Chemo-immunotherapy Using Pegylated Liposomal Doxorubicin and Interleukin-18 in Recurrent Ovarian Cancer: a Phase I Dose-escalation Study

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Abstract

Recombinant interleukin-18 (IL-18; SB-485232) is an immunostimulatory cytokine, with demonstrated antitumor activity in combination with pegylated liposomal doxorubicin (PLD) in preclinical models. This phase I study evaluated the safety, tolerability and biological 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 ≥3 prior chemotherapy regimens. SB-485232 up to 100 μg/kg with PLD had an acceptable safety profile. Common drug-related adverse events (AEs) were grade 1 or 2 (no grade 4 or 5 AEs). Concomitant PLD administration did not attenuate the biological activity of IL-18, with maximal SB-485232 biological activity already observed at 3 μg/kg. Ten of 16 enrolled subjects (63%) completed treatment, while 5 (31%) subjects progressed on treatment. A 6% partial objective response rate, and a 38% stable disease rate were observed. We provide pilot data suggesting 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.
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 mortality in women in the US (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 survival benefit as second-line treatment for platinum-resistant ovarian cancer (2). Pegylated liposomal doxorubicin (PLD) was shown to have a 14-20% response rate as monotherapy in recurrent ovarian cancer (3, 4) and has received US Food and Drug Administration 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 over 1800 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 interferon gamma (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 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 demonstrated 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 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 6 cycles of chemoinmunotherapy (16). Furthermore, adverse biological interactions might have also accounted for the lack of benefit in the combination with paclitaxel, since the accompanying steroids can suppress effector T cell function and induce T regulatory (Treg) cell 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, i.e. 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+ 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 2 (IL-2), IL-12, or tumor necrosis factor-alpha (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 chemoinmunotherapy combinations. In a mouse model of ovarian cancer, we reported that tumor cells surviving the direct toxicity of PLD, upregulated surface expression of major histocompatibility complex (MHC) class I molecules and the death receptor Fas and became susceptible to immune attack, enhancing recognition and killing by activated T and NK cells (28).

IL-18 is an immunostimulatory cytokine known to induce the production of Th1-type cytokines and chemokines like 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 up-regulates 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. While IL-18 or PLD monotherapy had a moderate antitumor effect, in combination, they significantly restricted tumor growth, augmented overall survival 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 cancer patients with advanced solid tumors and lymphomas, and was found to be biologically active and well-tolerated without reaching a maximally tolerated dose (MTD) even at 1000 μg/kg (35, 36). The biological 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 to 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 IV every four weeks for a minimum of four cycles, per manufacturer’s recommendation. Several prospective non-randomized 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) (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. Since 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 progression), 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 phase II trials, biological activity and clinical efficacy were documented at the lowest dose of 10 μg/kg, with a partial response 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 day 2 and day 9 was chosen, given the similar pharmacokinetic and pharmacodynamic effects shown with dosing over 5 consecutive days vs. dosing once a week. Also, a weekly dosing regimen allowed for IL-18 binding protein 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 biological activity; we gathered pilot pharmacodynamic 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, non-randomized, dose-escalation, safety and tolerability phase I clinical study (GSK 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 prior to enrollment. Study drug SB-485232, a recombinant form of human IL-18 (rhIL-18) supplied by GSK (Research Triangle Park, NC), 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 intravenously [IV]) 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 per cohort) to 3, 10, 30, and 100 μg/kg. SB–485232 was administered IV 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 partial response after completing four cycles of experimental treatment, were allowed to continue PLD monotherapy during any follow-up period, 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 prior to initiation, one hour after initiation, immediately prior to termination of infusion (2 hours), and at 4, 6, 8, 48, and 168 hours after initiation of SB-485232 infusion on Day 2 and Day 4 of Cycle 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 (λz) 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)/λz and clearance (CL) was calculated as dose/AUC_(0-t).

**Pharmacodynamics and biomarkers**

Blood samples were collected for quantification of plasma cytokines and chemokines including GM-CSF, CXCL10 (IP-10), CXCL9 (MIG), CCL2 (MCP-1), IFN-γ, TNF-α, IL-1, IL-2, IL-6,
IL-10, IL-12, IL-8, prior to initiation of PLD infusion on Day 1 of Cycles 1 to 4, and prior to and 4 hours after initiation of each SB-485232 infusion. Also, blood samples were collected for quantification of plasma IL-18 binding protein (IL-18BP) prior to initiation of PLD infusion on Day 1 of Cycle 1, and prior to initiation of each SB-485232 infusion. Blood samples were collected for flow cytometry analysis prior to initiation of PLD infusion (Cycles 1 and 4), prior to initiation of SB-485232 infusion, and 4, 48, and 168 hours after initiation of SB-485232 infusion on Day 2 of Cycle 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 magnetic resonance imaging [MRI]). Radiologic assessments (using the same methodology as was used at baseline) were performed 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 [CR], partial response [PR], stable disease [SD], and progressive disease [PD]) 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, standard deviation, 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 ≥3 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 (AEs). 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 AEs were Grade 1 or 2 chills (81%), nausea (75%), anemia (63%), fatigue (56%), hyperglycemia (50%), or pyrexia (50%), which did not appear to be dependent on the dose of SB-485232 (Table 2). Hematological 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 AEs 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 AEs, 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 AEs was 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 AEs 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 AEs were reported. Four subjects experienced 10 non-fatal, serious AEs. 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 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.

Cardiac toxicity was evaluated by 12-lead ECGs before, during, and after treatment, and by pre- and post-treatment 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 post-baseline 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), while 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 AE 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 due to disease progression (at the dose of 1, 10, or 100 μg/kg of SB-485232, respectively), while another subject (at the dose of 30 μg/kg SB-485232) had evidence of SD 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\(\text{max}\) and AUC values appeared to be less than dose-proportional (Fig. 1). This non-linear 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 dosing of SB-485232 (3 to 100 \(\mu\)g/kg) in combination with 40 mg/m\(^2\) PLD.

**Biological 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 post-dose (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 biological effects were seen with NK cells, both in cell counts and changes in activation status. CD56\(^{\text{dim}}\) CD16\(^+\) NK cells showed a dose-dependent increase in the percentage of activated cells expressing CD69 at the 4-hour and 48-hour time points (Fig. 3a). Interestingly, the maximal biologic effect with activated CD56\(^{\text{dim}}\) CD16\(^+\) NK cells was observed at a dose as low as 3 \(\mu\)g/kg in this study. The level of activated CD56\(^{\text{dim}}\) CD16\(^+\) NK cells induced after dosing were similar after Cycle 1 and 4, demonstrating that the biological 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+ T regulatory cells, monocytes or neutrophils (data not shown).

Almost all of the measured soluble cytokine and chemokine biomarkers showed strong responses 4 hours after SB-485232 dosing, with a several-fold increase from pre-dose levels. In individuals dosed with 3-100 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 post-dose. Serum levels of IFN-γ, CCL2, CXCL9 and CXCL10 were elevated several fold from baseline (Fig 4b). 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 3 μg/kg dose, while CCL2 reached a maximum at 10 μg/kg dose (Fig. 4a). The levels of TNF-α and GM-CSF were not significantly changed at 1 and 3 μg/kg dose, 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 towards a plateau at the 10 μg/kg dose (Fig 4). Importantly, cytokine or chemokine responses to SB-485232 were not attenuated over the 4 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% (1/16; 95% confidence interval CI = 0%, 18.1%) and the SD rate was 38% (6/16). 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). Based on this analysis, eight of sixteen subjects had either stable overall tumor burden or a reduction of the overall tumor
burden. Three heavily pretreated subjects had SD 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 (6 cycles of PLD) during the follow-up period. Subject 5004 (3 μg/kg, 4 lines of prior therapy) had a PR lasting over 9.6 months. Subject 2001 (1 μg/kg, 5 lines of prior therapy) had SD over the 13-month follow-up period and had a significant reduction in CA-125 (Fig. 5b). Subject 5008 (10 μg/kg, 5 lines of prior therapy) had SD for 6.3 months. Subject 5005 (3 μg/kg, 6 lines of prior therapy) had the greatest reduction in tumor volume (53%) but was considered to have PD 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 chemo-immunotherapy 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, since 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 1000 μg/kg, and has demonstrated immunomodulatory activity (35, 36). The biological activity of IL-18 in vivo in cancer patients included lymphocyte activation and the detection 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 (30 mg/m² every 28 days) alone or in combination with carboplatin (AUC 5) 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 biological 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%) were platinum-resistant and had received >3 prior regimens. Importantly, there was no positive drug interaction in terms of toxicity, and the most common AEs 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 Th1 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 CD56dim NK cells. CD69 is an important marker of NK
cell activation. In previous murine studies using EL4 T cell lymphoma, where combination of IL-18 with doxorubicin showed synergistic anti-tumor 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 μg/kg dose, we observed an optimal Th1 cytokine and chemokine activation profile, including a significant increase in serum IFN-γ 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 appeared to plateau at the dose of 3 μg/kg. Additionally, 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 μg/kg dose and consistently increased at the 10 μg/kg dose, further supporting the 3 μg/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 μg/kg; one subject had a PR, and the other experienced PD 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 biological 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 four hours post exposure to SB-485232. This was interpreted as likely due to cell activation and margination rather than PLD-induced depletion of activated T cells, since 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 repeat 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/m$^2$ appears to be suitable for combining with immunostimulatory drugs, and the
optimal expansion phase dose of SB-485232 appears to be 3 $\mu$g/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 pro-
inflammatory Th1 cytokine and chemokine profile, which is more similar to the biological
effects observed in our patients. Furthermore, plasma levels of SB-485232 were >10 ng/mL for
subjects in the lowest dose cohort receiving 1 $\mu$g/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 cancer patients.

In conclusion, the present study demonstrates the safety, tolerability and biological
efficacy of SB-485232 in combination with PLD. The evidence that sufficient biological activity
is observed at a low dose of SB-485232 (3 $\mu$g/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.

References


(2) Herzog TJ, Pothuri B. Ovarian cancer: A focus on management of recurrent disease. Nat

platinum therapy in patients with ovarian cancer previously treated with cisplatin. J Clin

study of liposomal doxorubicin in platinum- and paclitaxel-refractory epithelial ovarian
(gamma) expression is an independent prognostic factor in ovarian cancer. Am J Obstet

(6) Odunsi K, Sabbatini P. Harnessing the immune system for ovarian cancer therapy. Am J


Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. New Engl J

(10) 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

of immunomodulation: Combinatorial immunochemotherapy opportunities for the next

interaction between cisplatin and human recombinant interaction gamma in human

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

(14) 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.

Interferon-gamma in the first-line therapy of ovarian cancer: A randomized phase III

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


(41) Struemper H, Koch K, Bauman J, Robertson M, Toso J. Physiologically-based model of recombinant human IL-18 pharmacokinetics. 2011 American Conference on Pharmacometrics (ACoP) 2011;San Diego, CA; USA.


### Table 1

Most frequently reported AEs (at least 4 subjects) regardless of causality

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<tr>
<th>Preferred term</th>
<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>
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AE, adverse event.
Table 2.

Adverse events with maximum grade 3

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<th>3 μg/kg N=4</th>
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<th>30 μg/kg N=3</th>
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AE, adverse event; PLD, pegylated liposomal doxorubicin.
Figure legends

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

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

Figure 3
\(a\), Percentage change from pre-dose levels of activated CD56$^{DIM}$ CD16$^+$ CD69$^+$ NK Cells at 4 and 48 hours after receiving SB-485232 averaged over available cycles. \(3b\), 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, pre-dose, 4 and 48 hours after receiving SB-485232.

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, IL-8; % change for IL-10).

Figure 5
\(a\), Waterfall plot of maximum percentage reduction of tumor burden for subjects that completed Cycle 4. \(5b\). Waterfall plot of maximum percentage reduction of CA-125.
Figure 1
Profile of median SB-485232 plasma concentrations by dose group.
Figure 2 Absolute CD8$^+$ T cell counts by subject for Cycles 1 and 4 at pre-dose, 4, 48, and 168 hours after receiving SB-485232.
Figure 4

IFN-gamma

CXCL9

TNF-alpha

GM-CSF

CXCL10

CCL2

IL-2

IL-6

IL-8

IL-10
Plot of Maximum Percent Reduction from Baseline in Tumor Size by Subject and Best Overall Response

Subjects with missing information on tumor size at post-baseline scans are not included.

Subjects missing post-baseline CA-125 information are not included.
Cancer Immunology Research

Chemo-immunotherapy Using Pegylated Liposomal Doxorubicin and Interleukin-18 in Recurrent Ovarian Cancer: a Phase I Dose-escalation Study

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