INTRODUCTION

Epithelial ovarian cancer is the leading cause of disease-related deaths from gynecologic malignancies, and its incidence and mortality rates in Korea are increasing.\(^1\)\(^,\)\(^2\) In addition to well-known prognostic factors, such as stage, histology, grade, and residual disease after surgery,\(^3\) clinical studies are underway to identify potentially actionable mutations in ovarian cancer through a greater understanding of molecular mechanisms and to evaluate therapeutic agents of these mutations.\(^4\)\(^,\)\(^5\)

Next generation sequencing (NGS) is able to reveal genomic aberrations by harnessing its massively parallel sequencing capability to analyze multiple genes simultaneously in a single assay. Moreover, this technology has recently become more affordable, leading to large collaborative studies on whole genomes that have been able to document targetable genes and predictive biomarkers in cancer.\(^6\)\(^,\)\(^7\) As of March 2017, the National Health Insurance system in Korea has paid the cost of NGS panels for several types of solid tumors, including ovarian cancer, and the number of NGS tests has increased exponentially.

We reviewed retrospective data of 84 patients who under-
went NGS and reported our experiences with integrating an NGS panel into clinical practice in ovarian cancer. We identified potentially actionable genomic alterations and used them to evaluate the therapeutic utility of individual treatment options.

MATERIALS AND METHODS

Patient samples
Between March 1, 2017 and July 31, 2018, 84 tumor samples from ovarian cancer patients treated at Yonsei Cancer Center were subjected to NGS. The tumor samples were prepared from formalin-fixed, paraffin-embedded (FFPE) tissues. An expert pathologist (H.S.K.) reviewed hematoxylin and eosin-stained slides to ensure that ≥20% of the nucleated cells in the sample were derived from the tumor. Tumor specimens were macrodissected after a hematoxylin-eosin reference slide check to ensure the proportion of tumor content. For DNA and RNA extraction, two to five slides of resected specimens of a thickness of 5 μm were needed. A board-certified gynecological pathologist diagnosed all cases. We performed a retrospective review of patient medical records, including age, histologic type, stage as defined by the International Federation of Gynecology and Obstetrics, and the timing of the NGS test.

NGS
We performed NGS analysis of 84 FFPE cancers with sufficiently high tumor cellularity (>30%). Genomic DNA was extracted using a Maxwell CSC DNA FFPE Kit (Promega, Madison, WI, USA), according to the manufacturer’s instructions. The products were sequenced on a MiSeq System (Illumina, San Diego, CA, USA). Mutational and copy number analyses were performed using a TruSight Tumor 170 panel (Illumina) that covers, respectively, 170 genes and 59 genes for mutational and copy number analyses (Supplementary Table 1, only online). For mutational analysis, FASTQ files were uploaded on the Illumina BaseSpace software (Illumina) for variant interpretation. Only variants in coding regions and promoter regions or splice variants were retained. In addition, we retained only variants present in <1% of the population, according to ExAC and 1000 genomes, and also present in >5% of reads with a minimum read depth of 250. All retained variants were reviewed against reference websites [Catalogue of Somatic Mutations in Cancer (http://evs.gs.washington.edu/EVS/), Precision Oncology Knowledge Base (http://oncokb.org), and dbSNP (https://www.ncbi.nlm.nih.gov/snp)]. Only pathogenic variants were selected. In copy number analysis, only genes with a more than two-fold change relative to the average level were considered for amplification. We also performed total nucleic acid extraction to obtain ribonucleic acid (RNA). An Archer FusionPlex Solid Tumor Kit (ArcherDx, Boulder, CO, USA) was used to analyze the RNA for fusions and splice variants: the kit covers 55 genes. Specimens yielded more than 40 ng of DNA and RNA. DNA fragment sizes of at least 79 bp and RNA fragment sizes of at least 63 bp were selected for targeted sequencing. Our goal in this study was to assess the feasibility and utility of using the Illumina MiSeq platform to integrate a NGS panel into a real-world setting of ovarian cancer clinical practice.

Data interpretation
Actionable somatic alterations are defined as those that could be targeted by a drug available for on-label, off-label, or in clinical trials. These alterations were selected based on a literature search of the MD Anderson Knowledge Base for Precision Medicine (http://PCT.MDAnderson.org), The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/), and genes related to homologous recombination repair (HRR) (Supplementary Table 2, only online).

We used the following guidelines to classify these alterations into four tiers. Tier 1 comprised known tumor type-specific actionable somatic mutations of confirmed clinical utility in predicting responses to U.S. Food and Drug Administration (FDA)-approved therapies, prognoses, diagnoses, or increased risk of inherited cancer. Tier 2 included actionable somatic mutations in other tumor types or somatic mutations in targetable pathways with potential clinical significance, such as susceptibility to FDA-approved therapies, prognoses, diagnoses, or increased risk of inherited cancer. Alterations of unknown clinical significance were classified as Tier 3, and those considered benign or likely so were grouped in Tier 4.

Clinical implications
We defined the term clinical implication as the capability of NGS results to provide useful information about patients and their family members that could be used to diagnose, monitor, predict the occurrence of disease and to create informed choices about treatment options. These clinical implications were categorized into three categories to evaluate the clinical impact of NGS results: 1) those who received targeted therapy; 2) identification of potential candidates for targeted therapy; and 3) genetic counseling for the patient and other at-risk family members.

Ethical statement
This study was approved by the Institutional Review Board of Severance Hospital at Yonsei University College of Medicine (IRB No. 4-2018-0518).

RESULTS

Patient clinicopathologic characteristics
A total of 227 ovarian cancer patients were treated in our institution between March 1, 2017 and July 31, 2018, and 84 (37%) patients underwent NGS analysis. Table 1 illustrates the base-
Table 1. Clinicopathological Characteristics (n=84)

| Characteristic        | Values                     |
|----------------------|----------------------------|
| Age (yr) [median (range)] | 54 (34–77)                 |
| Histologic type      |                            |
| High grade serous    | 55 (65.1)                  |
| Low grade serous     | 4 (4.8)                    |
| Clear                | 10 (12.0)                  |
| Endometrioid         | 6 (7.3)                    |
| Mucinous             | 2 (2.4)                    |
| Other                | 7 (8.4)                    |
| FIGO stage           |                            |
| I                    | 12 (14.5)                  |
| II                   | 4 (4.8)                    |
| III                  | 33 (39.8)                  |
| IV                   | 35 (40.9)                  |
| Treatment type       |                            |
| PDS                  | 49 (57.8)                  |
| NAC                  | 35 (42.2)                  |
| Tissue tested        |                            |
| PDS                  | 39 (45.8)                  |
| Pre-NAC              | 8 (9.6)                    |
| IDS                  | 26 (31.3)                  |
| Relapse              | 11 (13.3)                  |

*FIGO*, International Federation of Gynecology and Obstetrics; PDS, primary debulking surgery; NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery. Values are presented as n (%) or median (range) unless otherwise indicated.

Genomic alterations

All patients had at least one genomic alteration. The mean number of mutations per patient was 10.5. Fifty-seven (67.9%) patients had more than one actionable alteration other than *TP53*. Of the 57 patients, 16 (28.6%) had a mutation in HRR-related genes (Fig. 1). In addition, we analyzed the distribution of patients with somatic BRCA mutations at each time point of NGS analysis. Of the 11 patients with somatic BRCA mutations, there were 2 patients (2/8, 25.0%) in the Pre-NAC group, 5 patients (6/39, 15.4%) in the PDS group, 2 patients (2/26, 7.7%) in the IDS group, and 2 patients (1/11, 9.1%) in the relapse group. The chemo-naive group (pre-NAC, PDS) and the chemotherapy group (IDS, relapse) comprised 8/47 (17.0%) and 3/37 (8.1%), respectively. We also reviewed the Germline BRCA status for the patients (Supplementary Table 3, only online). Of the 84 patients, 12 (14.3%) had germline *BRCA1*/2 mutation, 50 (59.5%) had no germline *BRCA1*/2 mutation, and 26 (31.0%) did not undergo the germline BRCA test. Table 2 shows the tumor molecular profiles and clinical utility of actionable somatic mutations. Among single nucleotide variants and indel in tiers 1 or 2, the most frequently identified mutations were in *TP53* (64%), *PIK3CA* (15%), and *BRCA1*/2 (17%) (Fig. 2A). Among copy number variations and fusions, the most frequently identified mutations were in *MYC* (27%), *TFR2* (24%), and *CCNE1* (13%) (Fig. 2B). The most commonly mutated genes among the HRR-related genes in tiers 1 or 2 included *BRCA1* (11%), *BRCA2* (9%), *ATM* (4%), and *CHEK1* (4%) (Fig. 2C). The most frequently mutated genes among the TCGA druggable genes in tiers 1 and 2 were *KRAS* (16%), *CCNE1* (13%), *ERBB2* (5%), *RICOR* (1%), and *ERBB3* (1%) (Fig. 2C). Identified mutations were categorized into six pathways or functional groups: cell cycle (*RB1*, *CCNE1*, *CDK2*, *CCND1*, *CDK4*, *CDK6*, *CDKN2A*, *MYC*, *SRC*, *JAK1*, *JAK2*, *STAT1*, *STAT3*), DNA damage response (*CHEK1*, *CHEK2*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *ATM*, *ATR*), p53 (*CDKN2A*, *MDM2*, *MDM4*, *TP53*), PI3K-AKT-mTOR signaling (*PI3KCA*, *PIK3RA*, *PTEN*, *AKT1*, *AKT2*, *MTOR*, *RICOR*, *TSC1*, *TSC2*), Ras-Raf, MEK-Erk/JNK signaling (*KRAS*, *HRAS*, *BRAF*, *RAF1*, *MAP2K1*, *MAP2K2*, *MAP3K1*, *MAP3K4*, *MAPK1*, *MAPK3*), and the RTK signaling family (*EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *PDGFR*, *PDGFRB*, *KIT*, *FGFR1*, *KDR*). We analyzed the numbers of mutations with six functional and targetable pathways (Fig. 2D). The most frequently identified mutations were *MYC* in the cell cycle pathway, *BRCA1* in the DNA damage response pathway, *TP53* in the p53 pathway, *PIK3CA* in the PI3K-AKT-mTOR pathway, *KRAS* in the Ras-Raf-MEK-Erk/JNK pathway, and *FGFR1* in the RTK signaling pathway.

Targeted therapies

Of 57 patients with more than one actionable alteration, 7 (8.3%) were treated with matched therapies; 49 underwent standard chemotherapy without matched therapy; and 1 patient was treated with immunohistochemistry matched therapy (Fig. 3). Among the patients treated with matched therapies, five were
## Table 2. Tumor Molecular Profiles and Clinical Utility of Targetable Somatic Mutations

| Patient | Cell type | Alterations | Actual change | Potential change | Clinical implication | Genetic counseling |
|---------|-----------|-------------|---------------|------------------|---------------------|--------------------|
| 1       | High grade serous | *BRCA1* c.1961delA, *BRCA2* c.1806delA | No            | Consider future PARP inhibitor, consider future PARP inhibitor | Yes                |                    |
| 2       | High grade serous | *BRCA1* c.1961delA, *BRCA2* c.1806delA | No            | Consider future PARP inhibitor, consider future PARP inhibitor | No                 |                    |
| 3       | High grade serous | *BRCA1* c.3548A>G | No            | Consider future PARP inhibitor | No                 |                    |
| 4       | High grade serous | *BRCA2* c.3896A>T | No            | Consider future PARP inhibitor | No                 |                    |
| 5       | Mucinous        | *ERBB2* Amplification | No            | Consider future ERBB inhibitor | No                 |                    |
| 6       | Seromucinous    | *BRCA2* c.2795_2796delAC, *KRAS* c.38G>A | No            | Consider future PARP inhibitor, consider future MAPK inhibitor | No                 |                    |
| 7       | Clear cell      | *PTEN* G850A | No            | Consider future PTEN inhibitor | No                 |                    |
| 8       | Endometrioid    | *PIK3CA* 241G>A, *PTEN* 195G-T | No            | Consider future AKT inhibitor, consider future PTEN inhibitor | No                 |                    |
| 9       | High grade serous | *BRAF* Amplification | No            | Consider future BRAF inhibitor | No                 |                    |
| 10      | High grade serous | *BRCA2* c.1805_1806insA, *KRAS* Amplification | No            | Consider future PARP inhibitor, consider future MAPK inhibitor | No                 |                    |
| 11      | Low grade serous | *KRA* c.338A, *MLH1* c.440_44insT | Enrolled in NCT02628067 (pembrolizumab) | Consider future MAPK inhibitor | Yes                |                    |
| 12      | High grade serous | *ATM* Amplification, *CCNE1* Amplification | No            | Consider future PARP inhibitor, consider future CDK inhibitor | No                 |                    |
| 13      | High grade serous | *BRCA2* c.1399A>T | Treated with Olaparib | No | Yes                             |                    |
| 14      | High grade serous | *MET* Amplification, *PIK3CA* Amplification | No            | Consider future MET inhibitor, consider future AKT inhibitor | No                 |                    |
| 15      | High grade serous | *CCNE1* Amplification | No            | Consider future CDK inhibitor | No                 |                    |
| 16      | High grade serous | *CCNE1* Amplification, *PIK3CA* Amplification | No            | Consider future CDK inhibitor, consider future AKT inhibitor | No                 |                    |
| 17      | Mixed (clear+endometrioid) | *CCNE1* Amplification | No            | Consider future CDK inhibitor | No                 |                    |
| 18      | High grade serous | *CCNE1* Amplification, *KRAS* Amplification, *PIK3CA* Amplification | No            | Consider future CDK inhibitor, consider future MAPK inhibitor, consider future AKT inhibitor | No                 |                    |
| 19      | High grade serous | *KRAS* c.36G>T, *NRAS* Amplification | No            | Consider future MAPK inhibitor, consider future MAPK inhibitor | No                 |                    |
| 20      | High grade serous | *CDK4* Amplification, *PIK3CA* Amplification | No            | Consider future CDK4 inhibitor, consider future AKT inhibitor | No                 |                    |
| 21      | High grade serous | *PIK3CA* Amplification | No            | Consider future AKT inhibitor | No                 |                    |
| 22      | Carcinosarcoma  | *FGFR1* Amplification, *KIT* Amplification | No            | Consider future FGFR inhibitor, consider future KIT inhibitor | No                 |                    |
| 23      | High grade serous | *BRCA1* c.3991C>T | No            | Consider future PARP inhibitor | Yes                |                    |
| 24      | High grade serous | *BRCA2* c.2798del_2799delCA, *PIK3CA* c.3813dupA | Treated with Olaparib | No | Yes                             |                    |
| 25      | High grade serous | *PIK3CA* c.10357>T, *ERBB2* Amplification | No            | Consider future AKT inhibitor, consider future ERBB inhibitor | No                 |                    |
| 26      | High grade serous | *BRCA2* c.2808_2811del | No            | Consider future PARP inhibitor | Yes                |                    |
| 27      | Clear cell      | *PIK3CA* c.3140A>T | No            | Consider future AKT inhibitor | No                 |                    |
| 28      | High grade serous | *RICCTOR* Amplification | No            | Consider future RICTOR inhibitor | No                 |                    |
| 29      | Mucinous        | *BRAF* c.1799T-A, *PIK3CA* c.13570T-A | No            | Consider future BRAF inhibitor, consider future AKT inhibitor | No                 |                    |
| 30      | Clear cell      | *PIK3CA* c.3140A>G, *FGFR1* Amplification | No            | Consider future AKT inhibitor, consider future FGFR inhibitor | No                 |                    |
| 31      | Clear cell      | *PIK3CA* c.3140A>G | No            | Consider future AKT inhibitor | No                 |                    |
| 32      | High grade serous | *PTEN* c.604dupA, *BRAF* Amplification, *CCNE1* Amplification, *KRAS* Amplification | No            | Consider future PTEN inhibitor, consider future BRAF inhibitor, consider future CDK inhibitor, consider future MAPK inhibitor | No                 |                    |
| Patient | Cell type          | Alterations                                                                 | Actual change | Clinical implication                                                                 | Genetic counseling |
|---------|--------------------|------------------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------------|--------------------|
| 33      | High grade serous  | *CCNE1* Amplification *EGFR* Amplification *FGFR1* Amplification *KRAS* Amplification *PIK3CA* Amplification | No            | Consider future CDK inhibitor, consider future EGFR inhibitor, consider future FGFR inhibitor, consider future MAPK inhibitor, consider future AKT inhibitor | No                 |
| 34      | Seromucinous       | *PIK3CA* c.1624G>A *KRAS* c.35G>T                                           | No            | Consider future AKT inhibitor, consider future MAPK inhibitor                         | No                 |
| 35      | Carcinosarcoma     | *KRAS* c.35G>T                                                               | No            | Consider future MAPK inhibitor                                                       | No                 |
| 36      | High grade serous  | *BRCA1* c.5093_5096del                                                     | Treated with Olaparib | No                                                                                   | No                 |
| 37      | High grade serous  | *BRAF* Amplification *ERBB2* Amplification                                  | No            | Consider future BRAF inhibitor, consider future ERBB inhibitor.                       | No                 |
| 38      | High grade serous  | *ATM* Amplification *CCNE1* Amplification *FGFR1* Amplification *PIK3CA* Amplification | No            | Consider future PARP inhibitor, consider future CDK inhibitor, consider future FGFR inhibitor, consider future AKT inhibitor | No                 |
| 39      | High grade serous  | *BRAF* Amplification *FGFR2* Amplification *MET* Amplification              | No            | Consider future BRAF inhibitor, consider future FGFR inhibitor, consider future MET inhibitor | No                 |
| 40      | Endometrioid       | *PIK3CA* c.113G>A *PTEN* c.540C>G                                           | No            | Consider future AKT inhibitor, consider future PTEN inhibitor.                       | No                 |
| 41      | High grade serous  | *KRAS* c.35G>T *ERBB2* Amplification                                        | No            | Consider future MAPK inhibitor, consider future ERBB inhibitor.                       | No                 |
| 42      | Clear cell         | *ERBB2* Amplification                                                       | No            | Consider future ERBB inhibitor                                                       | No                 |
| 43      | Clear cell         | *PIK3CA* c.3140A>G *FGFR* Amplification                                     | No            | Consider future AKT inhibitor, consider future FGFR inhibitor.                       | No                 |
| 44      | High grade serous  | *CCNE1* Amplification                                                       | No            | Consider future CDK inhibitor                                                        | No                 |
| 45      | High grade serous  | *ATM* Amplification *CHEK1* Amplification                                   | No            | Consider future PARP inhibitor, consider future CHEK1 inhibitor.                      | No                 |
| 46      | Endometrioid       | *BRCA2* c.6952C>T *PIK3CA* Amplification                                   | Treated with Olaparib | Consider future AKT inhibitor.                                                        | Yes                |
| 47      | Endometrioid       | *PIK3CA* Amplification                                                      | Treated with Olaparib based on germline BRCA mutation | Consider future AKT inhibitor.                                                        | Yes                |
| 48      | Seromucinous       | *KRAS* c.35G>T *PIK3CA* c.1810T>c                                          | No            | Consider future MAPK inhibitor, consider future AKT inhibitor.                        | No                 |
| 49      | Clear cell         | *PTEN* c.810G>T                                                            | No            | Consider future PTEN inhibitor                                                       | No                 |
| 50      | Endometrioid       | *PIK3CA* c.1624G>A *CCNE1* Amplification *CHEK1* Amplification *FGFR2* Amplification *KIT* Amplification *MDM2* Amplification *PIK3CA* Amplification | No            | Consider future AKT inhibitor, consider future CDK inhibitor, consider future PARP inhibitor, consider future FGFR inhibitor, consider future KIT inhibitor, consider future MDM2 inhibitor, consider future AKT inhibitor | No                 |
| 51      | Low grade serous   | *KRAS* c.35G>A *BRAF* Amplification                                         | No            | Consider future MAPK inhibitor, consider future BRAF inhibitor.                        | No                 |
| 52      | High grade serous  | *KRAS* c.38G>A *CCNE1* Amplification                                        | No            | Consider future MAPK inhibitor, consider future CDK inhibitor.                        | No                 |
| 53      | High grade serous  | *BRCA1* c.1399A>T                                                          | No            | Consider future PARP inhibitor                                                        | No                 |
| 54      | High grade serous  | *BRAF* Amplification *CCNE1* Amplification *PTEN* Amplification             | No            | Consider future BRAF inhibitor, consider future CDK inhibitor, consider future PTEN inhibitor | No                 |
| 55      | High grade serous  | *KRAS* Amplification                                                       | No            | Consider future MAPK inhibitor                                                        | No                 |
| 56      | Clear cell         | *PIK3CA* c.1624G>A                                                         | No            | Consider future AKT inhibitor                                                        | No                 |
| 57      | High grade serous  | *PIK3CA* c.1633G>A                                                         | Enrolled in NCT03017521 (ATK inhibitor) | No                                                                                   | No                 |

PARP, poly ADP ribose polymerase.
Fig. 2. Mutation, copy number variation profiling. (A) Single nucleotide variants and indels. Color legend of the variations represented, including frame-shift indel, inframe indel missense, and nonsense. Vertical lines indicate gene names; horizontal lines indicate cases with germline mutations. (B) Copy number variations and fusions. Color legend of the variations represented, including amplification, fusion. Vertical lines indicate gene names; horizontal lines indicate cases with germline mutations.

TP53 64%
PIK3CA 15%
KRAS 11%
ARID1A 9%
BRCA2 9%
BRCA1 8%
PTEN 7%
CTNNB1 4%
PIK3R1 3%
BRAF 1%
JAK2 1%
PIK3CD 1%
STK11 1%

Germline  BRCA1  BRCA2

Mutation type
- Missense
- Nonsense
- Frame-shift indel
- Inframe indel

Germline BRCA
- Mutation
- VOUS
- No mutation
- Not done

Tier
- Tier1
- Tier2

MYC 27%
TFRC 24%
CCNE1 13%
FGF23 13%
PIK3CA 13%
MDM4 11%
CCND3 8%
FGF8 8%
FGF7 8%
FGF6 8%
JAK2 8%
BRCA1 8%
ARID1A 7%
BRCA2 7%
PTEN 7%

Mutation type
- Amplification
- Fusion

Germline BRCA
- Not done
- Mutation
- No mutation
- VOUS

Count 0 20 40 60

Count 0 10 20

https://doi.org/10.3349/ymj.2019.60.10.914
treated with biomarker-driven therapy, and two were enrolled in biomarker matched clinical trials. BRCA1/2 mutations (n=5) were treated with poly ADP ribose polymerase (PARP) inhibitor; a MLH1 mutation with high microsatellite instability (n=1) was treated with a programmed cell death-1 (PD-1) inhibitor (ClinicalTrials.Gov Identifier: NCT02628067), and a PIK3CA mutation (n=1) was treated with an AKT inhibitor (ClinicalTrials.Gov Identifier: NCT03017521).

**Outcomes of matched therapy patients**
A total of 7 patients were treated with a targeted agent, and the median value of prior lines of chemotherapy was 2 (range, 2–4). Of the 7 patients, five received PARP inhibitor therapy, one received PD-1 inhibitor, and one received AKT inhibitor therapy. Among patients who were treated with PARP inhibitor, four were treated with a PARP inhibitor for maintenance therapy, and one was treated with a PARP inhibitor as a 4th-line monotherapy. Among patients who underwent maintenance therapy with a PARP inhibitor, three were on follow-up without recurrence for more than 5 months; one experienced disease progression at 7 months after initiation of maintenance therapy. One patient treated with a PARP inhibitor as a 4th-line monotherapy had stable disease at the time of the analysis and had been undergoing treatment for 7 months. A patient treated with a PARP inhibitor as a 4th-line mono-therapy had stable disease and had been undergoing treatment for 2 months.

**Clinical impact**
Clinically meaningful results are shown in Fig. 4. Clinically significant alterations were found in 57 (67.9%) patients. Of these 57, seven (8.3%) had matched targeted therapies, 53 (63.0%) had potentially actionable alterations, and eight (9.5%) and their at-risk family members without potentially actionable alterations received genetic counseling. Currently, the patients with potentially actionable alterations are currently either undergoing standard treatment or are in a state in which no disease is evi-
dent after treatment but remain candidates for matched therapy if there is a subsequent disease recurrence or progression. Several patients had multiple actionable alterations. These patients received matched therapy but had other alterations that may later make them potential candidates for matched therapy. Patients with actionable alterations that could make them future potential candidates for matched therapy are shown in Table 2.

**DISCUSSION**

In this study, we evaluated the clinical utility of NGS and identified clinically significant information beyond actionable alterations in ovarian cancer patients. In our review of NGS results for the 84 ovarian cancer patients in our institution, we found that 57 (67.9%) of them had one or more actionable alterations other than TP53 and that 16 (28.6%) of them had a mutation in HRR-related genes. Fifty-two (61.9%) patients had clinically significant alterations, seven (8.3%) were treated with matched targeted therapies, 48 (57.1%) had potentially actionable alterations, and eight (9.5%) received genetic counseling. In our study, 12 (14.3%) had germline BRCA1/2 mutations, and 50 (59.5%) had no germline BRCA1/2 mutation. In addition, the distribution of patients with somatic BRCA mutations at each time point of NGS were 8/47 (17.0%) in the chemo-naive group (pre-NAC, PDS) and 3/37 (8.1%) in the post chemotherapy group (IDS, relapse). Incorporation of NGS into standard clinical practice could provide a complementary tool with which to identify patients who might benefit from targeted therapies and genetic counseling.

NGS has been used in several studies to identify the actionable mutations of specific cancers and the clinical impact thereof. Oberg, et al. showed the feasibility of incorporating NGS into pediatric hematology-oncology. They found it was clinically significant in 66% of all cases in which it was used. Its benefits included avoidance of inappropriate treatments, confirmation of definitive diagnoses, and identification of pharmacogenomics modifiers. Heong, et al. described the feasibility of a molecular screening program in Asian cancer patients. Eighty-two percent of all patients had at least one reportable genomic alteration. Eight percent of the patients with reportable alterations were treated with matched therapies based on their specific molecular alteration. Nine of these patients (45%; 95% CI 23.1–68.5%) showed a clinical benefit, including three partial responses and six with stable disease. However, in the SHIVA

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**Fig. 3.** CONSORT diagram. *ClinicalTrials. Gov Identifier: NCT03509246; †ClinicalTrials.Gov Identifier: NCT03414047. NGS, next generation sequencing; IHC, immunohistochemistry; PD-L1, programmed cell death-ligand 1.

**Fig. 4.** Clinical impact of next generation sequencing panel.
Number of mutations can occur as the cancer develops. As a fur-
cell populations, an extremely complex etiology, and any num-
ovarian cancer patients. Ovarian cancers have heterogeneous
specific efficacious treatment agents is especially difficult with
Even after their detection, matching susceptible mutations with
hensive genomic analysis in ovarian cancer patients. Despite
our findings, only 8.3% of patients received targeted therapy.
Even after their detection, matching susceptible mutations with
specific efficacious treatment agents is especially difficult with
ovarian cancer patients. Ovarian cancers have heterogeneous
cell populations, an extremely complex etiology, and any num-
ber of mutations can occur as the cancer develops. As a fur-
complication, health insurers are not obligated to cover the
off-label use of expensive drugs.

Nevertheless, the incorporation of NGS into the treatment of
ovarian cancer may have a significant clinical impact, includ-
ing success in finding potential candidates for future targeted
therapies and genetic counseling. In our study, 57.1% of all pa-
tients were potential candidates for future targeted therapy, and
9.5% of all patients received genetic counseling for the patient
and their at-risk family members. In addition, the proportion
of somatic BRCA mutations varied according to the time point
of NGS analysis. Although the number of patients was small,
the difference in the proportion of patients with somatic BRCA
mutations in the two groups may be due to the effect of BRCA
reversion by platinum-based chemotherapy. Reversion muta-
tions in BRCA1/2 have been reported in ovarian cancer as a
mechanism of acquired resistance to platinum-based chemo-
therapies and PARP inhibitors. Based on these results, fur-
ther studies are needed to analyze the genetic alterations in se-
rial samples of chemo-naïve and post-chemotherapy patients.

Our study has some limitations. There are still cost-effective
issues with NGS that can limit clinical testing and prevent or
delay the initiation of targeted therapies, and failures, such as
insufficient collection of tissues or improper sequencing, can
lead to significantly increased turnaround times. In addition,
our follow-up period on patients was too short to demonstrate
definitively that matched targeted therapies based on the re-
ults of NGS yield better outcomes than empiric treatment
choices.

NGS may help guide immediate and future treatment op-
tions for patients with ovarian cancer. Implementation of NGS
served as a complementary tool to identify patients who may
benefit from targeted therapies and genetic counseling. Fur-
ther large-scale studies are needed to investigate the overall
clinical utility and feasibility of NGS in ovarian cancer.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research
Program through the National Research Foundation of Korea
funded by the Ministry of Education (2016R1D1A1B03931916)
and by the Bio & Medical Technology Development Program
of the National Research Foundation of Korea funded by the
Ministry of Science, ICT & Future Planning (2017M3A9E8029714).
This study was also supported by a faculty research grant of Yonsei
University College of Medicine (6-2018-0169).

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