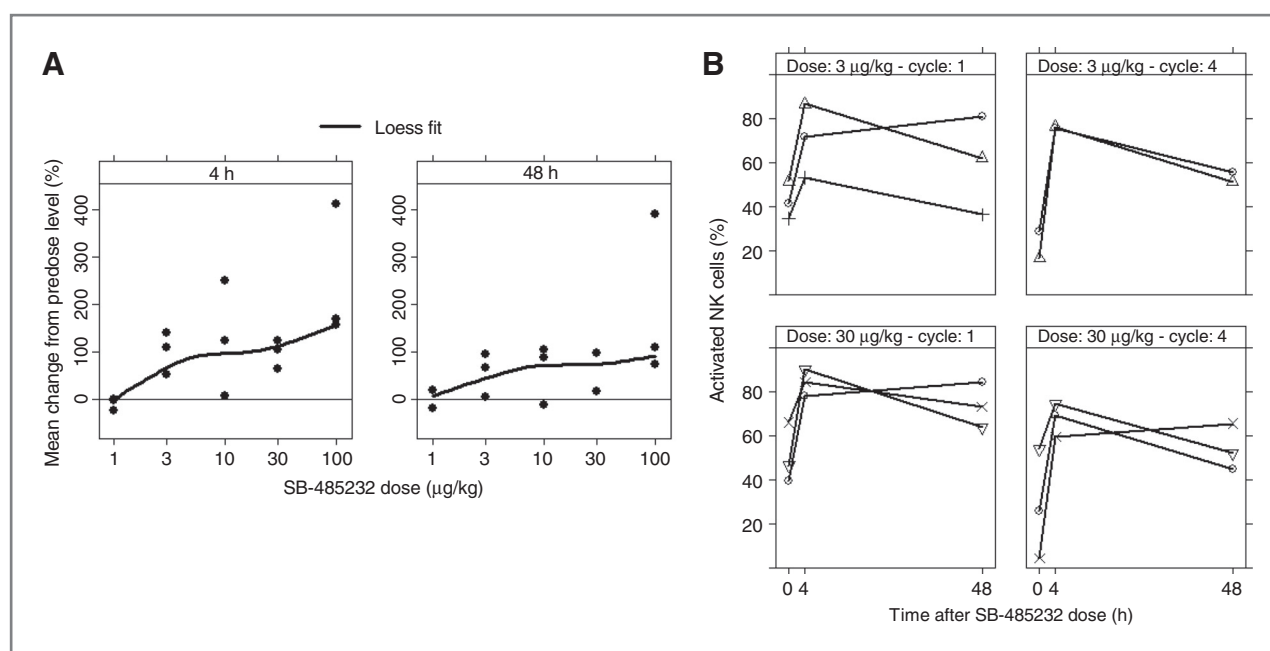


Figure 3. A, percentage change from predose levels of activated CD56^{DIM} CD16⁺ CD69⁺ NK cells at 4 and 48 hours after receiving SB-485232 averaged over available cycles. B, percentage of activated CD69⁺ cells of total CD56^{DIM} CD16⁺ NK Cells by subject after dosing with 3 and 30 μg/kg for cycle 1 and 4, predose, 4 and 48 hours after receiving SB-485232.

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; four lines of prior therapy) had a PR lasting over 9.6 months. Subject 2001 (1 μg/kg; five lines of prior therapy) had stable disease over the 13-month follow-up period and had a significant reduction in CA-125 (Fig. 5B). Subject 5008 (10 μg/kg; five lines of prior therapy) had stable disease for 6.3 months.

Subject 5005 (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 T_H1 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

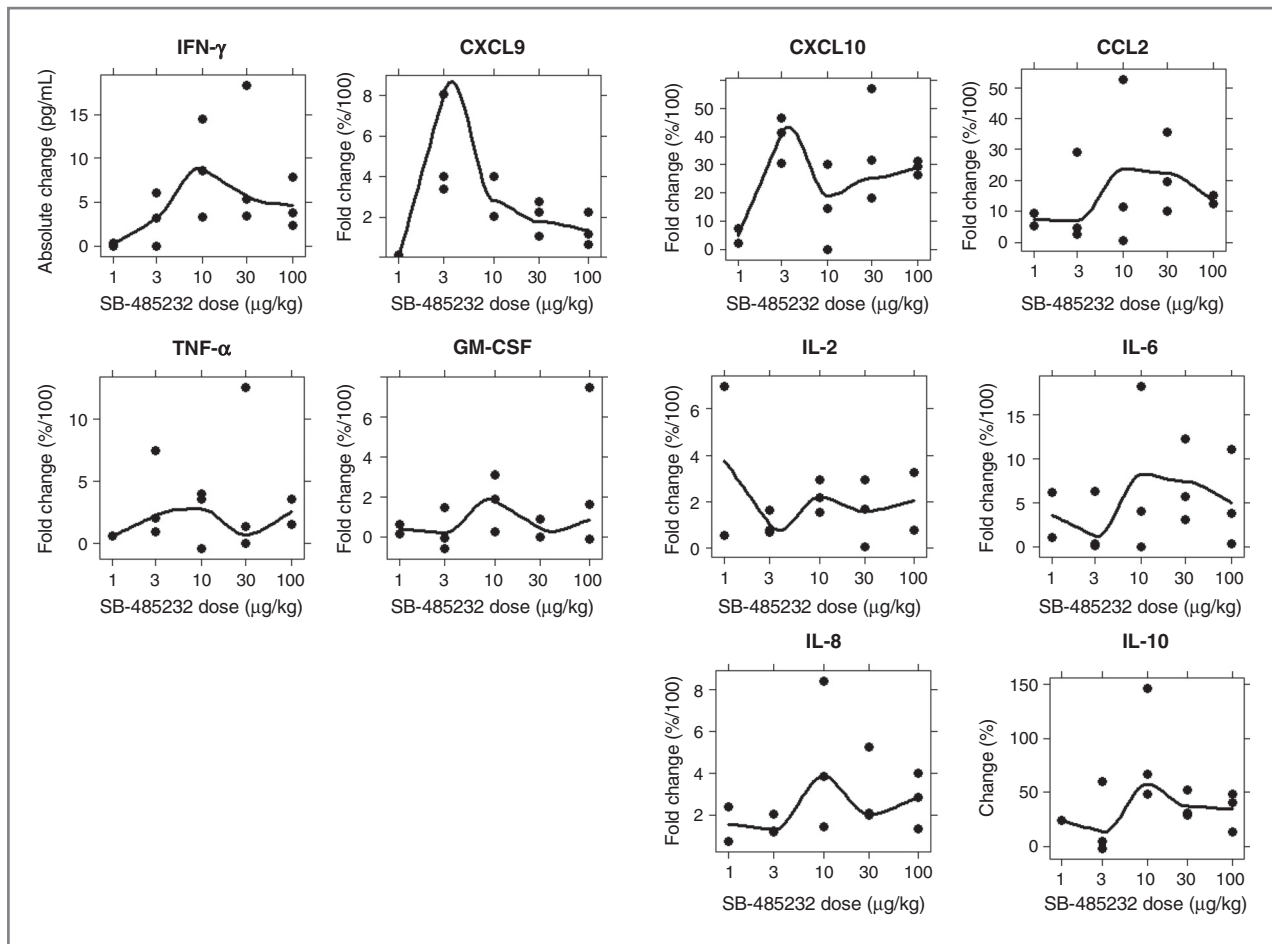


Figure 4. Mean cytokine and chemokine induction at 4 hours after receiving SB-485232 averaged over available cycles [absolute change for IFN- γ ; fold change (% change/100) for CXCL9, CXCL10, CCL2, TNF- α , GM-CSF, IL-2, IL-6, and IL-8; % change for IL-10].

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 T_H1 cytokines and inflammatory chemokines (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 EL4 T cell lymphoma, where combination

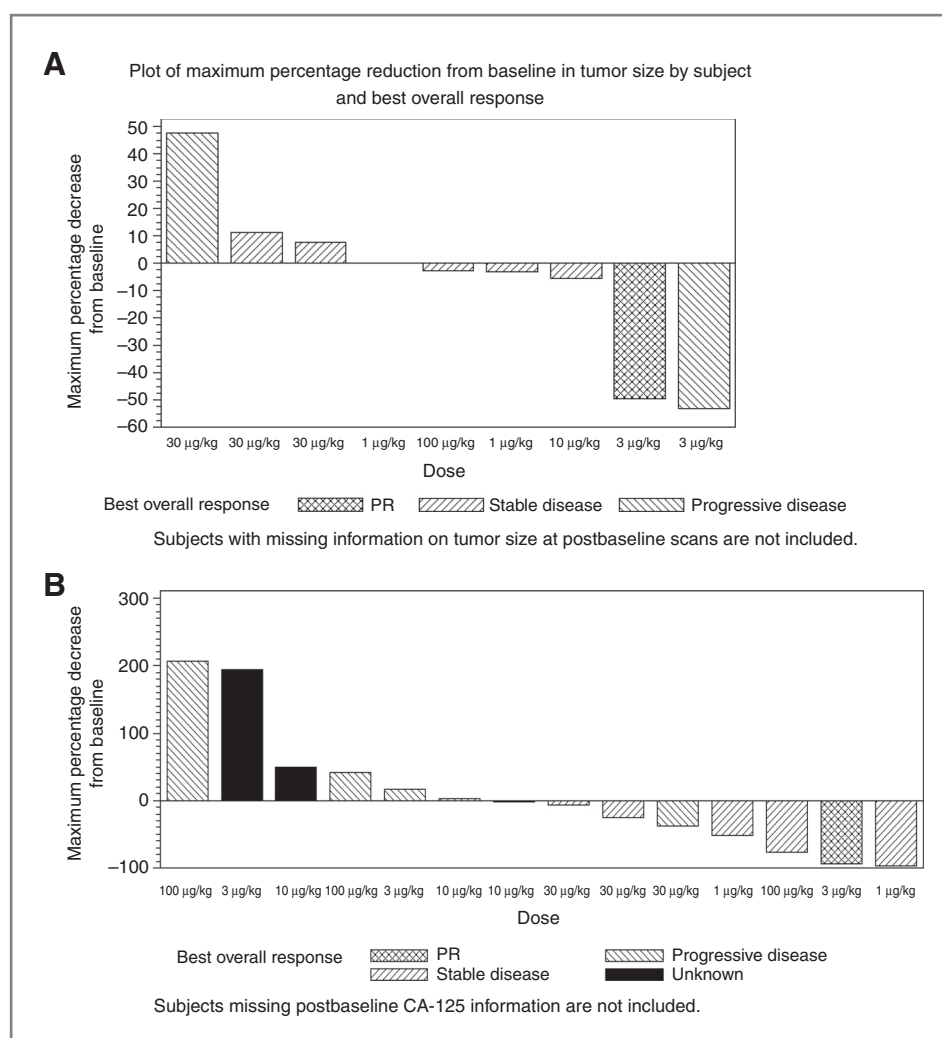


Figure 5. A, waterfall plot of maximum percentage reduction of tumor burden for subjects that completed cycle 4. B, waterfall plot of maximum percentage reduction of CA-125.

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 $\mu\text{g}/\text{kg}$ dose, we observed an optimal T_H1 cytokine and chemokine activation profile, including a significant increase in serum IFN- γ and maximal increase in serum CXCL9 and CXCL10, suggesting that effective T_H1 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 $\mu\text{g}/\text{kg}$. 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 $\mu\text{g}/\text{kg}$

dose and consistently increased at the 10 $\mu\text{g}/\text{kg}$ dose, further supporting the 3 $\mu\text{g}/\text{kg}$ dose level as the biologically optimal dose. The two subjects, who showed the most significant decrease in tumor burden, were both treated at 3 $\mu\text{g}/\text{kg}$; 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

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 T_H1 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 (43), 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. Toso is employed as Director in GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: F. Simpkins, C. Chu, J.S. Berek, S. Murray, J. Bauman, H. Struemper, Z. Jonak, O. Gardner, J. Toso, G. Coukos

Development of methodology: F. Simpkins, C. Chu, J.S. Berek, J. Bauman, H. Struemper, F. Germaschewski, Z. Jonak, O. Gardner, J. Toso, G. Coukos

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. Toso

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Simpkins, C. Chu, J.S. Berek, J. Lucci III, S. Murray, J. Bauman, H. Struemper, F. Germaschewski, Z. Jonak, O. Gardner, J. Toso, G. Coukos

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. Struemper, F. Germaschewski, Z. Jonak, O. Gardner, J. Toso, G. Coukos

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Bauman

Study supervision: J. Bauman, O. Gardner

Grant Support

This study was funded by GlaxoSmithKline.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 16, 2013; accepted July 18, 2013; published OnlineFirst July 31, 2013.

References

- Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. *CA Cancer J Clin* 2006;56:106–30.
- Herzog TJ, Pothuri B. Ovarian cancer: a focus on management of recurrent disease. *Nat Clin Pract Oncol* 2006;3:604–11.
- Markman M, Gordan AN, McGuire WP, Muggia FM. Liposomal anthracycline treatment for ovarian cancer. *Semin Oncol* 2004;31 (suppl 13):91–105.
- Gordon AN, Granai CO, Rose PG, Hainsworth J, Lopez A, Weissman C, et al. Phase II study of liposomal doxorubicin in platinum- and paclitaxel-refractory epithelial ovarian cancer. *J Clin Oncol* 2000;18:3093–100.
- Marth C, Fiegl H, Zeimet AG, Muller-Holzner E, Deibl M, Doppler W, et al. Interferon-(gamma) expression is an independent prognostic factor in ovarian cancer. *Am J Obstet Gynecol* 2004;191:1598–605.
- Odunsi K, Sabbatini P. Harnessing the immune system for ovarian cancer therapy. *Am J Reprod Immunol* 2008;59:62–74.
- Sabbatini P, Odunsi K. Immunologic approaches to ovarian cancer treatment. *J Clin Oncol* 2007;25:2884–93.
- Barnett B, Kryczek I, Cheng P, Zou W, Curiel TJ. Regulatory T cells in ovarian cancer: biology and therapeutic potential. *Am J Reprod Immunol* 2005;54:369–77.
- Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *New Engl J Med* 2003;348:203–13.
- Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol Oncol* 2012;124:192–8.
- Kandalaf LE, Singh N, Liao JB, Facciabene A, Berek JS, Powell J, et al. The emergence of immunomodulation: combinatorial immunotherapy opportunities for the next decade. *Gynecol Oncol* 2010;116:222–33.
- Nehme A, Albin N, Caliaro MJ, Guichard S, Jozan S, Julia AM, et al. Mechanism of interaction between cisplatin and human recombinant interaction gamma in human ovarian-cancer cell lines. *Int J Cancer* 1995;61:643–8.
- Marth C, Windbichler GH, Hausmaninger H, Petru E, Estermann K, Pelzer A, et al. Interferon-gamma in combination with carboplatin and paclitaxel as a safe and effective first-line treatment option for advanced ovarian cancer: results of a phase I/II study. *Int J Gynecol Cancer* 2006;16:1522–8.
- Clark S, McGuckin MA, Hurst T, Ward BG. Effect of interferon (gamma) and tumour necrosis factor (alpha) on sensitivity to cisplatin in ovarian carcinoma cell lines. *Cancer Immunol Immunother* 1994;39:100–4.
- Windbichler GH, Hausmaninger H, Stummvoll W, Graf AH, Kainz C, Lahodny J, et al. Interferon-gamma in the first-line therapy of ovarian cancer: a randomized phase III trial. *Br J Cancer* 2000;82:1138–44.
- Alberts DS, Marth C, Alvarez RD, Johnson G, Bidzinski M, Kardatzke DR, et al. Randomized phase 3 trial of interferon gamma-1b plus standard carboplatin/paclitaxel versus carboplatin/paclitaxel alone for first-line treatment of advanced ovarian and primary peritoneal carcinomas: results from a prospectively designed analysis of progression-free survival. *Gynecol Oncol* 2008;109:174–81.
- Xu L, Xu Z, Xu M. Glucocorticoid treatment restores the impaired suppressive of regulatory T cells in patients with relapsing-remitting multiple sclerosis. *Clin Exp Immunol* 2009;158:26–30.
- Aristimuno C, Navarro J, de Andres C, Martinez-Gines ML, Gimenez-Roldan S, Fernandez-Cruz E, et al. Expansion of regulatory CD8⁺

- T-lymphocytes and fall of activated CD8⁺ T-lymphocytes after iv methyl-prednisolone for multiple sclerosis relapse. *J Neuroimmunol* 2008;204:131–5.
19. Ashwell JD, Lu FWM, Vacchio MS. Glucocorticoids in T cell development and function. *Annu Rev Immunol* 2000;18:309–45.
 20. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005;202:1691–701.
 21. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med* 2007;13:54–61.
 22. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* 2013;38:729–41.
 23. Machiels JPH, Todd Reilly R, Emens LA, Ercolini AM, Lei RY, Weintraub D, et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. *Cancer Res* 2001;61:3689–97.
 24. Eralp Y, Wang X, Wang JP, Maughan MF, Polo JM, Lachman LB. Doxorubicin and paclitaxel enhance the antitumor efficacy of vaccines directed against HER 2/neu in a murine mammary carcinoma model. *Breast Cancer Res* 2004;6:R275–83.
 25. Ehrke MJ, Verstovsek S, Maccubbin DL, Ujhazy P, Zaleskis G, Berleth E, et al. Protective specific immunity induced by doxorubicin plus TNF-(alpha) combination treatment of EL4 lymphoma-bearing C57BL/6 mice. *Int J Cancer* 2000;87:101–9.
 26. Ewens A, Luo L, Berleth E, Alderfer J, Wollman R, Hafeez BB, et al. Doxorubicin plus interleukin-2 chemoimmunotherapy against breast cancer in mice. *Cancer Res* 2006;66:5419–26.
 27. Zhu S, Waguespack M, Barker SA, Li S. Doxorubicin directs the accumulation of interleukin-12-induced IFN(gamma) into tumors for enhancing STAT1-dependent antitumor effect. *Clin Cancer Res* 2007;13:4252–60.
 28. Alagkiozidis I, Facciabene A, Carpenito C, Benencia F, Jonak Z, Adams S, et al. Increased immunogenicity of surviving tumor cells enables cooperation between liposomal doxorubicin and IL-18. *J Transl Med* 2009;7:104.
 29. Lebel-Binay S, Berger A, Zinzindohoue F, Cugnene PH, Thiounn N, Fridman WH, et al. Interleukin-18: biological properties and clinical implications. *Eur Cytokine Netw* 2000;11:15–25.
 30. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukocyte Biol* 2003;73:213–24.
 31. Logan TF, Robertson MJ. Interleukins 18 and 21: biology, mechanisms of action, toxicity, and clinical activity. *Curr Oncol Rep* 2006;8:114–9.
 32. Osaki T, Peron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, et al. IFN-(gamma)-inducing factor/IL-18 administration mediates IFN-(gamma)- and IL-12- independent antitumor effects. *J Immunol* 1998;160:1742–9.
 33. Coughlin CM, Salhany KE, Wysocka M, Aruga E, Kurzawa H, Chang AE, et al. Interleukin-12, and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J Clin Invest* 1998;101:1441–52.
 34. Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, et al. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin- induced tumor apoptosis, respectively. *J Immunol* 1999;163:583–9.
 35. Robertson MJ, Mier J, Logan T, Koon H, Weisenbach J, Roberts S, et al. Tolerability and anti-tumor activity of recombinant human IL-18 (rhIL-18) administered as five daily intravenous infusions in patients with solid tumors [abstract]. *J Clin Oncol* 22:14s, 2004 (suppl; abstr 2553).
 36. Robertson MJ, Mier JW, Logan T, Atkins M, Koon H, Koch KM, et al. Clinical and biological effects of recombinant human interleukin-18 administered by intravenous infusion to patients with advanced cancer. *Clin Cancer Res* 2006;12:4265–73.
 37. Robertson MJ, Kirkwood JM, Logan TF, Koch KM, Kathman S, Kirby LC, et al. A dose-escalation study of recombinant human Interleukin-18 using two different schedules of administration in patients with cancer. *Clin Cancer Res* 2008;14:3462–9.
 38. Rose PG, Hawthorne Maxson J, Fusco N, Mossbrugger K, Rodriguez M. Liposomal doxorubicin in ovarian, peritoneal, and tubal carcinoma: a retrospective comparative study of single-agent dosages. *Gynecol Oncol* 2001;82:323–8.
 39. Tarhini AA, Millward M, Mainwaring P, Kefford R, Logan T, Pavlick A, et al. A phase 2, randomized study of SB-485232, rhIL-18, in patients with previously untreated metastatic melanoma. *Cancer* 2009;115:859–68.
 40. DOXIL (doxorubicin HCl liposome injection). Product Information June, 2008.
 41. Struemper H, Koch K, Bauman J, Robertson M, Toso J. Physiologically-based model of recombinant human IL-18 pharmacokinetics [abstract]. In: Proceedings of the 2011 American Conference on Pharmacometrics (ACoP); 2011 Apr 3–6; San Diego, CA: <http://www.go-acop.org/2011/posters>.
 42. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. *J Clin Oncol* 2001;19:3312–22.
 43. Alagkiozidis I, Facciabene A, Tsiatas M, Carpenito C, Benencia F, Adams S, et al. Time-dependent cytotoxic drugs selectively cooperate with IL-18 for cancer chemo-immunotherapy. *J Transl Med* 2011;9:77.
 44. Wagner U, Marth C, Largillier R, Kaern J, Brown C, Heywood M, et al. Final overall survival results of phase III GCIG CALYPSO trial of pegylated liposomal doxorubicin and carboplatin vs paclitaxel and carboplatin in platinum-sensitive ovarian cancer patients. *Br J Cancer* 2012;107:588–91.
 45. Green AE, Rose PG. Pegylated liposomal doxorubicin in ovarian cancer. *Int J Nanomedicine* 2006;1:229–39.
 46. Markman M. Pegylated liposomal doxorubicin: appraisal of its current role in the management of epithelial ovarian cancer. *Cancer Manage Res* 2011;3:219–25.
 47. Robertson MJ, Kline J, Weisenbach J, Struemper H, Murray S, Bauman J, et al. A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkins lymphoma. *J Immunol* 2013; 36:331–41.
 48. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res* 2009;15:7412–20.
 49. Koch KM, Jaworski D, Kirby L, Kathman S, Bell B, Robertson M, et al. 270 POSTER Recombinant human IL-18 (iboctadekin) PKPD and clinical activity in phase I-II. *Eur J Cancer Suppl* 2006;4:86.
 50. Terme M, Ullrich E, Aymeric L, Meinhardt K, Desbois M, Delahaye N, et al. IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res* 2011;71:5393–9.
 51. Terme M, Ullrich E, Aymeric L, Meinhardt K, Coudert JD, Desbois M, et al. Cancer-induced immunosuppression: IL-18-elicited immunoinhibitory NK cells. *Cancer Res* 2012;72:2757–67.