in vivo *36*: 1949-1958 (2022)
doi:10.21873/invivo.12917

A Phase II Trial to Evaluate the Efficacy of Bortezomib and Liposomal Doxorubicin in Patients With BRCA Wild-type Platinum-resistant Recurrent Ovarian Cancer (KGOG 3044/EBLIN)

YONG JAE LEE[1*], AERAN SEOL[2*], MARIA LEE[2], JAE-WEON KIM[2], HEE SEUNG KIM[2], KIDONG KIM[3], DONG HOON SUH[3], SUNGHOON KIM[1], SANG WUN KIM[1] and JUNG-YUN LEE[1]

[1]*Department of Obstetrics and Gynecology, Institute of Women's Life Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea;*
[2]*Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea;*
[3]*Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea*

Abstract. *Background/Aim: The majority of targeted therapies are focused on BRCA mutations, homologous recombination repair deficiency, and BRCA wild-type platinum-sensitive recurrent ovarian cancer. There is a growing need for platinum-resistant patients without BRCA mutations. Herein, we conducted a phase II multicenter study evaluated the efficacy and safety of bortezomib plus pegylated liposomal doxorubicin (PLD) in patients with BRCA wild-type platinum-resistant recurrent ovarian cancer (NCT03509246). Patients and Methods: Ovarian cancer patients with wild-type BRCA who experienced platinum-resistant recurrence after three or less prior treatment cycles from three Institutions were included. All patients received bortezomib, 1.3 mg/m^2 subcutaneously (days 1, 4, 8, and 11), and PLD, 40 mg/m^2 intravenously (day 4), every 4 weeks. The primary endpoint was best objective response rate (ORR), and secondary endpoints included disease control rate, progression-free survival (PFS), overall survival, and safety. Targeted sequencing was performed to evaluate biomarkers and their potential association with response to treatment. Results: The trial was terminated after 23 patients were recruited because of slow accrual. The median follow-up was 29.5 months. The overall ORR was 8.7% (2/23); partial response was observed in two patients. The median duration of response was 10.5 months, and median PFS was 2.9 months. Treatment-related adverse events (TRAEs) of grade 3/4 were reported in 43.5% of patients. One patient who exhibited TRAEs discontinued treatment. However, grade 4/5 TRAEs were not observed. Mutations in TP53 and CDK12 were detected in 67% (14/21) and 24% (12/21) of patients, respectively. Two patients with partial response harbored mutations in genes related to homologous recombination repair deficiency, including BRCA2, ATM, and CDK12. Conclusion: The combination of bortezomib and PLD was well tolerated; however, antitumor activity was not sufficient to warrant further investigation in ovarian cancer.*

Ovarian cancer is one of the most lethal gynecological cancers in women (1). Although the majority of patients with ovarian cancer initially respond to front-line therapy involving maximal cytoreductive surgery combined with platinum-based chemotherapy, unfortunately, most patients relapse during or after treatment with eventual drug-resistant disease (2). Platinum resistance is defined as disease relapse within six months of the last platinum-based chemotherapy dose (3). Non-platinum-based single-agent chemotherapy is the standard of care. Patients with platinum-resistant disease typically have low response rates to subsequent chemotherapy (<10%), median progression-free survival (PFS) of approximately 3 months, and median overall survival (OS) of approximately 12 months (4, 5).

To achieve higher responses, novel agents or treatment regimens need to be developed. Platinum-resistant disease can

*These Authors contributed equally to this study.

Correspondence to: Jung-Yun Lee, MD, Ph.D. Department of Obstetrics and Gynecology, Institute of Women's Life Medical Science, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea. Tel: +82 222282230, Fax: +82 23138357, e-mail: jungyunlee@yuhs.ac

Key Words: Bortezomib, pegylated liposomal doxorubicin, antitumor activity, platinum-resistant recurrent ovarian cancer, BRCA.

be treated with non-platinum-based chemotherapy or molecularly targeted agents, such as poly-ADP-ribose polymerase inhibitors (PARPis) or immunotherapy. PARPis have emerged as novel agents for patients with recurrent ovarian cancer in various settings, including for treatment of *BRCA* mutation-related relapsed disease (6, 7) or as maintenance therapy in patients with platinum-sensitive recurrence (8, 9). However, PARPis are not very effective in patients with wild-type *BRCA*. The addition of bevacizumab to chemotherapy showed PFS benefit for patients with platinum-resistant disease regardless of *BRCA* mutation (4). The efficacy of immune checkpoint inhibitors has been disappointing in platinum-resistant settings (10, 11). The introduction of targeted agents and immunotherapies have not yet shown major advances beyond the addition of bevacizumab to chemotherapy in patients with platinum-resistant disease.

The Cancer Genome Atlas suggested 22 druggable targets in 2011. Among them, *CCNE1* amplification is found in approximately 20%, and *BRCA* mutation has been reported to be mutually exclusive (12). Preclinical data supported that *CCNE1*-amplified tumor cells show specific sensitivity to the proteasome inhibitor bortezomib and suggested the possibility of a unique therapeutic approach in high-grade serous ovarian cancer (HGSC) (13). Bortezomib is one of the most commonly used drugs in multiple myeloma (14). In a phase III trial, combination of bortezomib and pegylated liposomal doxorubicin (PLD) had a synergistic effect on survival compared to PLD monotherapy in patients with relapsed or refractory multiple myeloma (15). PLD is commonly used for the treatment of platinum-resistant ovarian cancer. Therefore, we hypothesized that combining bortezomib and PLD would be effective and safe in patients with *BRCA* wild-type platinum-resistant ovarian cancer.

Patients and Methods

Study design and participants. A phase II, single-arm, multicenter study was conducted at three cancer centers across the Korean Gynecologic Oncology Group. Eligible patients were women aged 19 years or older, who had platinum-resistant (defined as progression within 6 months after the last platinum-based chemotherapy) or refractory (defined as progression within 1 month after the last platinum-based chemotherapy) recurrent ovarian cancer with *BRCA* wild-type and a histologically confirmed diagnosis of high-grade serous, endometrioid, carcinosarcoma, mixed Müllerian with high-grade serous component, clear-cell, or low-grade serous, primary peritoneal cancer, or fallopian tube cancer. Other inclusion criteria were an Eastern Cooperative Oncology Group performance status of 0-2, adequate bone marrow function (absolute neutrophil count ≥1,500 cells/ml, hemoglobin level ≥9.0 g/dl, and platelet count ≥100,000/ml), adequate renal function [creatinine level ≤1.5× the upper limit of normal (ULN)], and hepatic function (alanine and aspartate aminotransferase concentrations ≤3× ULN or ≤3× ULN in case of liver metastases and total bilirubin level ≤1.5× ULN).

Key exclusion criteria included prior use of four or more lines of anticancer therapies, suspected deleterious mutation (germline or somatic) in *BRCA*, known additional malignancy that is progressing or has required active treatment within the last 3 years, and active infection, including tuberculosis, hepatitis B, hepatitis C, or human immunodeficiency virus. All inclusion and exclusion criteria are listed in Table I.

This study was approved by the institutional review boards of the participating centers (IRB No., Yonsei University Severence Hospital 4-2017-1189; Seoul National University Bundang Hospital 1712-034-905; Seoul National University College of Medicine B-1709-420-004). All participants provided informed consent, and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines.

Procedures. Patients received bortezomib, 1.3 mg/m^2, as a subcutaneous injection (days 1, 4, 8, and 11), and PLD, 40 mg/m^2, intravenous infusion, for more than 60 min on day 4 of a 4-week cycle. Patients could receive up to six cycles and continued treatment until disease progression or unacceptable side-effects.

Dose modifications (interruptions and reductions) were allowed for the management of adverse events, and dose re-escalation was not allowed. Two dose reductions were allowed for both bortezomib (first reduction to 1.1 mg/m^2, second reduction to 0.9 mg/m^2) and PLD (first reduction to 30 mg/m^2, second reduction to 20 mg/m^2) in the event of toxicity. Bortezomib and PLD were interrupted in patients with grade 4 toxicities. For grade 3 toxicities, patients underwent dose reduction, and for grade 2 or below toxicities, patients were maintained on the same dose. At first occurrence of grade 3/4 toxicities, bortezomib and PLD were delayed up to 3 weeks until recovery to grade 1 or improvement. When non-hematologic toxicities were recovered, but the hematologic toxicities were grade 2, patients underwent one dose reduction of PLD, and when hematological toxicities were grade 3, patients underwent permanent discontinuation. When non-hematologic toxicity except for hair loss and neurotoxicity did not recover to grade 1 or lower by 3 weeks from the scheduled administration time, patients underwent permanent discontinuation. When non-hematologic toxicities and hematologic toxicities were recovered, but neurotoxicity was grade 2, patients underwent one dose reduction of bortezomib, and for grade 3/4, patients underwent permanent discontinuation. When neutropenic fever occurred in the previous cycle, patients underwent one dose reduction of PLD.

Measurable disease and tumor response were assessed using RECIST version 1.1. Tumor response was assessed using contrast CT scans after three and six cycles of chemotherapy. Additional chest CT, pelvic MRI, and whole-body PET-CT were recommended if investigators considered it necessary for the evaluation of tumor response. Safety and adverse events were assessed on the day of each cycle and were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0.

Outcomes. The primary endpoint was the objective response rate (ORR) according to RECIST version 1.1, which included the patients with measurable disease who had a complete or partial response. Secondary endpoints were PFS, OS, duration of response, proportion of disease control, and safety. PFS was defined as the time from the start of treatment until the first documented sign of disease progression or death from any cause, and OS was defined as the time

Table I. *Inclusion and exclusion criteria used to select participants for the clinical trial.*

Inclusion criteria
(1) Patients over 19 years of age (2) Patients diagnosed with epithelial ovarian cancer, fallopian tube cancer, or peritoneal cancer based on histologic findings obtained from biopsy/surgery and having a histologic type of high-grade serous cancer. (3) In the absence of a mutation of the BRCA gene (no germline mutation should be identified, not in the case of a somatic mutation) (4) Recurrence within 6 months after platinum-based chemotherapy. (5) ECOG performance 2 points or less. (6) Blood tests performed within 2 weeks of enrollment meet the following results: - Neutrophil >1,500/mm^3 - Platelet >100,000/mm^3 - Hemoglobin >9.0 g/dl - Total bilirubin <1.5×upper limit of normal (ULN) - AST/ALT <3.0×ULN (or <5×ULM in case of liver metastases) - Creatinine <1.5×ULN; Electrolytes should be within normal limits. (7) Patients who understand the content of the study description and voluntarily agree in writing. (8) Patients who are willing and able to adhere to the visit schedule, treatment plan, laboratory tests, and other testing procedures.
Exclusion criteria
(1) Patients previously treated with three or more anticancer regimens. Maintenance therapy is not considered a separate regimen (*e.g.*, >paclitaxel-carboplatin-bevacizumab therapy). In the combined chemotherapy, when one drug is subtracted due to toxicity, the regimen is not counted as a change (*e.g.*, >paclitaxel-carboplatin chemotherapy, paclitaxel was discontinued due to neurotoxicity and carboplatin alone was not considered as a change of regimen). (2) Patients diagnosed with other tumors other than ovarian cancer for the last 5 years (not CIS). (3) Pregnant woman. (4) Patients with uncontrolled infection. (5) In the case of congenital immune disease or acquired immune deficiency syndrome. (6) Women in lactation. (7) History with Grade 3 or higher peripheral neuropathy. (8) History of hypersensitivity reactions to PLD or bortezomib. (9) If the physician is judged to have any serious illness or medical condition for which the patient is not suitable for the study. (10) Patients with confirmed BRCA somatic mutations. (11) Patients with acute diffuse infiltrative lung disease and cardiovascular disease.

from the first treatment until death from any cause. Duration of response was assessed in patients who achieved a response and was defined as the time from the first documented response until the time of documented progression or death from any cause. Disease control was defined as the proportion of patients who achieved a complete or partial response or stable disease.

Statistical analysis. We used the A'Hern single-stage design to calculate sample size. Previously reported data showed that the ORR of single-agent chemotherapy was 11.8% and that of single-agent chemotherapy combined with bevacizumab was 27.3% in platinum-resistant recurrent ovarian cancer (4). We expected that the objective response for bortezomib plus PLD would be 15%. If a total of 8 or more responses were observed, the treatment regimen was considered a success. In a single-stage phase II design with a one-sided 5% level of significance and 80% power, a single arm requires approximately 40 patients. Allowing for a follow-up loss rate of 10%, the total sample size was expected to be 44 patients.

We analyzed efficacy and safety in the intention-to-treat patients; all patients received at least one treatment dose. We calculated the proportions of patients achieving responses and associated 95% CIs using the Clopper-Pearson method as well as the median duration of response and PFS and associated 95% CIs using the Kaplan-Meier method. Survival analysis was performed using the Kaplan-Meier method with a log-rank test. Based on a decision of the Data Safety Monitoring Board, this study was terminated before completion of the planned patient recruitment because of poor accrual, and we analyzed data that were collected by the cutoff date of May 31, 2021. Statistical analyses were performed using the SPSS statistical software (version 21.0; IBM, Armonk, NY, USA).

Library preparation and next-generation sequencing (NGS). An NGS panel was designed for the detection of mutations in 225 ovarian cancer-related genes, including 15 homologous recombination deficiency genes (Table II). Custom RNA probes were designed for target-enrichment sequencing (Celemics, Seoul, Republic of Korea) and covered all unions of reported exons of the 225 genes (total count of regions was 3,690). The panel covered 637,421 bases of the human genome (hg19). All transcripts of genes reported in the UCSC database were included as targets to comprehensively detect SNVs, small insertion-deletion mutations, and structural variants.

For exploratory genomic analyses, tumor samples were prepared from formalin-fixed, paraffin-embedded tissues before treatment. Eighteen specimens were included in this study. Genomic DNA was sheared and processed by end-repair, dA-tailing, adapter

Table II. *Gene contents in the NGS panel that covers 225 ovarian cancer-related genes, including 15 homologous recombination deficiency genes.*

Gene							
MTOR	FOXL2	FAM135B	IDH2	U2AF1	SOX2	KRAS	AR
RUNX3	ATR	SMARCA2	BLM	MAPK1	ATP11B	CDK2	
ARID1A	PIK3CA	JAK2	TSC2	MN1	DCUN1D1	ERBB3	
MPL	ETV5	PAX5	TNFRSF17	EWSR1	FGFR3	CDK4	
MAGOH	TACC3	TJP2	MAPK3	NF2	KIT	MDM2	
JAK1	PDGFRA	GNAQ	CDH1	BCOR	TERT	FLT3	
FUBP1	KDR	NTRK2	FANCA	DDX3X	RICTOR	BRCA2	
RBM15	NFKB1	PTCH1	MAP2K4	KDM6A	FGFR4	GAS6	
NRAS	TET2	SET	NF1	ARAF	VEGFA	PNP	
PRCC	FBXW7	ABL1	STAT3	CLCN5	HSP90AB1	NKX2-1	
NTRK1	ADAM29	TSC1	ETV4	MED12	IL6	RAD51B	
DDR2	FAT1	NOTCH1	SPOP	NONO	EGFR	AKT1	
ABL2	PIK3R1	GATA3	PRKAR1A	ATRX	CDK6	IGF1R	
TPR	APC	RET	CANT1	BTK	MET	PALB2	
H3F3A	CSF1R	PTEN	RNF213	PHF6	NOS3	TP53	
RHOB	PDGFRB	IFITM1	SMAD4	MYCL	RHEB	TIAF1	
DNMT3A	FAT2	IFITM3	STK11	RAD54L	PPP2R2A	MYO18A	
MSH2	GABRA6	HRAS	GNA11	BCL9	FGFR1	RAD51D	
XPO1	NPM1	WT1	MAP2K2	MCL1	POLB	CDK12	
NFE2L2	NSD1	FAT3	MAP2K7	MDM4	MYC	ERBB2	
SF3B1	FOXO3	CBL	SMARCA4	AKT3	PTK2	BRCA1	
IDH1	ROS1	FLI1	NOTCH3	MYCN	MAPK15	RAD51C	
ERBB4	ESR1	PTPN11	JAK3	ALK	CD274	RPS6KB1	
VHL	CARD11	HNF1A	CEBPA	EML4	PDCD1LG2	BRIP1	
PPARG	RAC1	CDX2	KMT2B	EPCAM	CDKN2A	RPTOR	
RAF1	ETV1	RB1	AXL	FANCL	FGFR2	CCNE1	
MLH1	SMO	APEX1	ERCC2	ACVR1	CD44	AKT2	
MYD88	BRAF	MAX	PPP2R1A	MSTN	CCND1	CSNK2A1	
CTNNB1	EZH2	DICER1	SRC	STAT1	BIRC3	BCL2L1	
RHOA	FZD6	KNSTRN	GNAS	STAT4	BIRC2	ZNF217	
BAP1	CSMD3	MAP2K1	RUNX1	BARD1	ATM	SMARCB1	
GATA2	NDRG1	NTRK3	ERG	MECOM	CHEK1	CHEK2	

ligation, and pre-PCR for indexation of the NGS library for Illumina sequencing. Genomic DNA and capture probes were hybridized to the capture target regions using a Celemics Target Enrichment Kit. Captured regions were amplified by post-PCR for enrichment. The captured library was then sequenced on an Illumina NextSeq550 instrument (Illumina, San Diego, CA, USA), generating 2×150-bp reads.

Results

Patient characteristics. Between May 2018 and Jan 2020, 23 patients were enrolled in the study (Figure 1) and included in the intention-to-treat analysis. The median duration of follow-up at the time of data analysis (data cutoff point was Jan 31, 2021) was 29.5 months. At the data cutoff point, all 23 (100%) patients had discontinued the study; 19 (82.6%) patients discontinued of disease progression, 1 (4.4%) patient discontinued because of adverse events, and 3 (13%) patients were lost to follow-up. The clinical characteristics of the patients are shown in Table III.

Outcomes. Of the 23 patients, 19 patients were evaluable for objective response using the RECIST criteria. The overall confirmed ORR was 8.7% (2/23); partial response was observed in two patients. Disease control was achieved in 69.6% (16/23) patients (Table IV). Tumor shrinkage was noted in 9 (39.1%) of 23 patients who had at least one post-baseline efficacy assessment (Figure 2). The mean best percentage change of the target lesion size from the baseline was –0.6% (SD 19.1). The median duration of response was 10.5 months. At the data cutoff point, all 23 (100%) patients had disease progression and 9 (39.1%) patients had died. Furthermore, the median PFS was 2.9 months, and median OS was 19.0 months (Figure 3).

Treatment-related adverse events (TRAEs) (any grade) occurred in 21 (91.3%) patients (Table V). One patient (4.3%) discontinued the study because of an adverse event due to diarrhea. Grade 3/4 TRAEs were reported in 43.5% of patients. The most common grade 3/4 adverse event was diarrhea [two (8.7%)], followed by abdominal pain [one (4.3%)], acute pyelonephritis [one (4.3%)], anemia [one (4.3%)], anorexia

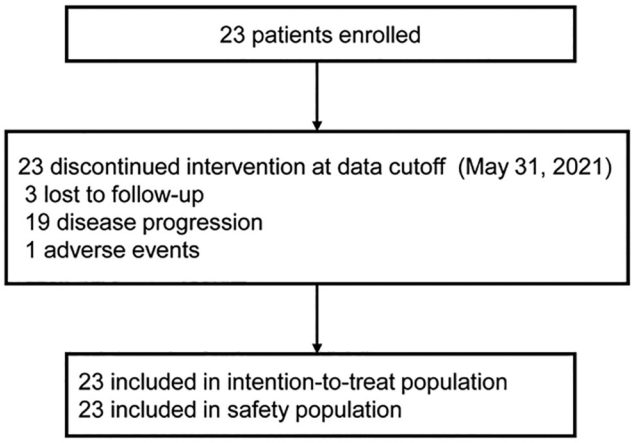

Figure 1. *Trial design*.

Table III. *Characteristics of enrolled patients*.

Characteristic	No. (%) (N=23)
Age, median (range), y	59 (36-81)
Histology subtype	
High-grade serous	23 (100)
Best response	
Partial response	2 (8.7)
Stable disease	14 (60.9)
Progressive disease	3 (13.0)
Not evaluable	4 (17.4)
ECOG performance status	
0	6
1	15
2	2
CA-125, median (range), U/mL	393.3 (11.6-2221.6)
No. of prior lines of chemotherapy, median (range)	1 (1-4)

ECGO, Eastern Cooperative Oncology Group.

Table IV. *Best response, overall response, and disease control to bortezomib plus liposomal doxorubicin treatment*.

Treatment response	No. (%)
Complete response	0
Partial response	2 (8.7%)
Stable disease	14 (60.9%)
Disease progression	3 (13.0%)
Not evaluable	4 (17.4%)
Overall response	2 (8.7%)
Disease control	16 (69.6%)

[one (4.3%)], herpes zoster infection [one (4.3%)], mucositis [one (4.3%)], neutropenia [one (4.3%)], and thrombocytopenia [one (4.3%)]. Grade 4/5 TRAEs were not observed. Serious TRAEs, including abdominal pain, acute pyelonephritis, diarrhea, dyspepsia, hepatic enzyme level elevation, herpes zoster infection, stomatitis, thrombocytopenia, were reported in eight patients. Dose reduction occurred in two (8.7%) patients, and all patients were subjected to a PLD dose reduction. Dose interruption occurred in 12 (52.2%) patients.

Exploratory gene expression profiles obtained from 21 samples from the patients enrolled in the study are shown in Figure 4. Missense mutations accounted for the majority (60.5%) of identified variants, followed by nonsense mutation (32.6%), splice-site mutation (4.7%), and frameshift deletion (2.3%). *TP53* and cyclin-dependent kinase 12 (*CDK12*) mutations were detected in 67% (14/21) and 24% (12/21) of patients, respectively. Other genes, including *FAT1*, *MYO18A*, and *NTRK1* were also identified. However, mutation in *CCNE-1* was not detected.

Cases of interest. There were two cases with partial remission with a durable response in this study. As a representative case, patient #6, with *BRCA2*, *ATM*, and *CDK12* mutations, demonstrated the best overall radiologic response of PR with a durable response to bortezomib and Caelyx of 5.8 months and received six cycles of PLD plus bortezomib. Patient #8, with *CDK12* mutation, demonstrated the best overall radiologic response of PR with a durable response to bortezomib and Caelyx of 8.2 months and received six cycles of PLD plus bortezomib.

Discussion

In this study, bortezomib plus PLD did not seem to provide substantial benefit in the treatment of patients with *BRCA* wild-type platinum-resistant recurrent ovarian cancer. The median PFS was 2.9 months, and the median duration of response was 3.0 months. Furthermore, bortezomib plus and PLD was found to have a manageable toxicity profile.

The incorporation of targeted therapies based on genetic testing has significantly altered the treatment landscape for patients with ovarian cancer. These targeted therapies are mainly focused on first-line therapy or platinum-sensitive recurrent ovarian cancer. However, effective treatments for platinum-resistant or platinum-refractory ovarian cancer remain limited. Non-platinum single-agent chemotherapies, including PLD, gemcitabine, topotecan, oral etoposide and paclitaxel, are used for platinum-resistant recurrent ovarian cancer patients. However, response rates to these agents are approximately 10%, and the PFS of these regimens is only approximately 3 months (16). PLD is the most commonly used chemotherapeutic agent in platinum-resistant ovarian cancer patients. Hence, optimal treatment strategies are warranted to enhance the effectiveness of PLD. Recently, addition of bevacizumab to these non-platinum single agents improved the PFS and increased the response rate (14);

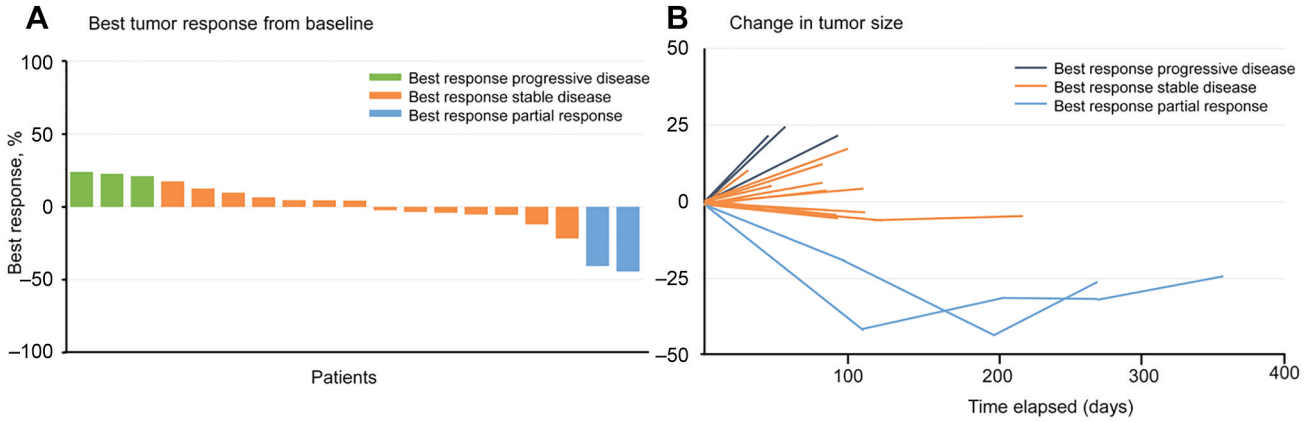

Figure 2. *Best percentage change from the baseline in target lesion size in patients. (A) Waterfall plot showing the percentage change in tumor size from baseline constituting the best response for each patient. (B) Spider plot showing responses for all patients.*

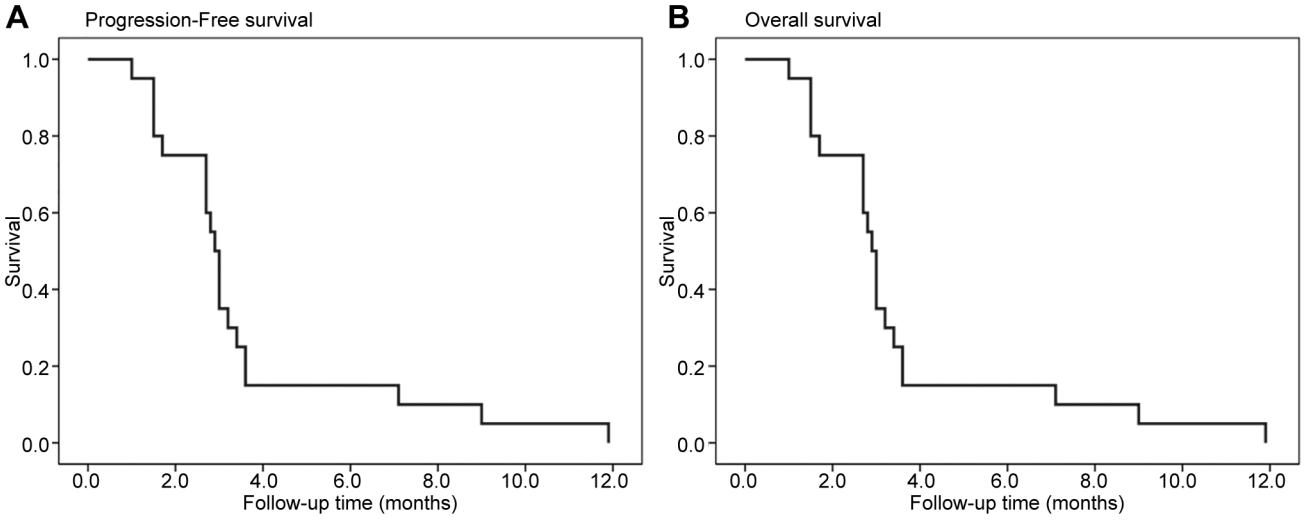

Figure 3. *Kaplan-Meier curves of progression-free survival (A) and overall survival (B).*

however, the effect of bevacizumab in patients with platinum-resistant ovarian cancer is still unsatisfactory.

PARPis are approved for the treatment of patients with ovarian cancer harboring *BRCA* mutations and olaparib plus bevacizumab for first-line maintenance treatment of homologous recombination repair deficiency, as well as in *BRCA* wild-type platinum-sensitive recurrent ovarian cancer. However, studies on targeted therapy and optimal therapeutic strategies in platinum-resistant patients without *BRCA* mutations are scarce. Recently, it has been reported that ovarian cancer patients with *CCNE-1* amplification express mutually exclusive *BRCA* mutations and exhibit poor prognosis (13). Furthermore, the results showed that these patients were sensitive to bortezomib and suggested the possibility of unique therapeutic strategies in HGSC (13). PLD can activate anti-apoptotic pathways *via* the activation of nuclear factor-κB (NF-κB), which potentially limited the effectiveness of the drug (17). Agents that inhibit the proteasome, such as bortezomib, are particularly effective in blocking the NF-κB pathway and provide a rationale for the combination of bortezomib plus PLD (18). Based on the aforementioned results, we designed this study with a combination of bortezomib plus PLD in platinum-resistant recurrent ovarian cancer patients without *BRCA* mutations.

In the AURELIA trial (4), a combination of bevacizumab and chemotherapy yielded an ORR of 30.0%, and the median PFS was 6.7 months in patients with platinum-resistant recurrent ovarian cancer. In the MITO 11 study (19), the addition of pazopanib to weekly paclitaxel for patients with

Table V. *Incidence and severity of drug-related treatment adverse events.*

Event	Patients (N=23)				
	Total n (%)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Abdominal pain	5 (21.7)	3 (13.0)	1 (4.3)	1 (4.3)	0 (0)
Acute pyleonephritis	2 (8.7)	0 (0)	1 (4.3)	1 (4.3)	0 (0)
Alopecia	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Anemia	5 (21.7)	0 (0)	4 (17.4)	1 (4.3)	0 (0)
Anorexia	4 (17.4)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)
Blurred vision	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Chest pain	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Constipation	3 (13.0)	3 (13.0)	0 (0)	0 (0)	0 (0)
Diarrhea	6 (26.1)	3 (13.0)	1 (4.3)	2 (8.7)	0 (0)
Dizziness	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Dyspepsia	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)
Fatigue	4 (17.4)	4 (17.4)	0 (0)	0 (0)	0 (0)
Fever	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)
Hepatic enzyme elevation	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)
Herpes zoster	3 (13.0)	1 (4.3)	1 (4.3)	1 (4.3)	0 (0)
Infusion related reaction	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)
Insomnia	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)
Mucositis	3 (13.0)	1 (4.3)	1 (4.3)	1 (4.3)	0 (0)
Myalgia	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Nausea	7 (30.4)	5 (21.7)	2 (8.7)	0 (0)	0 (0)
Neutropenia	13 (56.5)	0 (0)	12 (52.2)	1 (4.3)	0 (0)
Palmar-plantar erythyrodysesthesia syndrome	3 (13.0)	2 (8.7)	1 (4.3)	0 (0)	0 (0)
Peripheral sensory neuropathy	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)
Pleural effusion	1 (4.3)	0 (0)	1 (4.3)	0 (0)	0 (0)
Pruritis	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Purpura	7 (30.4)	6 (26.1)	1 (4.3)	0 (0)	0 (0)
Skin rash	4 (17.4)	2 (8.7)	2 (8.7)	0 (0)	0 (0)
Stomatitis	3 (13.0)	3 (13.0)	0 (0)	0 (0)	0 (0)
Thrombocytopenia	5 (21.7)	1 (4.3)	3 (13.0)	1 (4.3)	0 (0)
Vomiting	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Serious treatment related adverse event (TRAE)	8 (34.8)				
TRAE - Grade 3-4	10 (43.5)				
TRAE leading to dose reduction	2 (8.7)				
TRAE leading to dose interruption	12 (52.2)				
TRAE leading to dose discontinuation	1 (4.3)				

platinum-resistant or platinum-refractory ovarian cancer yielded an ORR of 56% and a median PFS of 6.3 months. In a previous phase II trial (20), the addition of bortezomib to PLD did not seem to provide substantial benefit in recurrent ovarian cancer. The ORR was 16% in 56 recurrent ovarian cancer patients, and the platinum-resistant group was closed at the interim analysis for lack of efficacy. The median duration of response was 4.8 months. In our study, the PFS was 2.9 months, and the median duration of response was 3.0 months.

Myelosuppression, gastrointestinal complaints, and dermatologic toxicities are considered to be the most common adverse events related to the combination of bortezomib and PLD (15). In our trial, the most frequent TRAEs were neutropenia, nausea, and dermatologic toxicities, which were predominantly of low grade. Grade 3 or higher-grade hematological toxicities (4.3% neutropenia and 4.3% thrombocytopenia) were generally consistent with those reported in previous studies in ovarian cancer (7% neutropenia and 10.3% thrombocytopenia). In our study,

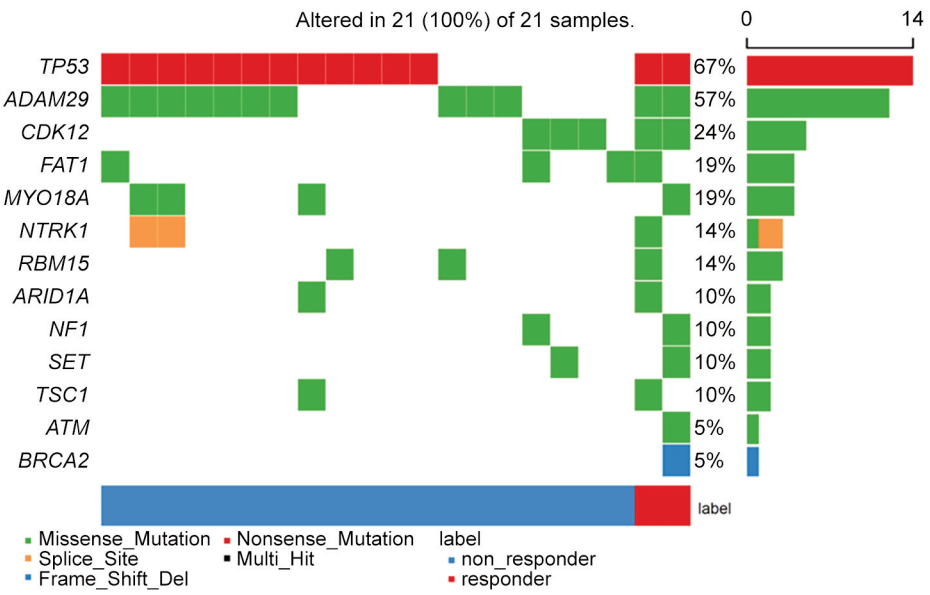

Figure 4. *Mutation profiles*.

gastrointestinal complaints were the most frequently reported grade 3/4 TRAEs, and no treatment-related death was recorded. Peripheral neuropathy, an established toxicity (of low grade) of bortezomib, was reported in 8.7% of patients. PLD-related toxicities were less frequent than in previous reports. No cardiac toxicities occurred, grade 3 mucositis was experienced only by one patient, and no grade 3 palmar-plantar erythyrodysesthesia syndrome was reported.

We performed NGS of tumor samples to identify gene expression profiles related to the bortezomib-PLD response. We observed partial response in two patients with somatic mutations in *BRCA2*, *ATM*, and *CDK12*. Ovarian cancers with somatic mutations in BRCA1/2, ATM and CDK12 genes could cause a defect in double-strand break repair by homologous recombination repair, which can be defined as a BRCAness (21). In addition, the incorporation of boltezomib may have affected BRCAness state. Proteasome is involved in homologous recombination repair, and inhibition of proteasome blocks the recruitment of DNA repair components BRCA1, FANCD2, and RAD51 (22). Neri *et al*. (23) showed that bortezomib induces a functional state of BRCAness in multiple myeloma cells. PLD is known to act as single-stranded and double-stranded DNA breaks by interfering with topoisomerase II-mediated repair. Previous study showed that HGSC patients with BRCA1/2 mutations experience superior sensitivity to PLD (24). In our study, sensitivity to PLD may have increased by inducing increased BRCAness with the somatic mutation in BRCA1/2, ATM and CDK12 genes. The NGS results in our study are only hypothesis generating and should be validated in studies of bortezomib with or without the addition of PLD. Further study is needed to investigate the predictive biomarker of therapeutic response of PLD with bortezomib.

Our study had several limitations. First, patient recruitment was closed earlier than the predesigned protocol. Second, the sample size was small, which limits the conclusions. Third, we observed no *CCNE-1* amplification in the sequencing data; therefore, we could not determine whether bortezomib plus PLD is effective in *BRCA* wild-type patients with *CCNE-1* amplification.

In conclusion, we observed no new toxicity profile for bortezomib plus PLD in patients with *BRCA* wild-type platinum-resistant recurrent ovarian cancer; however, insufficient antitumor activity of this combination does not warrant further phase III investigations.

Conflicts of Interest

The Authors have no competing interests to disclose in relation to this study.

Authors' Contributions

KDK, ML, JYL, YJL and ARS designed the study and collected the data. YJL and JYL analyzed and interpreted the data. YJL, ARS, and JYL wrote the manuscript. JWK, HSK, KDK, DHS, SHK, SWK revised the manuscript. All Authors contributed to the article and approved the submitted version.

Acknowledgements

This study was funded by Samyang Biopharmaceuticals Corporation.

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Received February 25, 2022
Revised April 12, 2022
Accepted April 28, 2022