学位論文　　博士（医学）

Molecular analysis of ascitic fluid cytology reflects genetic changes of malignancies of the ovary equivalent to surgically resected specimens

（腹水細胞診を用いた分子生物学的解析は外科的切除標本と同等に卵巣癌の遺伝子変化を反映する）

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Molecular analysis of ascitic fluid cytology reflects genetic changes of malignancies of the ovary equivalent to surgically resected specimens

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BACKGROUND: The objective of this study was to identify the clinical utility of genomic analysis of ascitic fluid cytology (AC) in patients with epithelial ovarian cancer. **METHODS:** Targeted next-generation sequencing was used to analyze 66 samples from 33 patients who had ovarian (n = 23), fallopian tube (n = 2), and peritoneal (n = 8) carcinoma, and the concordance rate of molecular profiles was compared between surgically resected, formalin-fixed, paraffin-embedded (FFPE) tissues and AC samples. **RESULTS:** In total, 159 mutations were identified (54 oncogenic mutations and 105 nononcogenic mutations) in 66 DNA samples (33 FFPE tissues and 33 AC samples) from 33 patients. Of the 159 mutations, 57 (35.8%) were shared between surgically resected FFPE tissues and AC samples. However, the concordance rate of the molecular profiles between the 2 was significantly higher for oncogenic mutations compared with nononcogenic mutations (85.1% vs 10.5%; $P < .01$). Indeed, the AC samples covered all oncogenic mutations (n = 46) that were detected in surgically resected specimens and identified additional mutations (n = 8). **CONCLUSIONS:** The current results indicated that genomic analysis of AC can identify all of the genetic changes associated with epithelial ovarian cancer to understand tumor characteristics without interventional surgery or biopsy and may play an important role in developing personalized precision medicine. ***Cancer Cytopathol* 2022;130:640-649**. © *2022 American Cancer Society.*

KEY WORDS: ascites; cytology; genomics; mutation; ovarian cancer.

INTRODUCTION

Epithelial ovarian cancer (EOC) is a common and fatal gynecologic malignancy in Japan, with 13,049 new cases and an estimated 4784 deaths in 2018.[1] High-grade serous carcinoma (HGSC) accounts for the majority of the histologic subtype of EOC.[2] Most women with HGSC present at an advanced stage, which is associated with a poor 5-year overall survival rate of approximately 30% compared with 90% in women who present with early stage ovarian cancers.[3]

The treatment for advanced-stage EOC includes primary debulking surgery (PDS) followed by adjuvant chemotherapy or neoadjuvant chemotherapy (NAC), then followed by interval debulking surgery.[4] In addition, highly invasive surgery is sometimes required in these patients to make a definitive diagnosis.

Ascitic fluid cytology (AC) is a minimally invasive, rapid, and clinically useful method for diagnosis, but it is difficult to evaluate the nature of the tumor using morphologic features alone. Recently, we reported the results of a genomic analysis using archival cytologic specimens with details.[5,6] Although there are several reports of genomic

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analyses of ascitic fluid,[7,8] whether the genomic profile of AC is compatible with that of surgically resected tissue has not been fully evaluated.

In the current study, we determined the concordance rate of molecular profiles between surgically resected tissue samples and peeling samples of AC in patients with EOC and evaluated its application.

MATERIALS AND METHODS

Patients

Patients (n = 369) with ovarian, fallopian tube, or peritoneal carcinoma were treated at our hospital from 2008 to 2021. We performed a molecular biologic analysis on 33 patients from whom written informed consent for genomic analysis was obtained. In all patients, the diagnosis was based on perioperative intraperitoneal findings and pathologic evaluation. The median patient age was 62 years (range, 41-78 years), and the patients had ovarian (n = 23), fallopian tube (n = 2), and peritoneal (n = 8) carcinomas (Table 1). The histologic subtypes of these 33 patients included 31 serous carcinomas, 1 clear cell carcinoma, and 1 endometrioid carcinoma. All patients were staged according to International Federation of Gynecology and Obstetrics 2014 criteria and clinical data. Almost all patients (n = 32) were diagnosed with stage III or IV disease.

TABLE 1. Clinicopathologic Features of 33 Patients

Characteristic	No. of Patients (%)
Age: Median [range], y	62 [41-78]
Primary site	
Ovary	23 (70.0)
Fallopian tube	2 (6.0)
Peritoneum	8 (24.0)
Histology	
Serous carcinoma	31 (94.0)
Clear cell carcinoma	1 (3.0)
Endometrioid carcinoma	1 (3.0)
FIGO stage	
I/II	1 (3.0)
III/IV	32 (97.0)

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

Preparation of Formalin-Fixed, Paraffin-Embedded Tissues and AC Samples for DNA Extraction

Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from surgically resected specimens. Laser capture microdissection (LMD) was performed to obtain neoplastic epithelial cells using an Arcturus XT laser capture microdissection system (Thermo Fisher Scientific) in all 33 FFPE samples.

Ascites fluid was collected at the same time as the surgically resected specimens. The preparation of DNA from cytology specimens was done as previously described.[9] Briefly, a glass slide was soaked in xylene for 1 to 7 days, then the coverglass was removed. The tumor-rich clusters were peeled off from the glass slides with a razor blade and processed (Fig. 1).

DNA Extraction and Quality Analysis

DNA was extracted using a GeneRead DNA FFPE Kit (Qiagen) as previously described.[10,11] DNA quality was assessed using 2 sets of primers targeting the *ribonuclease P* locus. We measured long-length (268 base pairs) and short-length (87 base pairs) DNA concentrations and estimated DNA fragmentation using the ratio of DNA (relative quantification [RQ]). The RQ value is the ratio of long-length to short-length amplicons and is an indicator of the amount of degradation of genomic DNA. Matched peripheral blood samples were collected from each patient. The buffy coat was isolated after centrifugation, and DNA was extracted using the QIAamp DNA Blood Mini Kit with a QIAcube system (Qiagen).

Selecting Genes and Primer Design

A panel of 52 genes that have repeatedly been identified as oncogenic in gynecologic malignancies was created in house.[12,13] These included the following genes, which are associated with 9 signaling pathways: DNA repair (*ATM*, *ATR*, *BRCA1*, *BRCA2*, *MLH1*, *MSH6*, *POLD1*, *POLE*, *RAD51B*), epigenetics (*ARID1A*, *ARID1B*, *ARID2*, *ARID5B*, *BAZ1A*, *CHD4*, *MLL*, *MLL3*, *SMARCA4*), RTK/RAS (*BRAF*, *ERBB2*, *FGFR2*, *KRAS*, *MAP3K4*, *MAPK1*, *NF1*, *NRAS*), transcriptional regulation (*CBFB*, *CTCF*, *DICER1*, *ELF3*, *NFE2L2*, *SOX17*), cell cycle (*CCNE1*, *CDK12*, *CDKN2A*, *RB1*, *TP53*), PIK3 (*PIK3CA*, *PIK3R1*, *PPP2R1A*, *PTEN*, *STK11*), NOTCH (*EP300*, *FBXW7*, *NOTCH3*), Wnt (*APC*, *CTNNB1*), and others (*ABCC9*, *CSMD3*, *CYP4X1*, *RPL22*, *SPOP*). The primer design for targeted sequencing was done using the Ion AmpliSeq Designer software (Thermo Fisher Scientific). This comprehensive in-house gynecologic cancer panel contained 2897 primer pairs and spanned 287,430 base pairs (see Supporting Tables S1 and S2).

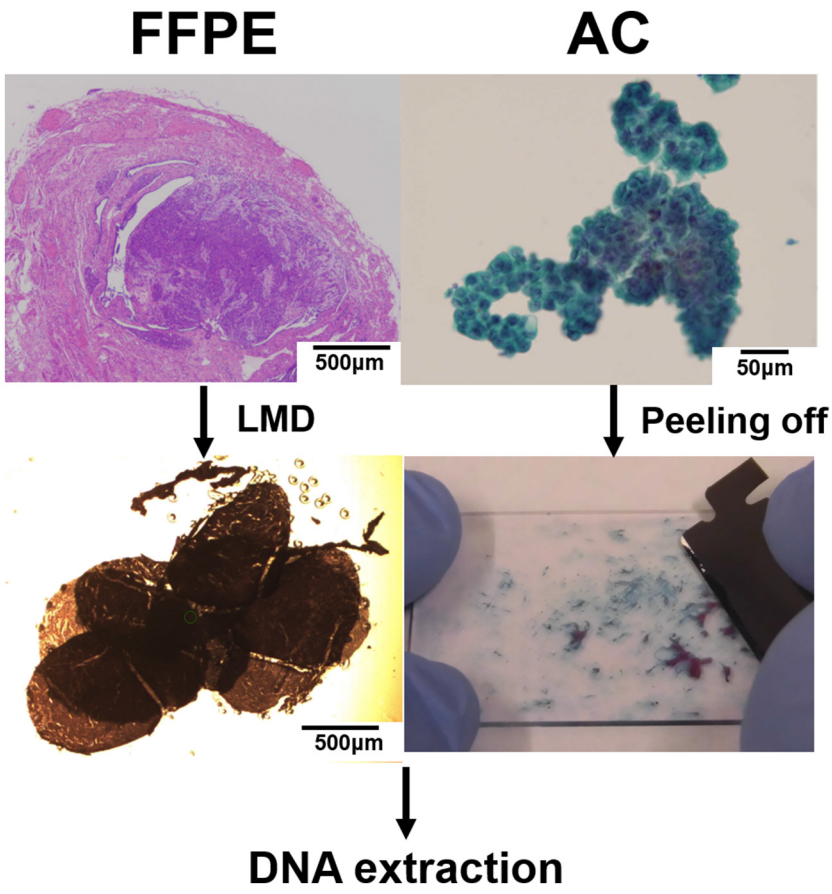

Figure 1. Representative images of DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissue samples and ascitic fluid cytology (AC) samples, including (*top left*) an FFPE tissue specimen with hematoxylin and eosin stain and (*top right*) an AC specimen with Papanicolaou stain of high-grade serous carcinoma. Because the surgically resected FFPE tissue contained large amounts of inflammatory cells and stromal tissue, laser capture microdissection (LMD) was performed to obtain neoplastic tissue, but AC samples were used by peeling off the specimen with a razor blade without LMD.

Targeted Sequencing

The construction of a next-generation sequencing library for targeted sequencing was done as previously described with minor modification.[14,15] The polymerase chain reaction (PCR) was performed with genomic DNA using a premixed primer pool and the Ion AmpliSeq HiFi Master Mix (Ion AmpliSeq Library Kit Plus) for 2 minutes at 99°C. This was followed by 15 to 23 cycles at 99°C for 15 seconds and at 60°C for 4 or 8 minutes, then a holding period at 10°C. The library concentration was determined using the Ion Library Quantification Kit. Emulsion PCR and chip loading were carried out using the Ion Chef with the Ion PI Hi-Q Chef Kit. Sequencing was conducted with an Ion Proton Sequencer (Thermo Fisher Scientific). Sequence data analysis was performed as previously described.[10] The buffy coat was used as a control to detect any variants.

In some cases, targeted sequencing was conducted on an Ion Torrent Genexus System according to the manufacturer's instructions (Thermo Fisher Scientific).[16,17] DNA concentration was determined using the Qubit dsDNA HS Assay Kit on a Qubit Fluorometer 3.0 (Thermo Fisher Scientific). The DNA was diluted to 1.1 ng/μL using nuclease-free water and amplified using the Gynecologic Panel. The Ion Torrent Genexus Library Strips and Templating Strips were incubated at room temperature for 30 minutes before loading into the sequencer. Sequencing reads were processed and the quality was assessed using Genexus software. The sequencing reads were mapped and aligned using the Torrent mapping alignment program. After initial mapping, a variant call and coverage analysis was performed.

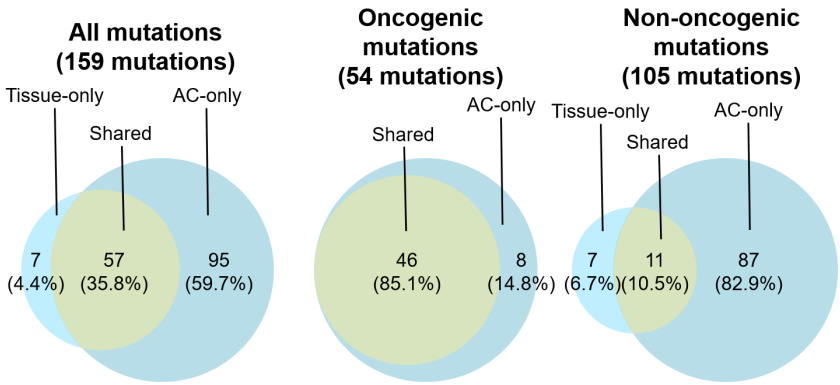

Figure 2. The frequency of shared mutations is illustrated between surgically resected tissue and ascitic fluid cytology (AC) samples. This is a Venn diagram of the proportion of shared mutations detected by sequencing of paired DNAs from formalin-fixed, paraffin-embedded tissue samples and AC samples.

Oncogenic and Actionable Mutations

Oncogenic and actionable (druggable and drug-matched) mutations were identified using the OncoKB database from the Memorial Sloan Kettering Cancer Center.[18] We defined *oncogenic mutations* as oncogenic or likely oncogenic mutations annotated in the OncoKB database and *nononcogenic mutations* as other mutations. When hypothetical and/or investigational drugs were available against oncogenic mutations, we designated them as *druggable* mutations. If US Food and Drug Administration (FDA)-approved drugs were available against 1 of those oncogenic mutations, we designated them *drug-matched mutations*. The former were compatible with the levels of evidence from 1 to 4, as defined by the OncoKB database, and the latter were compatible with levels of evidence of 1 and 2.

Statistical Analysis

The R commander EZR was used for statistical analysis, including the Fisher exact test and the Mann-Whitney U test. *P* values <.05 were considered statistically significant.

RESULTS

Quantity and Quality of Extracted DNA From FFPE and AC Samples

The quantity and quality of extracted DNA from FFPE and AC samples were determined by quantitative real-time PCR using 2 sets of primers that amplify long-length and short-length amplicons (see Supporting Table S3). The mean concentration of long-length DNA and the mean RQ value were 47.9 ng/μL (range, 0.4-483.6 ng/μL) and 0.30, respectively, in DNA extracted from FFPE tissues; whereas they were 6.0 ng/μL (range, 0.0-51.8 ng/μL) and 0.31, respectively, in DNA extracted from AC samples. As expected, there was a significant difference in the amount of extracted DNA (long/short) between FFPE tissues and AC samples ($P < .01$). However, there was no difference in the *quality* of the extracted DNA, which was represented by an RQ value ($P = .85$). This indicated that archival cytologic specimens could be used for genomic analysis.

Sequence Metrics of Extracted DNA From FFPE and AC Samples

We performed sequencing using an in-house panel targeting 52 significantly mutated genes in gynecologic cancers.[13] The mean coverage depth for the FFPE and AC samples was 1641 (range, 672-3854) and 1783 (range, 138-3213), respectively, indicating no significant difference ($P = .423$) (see Supporting Table S4). The mean on-target rate for both FFPE tissues and AC samples was 95% ($P = .909$). This also strengthens the applicability of AC samples for further analysis by molecular profiling.

Comparison of Genomic Analysis Between AC and FFPE Samples

We detected 159 mutations by genomic analysis in 33 AC and 33 FFPE surgically resected tissue samples. Among these mutations, 57 (35.8%) were shared between AC samples and FFPE tissues, 95 (59.7%) were detected in AC samples only, and 7 (4.4%) were detected in FFPE tissues only (Fig. 2).

We divided the 159 mutations into oncogenic (n = 54) and nononcogenic (n = 105) groups and compared the prevalence between the AC samples and FFPE tissues. Among 54 oncogenic mutations, the frequency of the shared mutations between AC samples and FFPE tissues was 85.1% (46 of 54 mutations), whereas the oncogenic mutations detected in FFPE tissues were all detected in AC samples. In addition, we were able to detect 8 oncogenic mutations in AC samples that were not detected in the FFPE tissues.

For nononcogenic mutations, the frequency of shared mutations between AC samples and FFPE tissues was only 10.5% (11 of 105 mutations). The concordance rate of the molecular profiles between FFPE tissues and AC samples was quite high for oncogenic mutations compared with the rate of nononcogenic mutations ($P < .01$) (Fig. 2).

The median variant allele frequency (VAF) of the shared mutations between AC samples and FFPE tissues was 36.7% (range, 3.4%-94.5%) and 58.4% (range, 3.7%-93.0%), respectively; whereas, for mutations detected only in AC samples, the VAF was 3.5% (range, 3.0%-87.4%; $P < .01$), and the VAF of mutations detected only in FFPE tissue samples was 4.0% (range, 3.0%-54.6%; $P < .01$) (Fig. 3).

For oncogenic mutations, the VAF of shared mutations between AC samples and FFPE tissues was 37.8% (range 3.4%-94.5%) and 60.6% (range 9.5%-93.0%), respectively; whereas, for mutations detected only in AC samples, the VAF was 3.3% (range 3.1%-51.2%; $P < .01$). For nononcogenic mutations, the VAF of shared mutations between AC samples and FFPE tissues was 36.0% (range, 5.4%-68.5%) and 36.5% (range, 3.7%-89.7%), respectively; whereas, for mutations detected only in AC samples, the VAF was 3.5% (range, 3.0%-87.4%; $P < .01$) and, for mutations detected only in FFPE tissues, the VAF was 4.0% (range, 3.0%-54.6%; $P < .01$) (Fig. 3). These data clearly indicate that the dominant clones, particularly oncogenic mutations, are readily detectable in AC specimens.

Numbers and Types of Oncogenic Mutations

The average number of mutations detected by genomic testing was higher in AC samples (4.6 mutations; range, 1-32 mutations) compared with FFPE tissues (2.0 mutations; range, 1-4 mutations). In AC samples, *TP53* mutation was identified in 31 of 33 cases (94%); in particular,

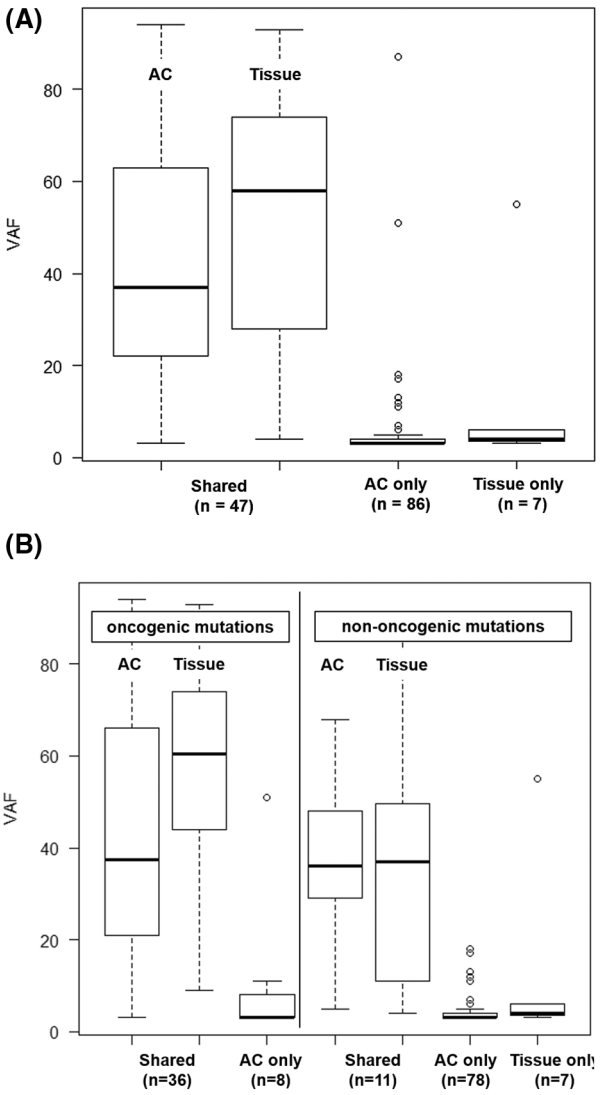

Figure 3. The variant allele frequency (VAF) of identified mutations is illustrated in ascitic fluid cytology (AC) and tissue samples. The median VAF of *shared mutations* in both AC and tissue samples was significantly higher than that of mutations detected in *AC only* or in *tissue only* (37% vs 4% and 58% vs 4%, respectively; $P < .01$, [A]). The median VAFs of shared oncogenic mutations and nononcogenic mutations also were significantly higher than those in AC only or in tissue only (B).

TP53 mutation was identified in all cases (31 of 31) of serous carcinoma. Other mutations that were frequently detected in AC samples were *BRCA1* (21%) and *ARID1A* (15%). In FFPE tissues, *TP53* mutations were also identified in 31 of 33 cases (94%), which was similar to the mutations identified in AC samples, and *BRCA1* mutations were identified in 15%. All 33 cases had at least 1 shared mutation between AC and FFPE tissues (Fig. 4).

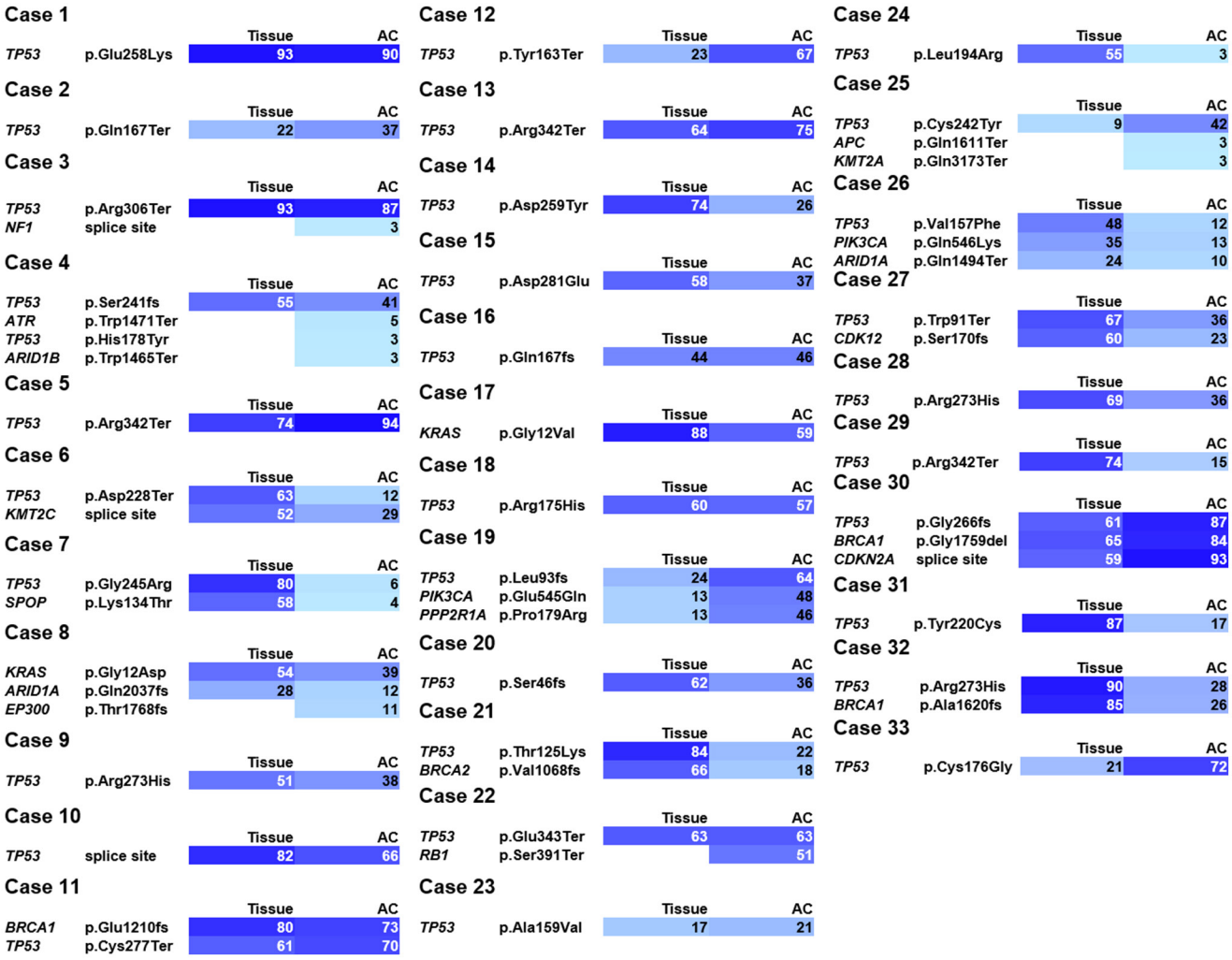

Figure 4. Oncogenic mutation profiles are illustrated between tissue samples (Tissue) and ascitic fluid cytology (AC) samples. This heat map illustrates the oncogenic mutation profiles detected in formalin-fixed, paraffin-embedded tissue and AC samples. Mutated genes with the corresponding amino acid changes are listed. The variant allele frequency (VAF) is shown as a percentage in each box and is indicated by the graduated color scale from 1% (light blue) to 100% (dark blue). A blank box indicates no identified mutations.

Signaling Pathways

Our in-house panel consisted of 52 genes involved in 9 different signaling pathways. With respect to the signaling pathways, most of the oncogenic mutations detected in tissue samples were abnormalities of the cell cycle (31 of 33 samples; 94%), whereas the others consisted of abnormalities in DNA repair (4 of 33 samples; 12%) and epigenetics (3 of 33 samples; 9%). In 30 cases, the detected mutations were mutually exclusive within the same pathway, whereas 23 cases had a single pathway abnormality, and 10 cases exhibited abnormalities in multiple pathways (Fig. 5).

Discovery of Actionable Mutations in AC Samples

We explored whether actionable mutations for drug treatment could be detected in the AC samples. This was done by finding matched drugs in the OncoKB database.[18] In total, in 33 AC samples from 33 patients with ovarian cancer, 54 oncogenic mutations were identified. Of those 54 mutations, 13 (24%) actionable mutations were detected in 12 patients (see Supporting Table S5). Of the 13 actionable mutations, we confirmed 8 (62%) FDA-approved, drug-matched mutations in 8 patients (see Supporting Table S6), which

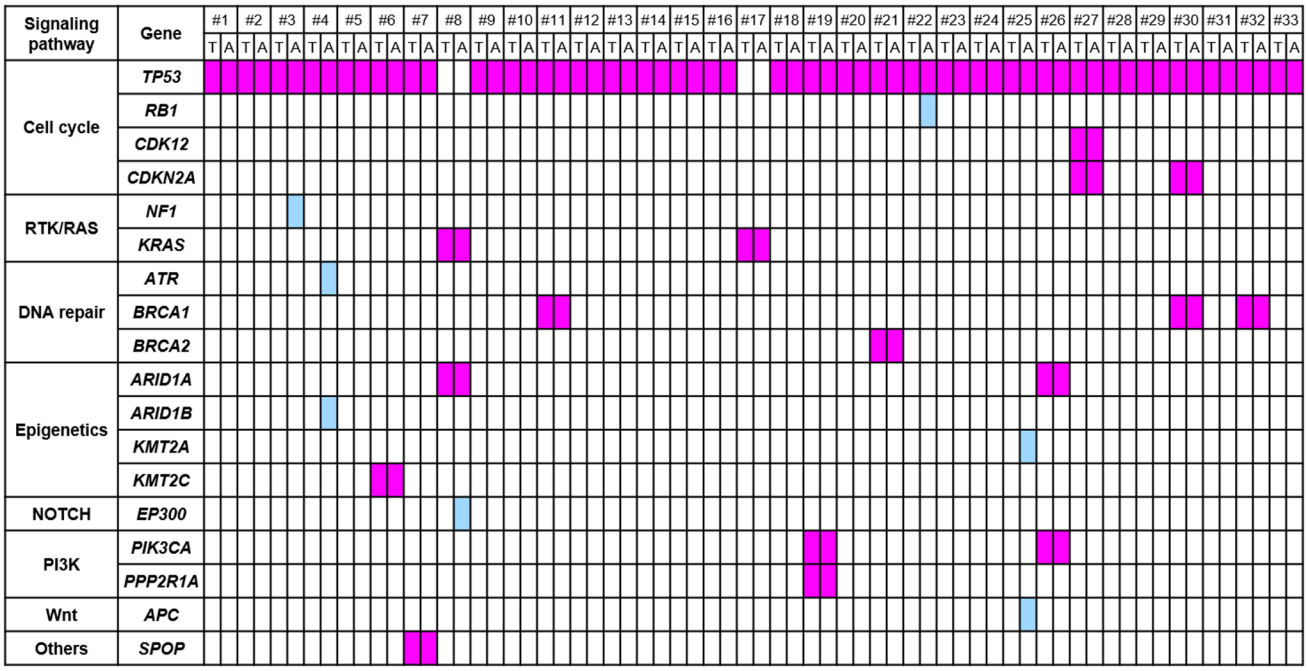

Figure 5. Classification of oncogenic mutations detected in tissue and ascitic fluid cytology (AC) is illustrated according to signaling pathway. This is an oncoplot of oncogenic mutations detected in formalin-fixed, paraffin-embedded (FFPE) tissues (T) and in AC samples (A) from 33 patients. The names of aberrant signaling pathways and mutated genes are listed on the left. Pink columns indicate shared mutations between tissue samples and AC samples, and light blue columns indicate mutations detected only in AC samples.

consisted of mutations in *BRCA1* (3 of 54 mutations; 6%), *PIK3CA* (2 of 54 mutations; 4%), *NF1* (1 of 54 mutations; 2%), *BRCA2* (1 of 54 mutations; 2%), and *CDK12* (1 of 54 mutations 2%). The remaining 5 actionable mutations, which currently do not have matched drugs, included mutations in *KRAS* (2 of 5 mutations; 40%), *ARID1A* (2 of 5 mutations; 40%), and *CDKN2A* (1 of 5 mutations; 20%).

For comparison, in 33 FFPE tissues from 33 patients with EOC, actionable and FDA-approved, drug-matched mutations were detected in 11 and 7 patients, respectively. This indicates that there was no difference in the discovery of actionable and FDA-approved, drug-matched mutations between AC samples and FFPE tissues.

DISCUSSION

Currently, surgical treatment for advanced EOC includes PDS followed by adjuvant chemotherapy or NAC as well as interval debulking surgery.[4] For patients with advanced EOC who are not eligible for PDS, NAC is recommended and should be introduced earlier. However, a significant disadvantage to NAC is the potential increased risk of platinum resistance. Rauh-Hain et al reported that significantly more patients who received NAC versus PDS developed platinum resistance (88.8% vs 55.3%).[19] Those authors stated that a better understanding of the mechanisms and risk factors associated with platinum resistance is needed for the development of drugs and improved treatment strategies that can circumvent drug resistance. Therefore, before the induction of NAC, it is important to obtain pathologic and genomic information from patients who have advanced EOC.

Compared with surgery, abdominal or vaginal paracentesis to obtain ascitic fluid is a far less invasive diagnostic procedure. In general, elucidating tumor features using AC is performed by cell morphologic evaluation and immunohistochemical staining. However, it can be difficult to make a definite diagnosis because it is subjective, and it is challenging to obtain sufficient samples from the tumor.

Few publications have compared the genomic profiles of ascites and resected tissues in ovarian cancer. Most of the studies focused on hot-spot mutations or only on *BRCA*-related abnormalities.[20-22]

In the current study, the genomic concordance rate of oncogenic mutations, not only for *BRCA* but for 52 potentially mutated genes, between AC samples and FFPE tissues was very high. In other words, we can understand the genomic characteristics of tumors in detail through a genomic analysis of AC samples. This may be useful, of course, for cases in which it is difficult to obtain surgical or biopsy specimens.

In this study, although the quantity of extracted DNA was limited, the quality obtained from AC samples was similar to that obtained from surgically resected FFPE tissues, and the sequence metrics were comparable. All oncogenic mutations detected by FFPE were also identified in AC samples. In addition, 8 oncogenic mutations that were not detected by FFPE were identified using AC. This may reflect that surgically resected specimens are localized lesions, whereas ascitic fluid may yield a more comprehensive picture of the whole abdominal cavity. It is unclear whether such mutations are clinically significant because many of them have low VAF. It is at least certain that AC may capture the heterogeneity of EOC better than FFPE.

TP53 mutations reportedly occur in 94% to 97% of patients with HGSC.[12,23-25] There are several reports that *TP53* hot-spot mutations are associated with drug resistance and prognosis.[26-29] The concordance rate of *TP53* mutations between tumor tissues and AC samples was 100% in this study.

We also observed that 100% of somatic and germline mutations in *BRCA1*/*BRCA2* were identified by genomic analysis of AC samples. Germline and somatic *BRCA* mutations have a positive impact on overall survival and platinum responsiveness in patients with EOC.[30,31] Moreover, it is important to identify *BRCA1*/*BRCA2* mutations for the selection of treatment and predicting prognosis in patients with EOC because *BRCA1*/*BRCA2*-associated EOC is sensitive to poly-(ADP ribose)-polymerase inhibitors.[32-34] Because immunohistochemical staining of *BRCA1*/*BRCA2* protein is not generalized, genomic analysis of AC samples may be a valuable tool for routine use in *BRCA* testing for patients who have advanced EOC.

Among the other drug-matched mutations identified, the FDA has already approved selumetinib (an MEK inhibitor) for *NF1*-mutated neurofibroma,[35] alpelisib (a PI3Kα-specific inhibitor) with fulvestrant for *PIK3CA*-mutated breast cancer,[36] and olaparib (a poly-[ADP ribose]-polymerase inhibitor) for *CDK12*-mutated prostate cancer.[37]

Unfortunately, in 25 patients (76%) from the current study, no approved drugs are currently indicated. In particular, although oncogenic mutations in *TP53* were identified with high frequency (31 of 33 patients; 94%) in this study, it remains difficult to find efficient and safe compounds that specifically target tumors with mutated *TP53*.[38] Recently, with respect to *TP53* mutations, BAY1895344 (an *ATR* inhibitor) has exhibited strong efficacy in tumors with DNA damage response deficiency.[39] There is also a phase 1 trial of AMG650 in patients with advanced solid tumors who have *TP53* mutations (jRCT203120176; https://rctportal.niph.go.jp/en).

KRAS mutations were also identified in this study, although less frequently (2 of 33 patients; 6%). Currently, there are no approved drugs for *KRAS* mutations in ovarian cancer, but several clinical trials, including ABBV-621 (TRAIL receptor agonist; ClinicalTrials.gov identifier NCT03082209), BI 1701963 (an *SOS1* inhibitor; https://rctportal.niph.go.jp/en), and BI 3011441 (an *MEK* inhibitor; jRCT2031200385) are in progress. MRTX1133, an inhibitor of *KRAS* G12D detected in case 8, is also under development.[40] If these investigational drugs show clinical benefits, it may become mandatory to obtain genomic profiles for all patients.

There are several limitations to this study. First, we selected class V and tumor cell-rich cytology samples for AC analysis. We did not investigate whether the genomic profile of the tumor could be adequately established in cases with few cancer cells for the AC sample. Second, we evaluated a small number of samples with EOC. Further analysis of histologic subtypes other than HGSC should be conducted. In addition, this is a single-center retrospective study, and there is a case selection bias. Conversely, although the number of samples analyzed was relatively small, the conclusions were straightforward. The genomic profiles of AC samples were shown to reflect the profiles of surgically resected FFPE tissues from patients with EOC. Further studies in a larger cohort are needed to establish the utility of the AC samples.

CONCLUSION

The current results indicate that genomic analysis of AC is useful for understanding the tumor characteristics in patients who have EOC without interventional surgery.

This ultimately may play an important role in the development of personalized precision medicine.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Takahiro Nozaki: Conceptualization, data curation, formal analysis, investigation, project administration, resources, validation, visualization, writing–original draft, and writing–review and editing. **Ikuko Sakamoto:** Conceptualization, formal analysis, investigation, resources, and writing–review and editing. **Keiko Kagami:** Formal analysis, investigation, and resources. **Kenji Amemiya:** Funding acquisition, investigation, resources, and validation. **Yosuke Hirotsu:** Data curation, funding acquisition, investigation, methodology, resources, software, and validation. **Hitoshi Mochizuki:** Resources and software. **Masao Omata:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, and writing–review and editing.

REFERENCES

1. Ministry of Health, Labor, and Welfare of Japan. Vital Statistics in Japan, tabulated by the Cancer Information Service. National Cancer Center, Japan; 2022. Accessed January 7, 2022. https://ganjoho.jp/reg_stat/statistics/dl/index.html#mortality
2. Bowtell DD, Bohm S, Ahmed AA, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer.* 2015;15:668-679.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin.* 2019;69:7-34.
4. Karam A, Ledermann JA, Kim JW, et al. Fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup: first-line interventions. *Ann Oncol.* 2017;28:711-717.
5. Amemiya K, Hirotsu Y, Goto T, et al. Touch imprint cytology with massively parallel sequencing (TIC-seq): a simple and rapid method to snapshot genetic alterations in tumors. *Cancer Med.* 2016;5:3426-3436.
6. Kunimasa K, Hirotsu Y, Amemiya K, et al. Genome analysis of peeling archival cytology samples detects driver mutations in lung cancer. *Cancer Med.* 2020;9:4501-4511.
7. Kim S, Kim S, Kim J, et al. Evaluating tumor evolution via genomic profiling of individual tumor spheroids in a malignant ascites. *Sci Rep.* 2018;8:12724.
8. Choi YJ, Rhee JK, Hur SY, et al. Intraindividual genomic heterogeneity of high-grade serous carcinoma of the ovary and clinical utility of ascitic cancer cells for mutation profiling. *J Pathol.* 2017;241:57-66.
9. Ohyama H, Hirotsu Y, Amemiya K, et al. Detection of actionable mutations in archived cytological bile specimens. *J Hepatobiliary Pancreat Sci.* 2021;28:837-847.
10. Hirotsu Y, Yokoyama H, Amemiya K, et al. Genomic profile of urine has high diagnostic sensitivity compared to cytology in non-invasive urothelial bladder cancer. *Cancer Sci.* 2019;110:3235-3243.
11. Hirotsu Y, Kojima Y, Okimoto K, Amemiya K, Mochizuki H, Omata M. Comparison between two amplicon-based sequencing panels of different scales in the detection of somatic mutations associated with gastric cancer. *BMC Genomics.* 2016;17:833.
12. Bell D, Berchuck A, Birrer M, et al. Integrated genomic analyses of ovarian carcinoma. *Nature.* 2011;474:609-615.
13. Hirotsu Y, Otake S, Ohyama H, et al. Dual-molecular barcode sequencing detects rare variants in tumor and cell free DNA in plasma. *Sci Rep.* 2020;10:3391.
14. Sakamoto I, Hirotsu Y, Nakagomi H, et al. *BRCA1* and *BRCA2* mutations in Japanese patients with ovarian, fallopian tube, and primary peritoneal cancer. *Cancer.* 2016;122:84-90.
15. Oyama T, Goto T, Amemiya K, Hirotsu Y, Omata M. Squamous cell carcinoma of the lung with micropapillary pattern. *J Thorac Oncol.* 2020;15:1541-1544.
16. Hirotsu Y, Omata M. Detection of R.1 lineage severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with spike protein W152L/E484K/G769V mutations in Japan. *PLoS Pathog.* 2021;17:e1009619.
17. Hirotsu Y, Omata M. Discovery of a SARS-CoV-2 variant from the P.1 lineage harboring K417T/E484K/N501Y mutations in Kofu, Japan. *J Infect.* 2021;82:276-316.
18. Chakravarty D, Gao J, Phillips S, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol.* 2017;2017:PO.17.00011.
19. Rauh-Hain JA, Nitschmann CC, Worley MJ, et al. Platinum resistance after neoadjuvant chemotherapy compared to primary surgery in patients with advanced epithelial ovarian carcinoma. *Gynecol Oncol.* 2013;129:63-68.
20. Gornjec A, Novakovic S, Stegel V, et al. Cytology material is equivalent to tumor tissue in determining mutations of BRCA1/2 genes in patients with tubo-ovarian high grade serous carcinoma. *BMC Cancer.* 2019;19:296.
21. Fumagalli C, Rappa A, Casadio C, et al. Next-generation sequencing-based BRCA testing on cytological specimens from ovarian cancer ascites reveals high concordance with tumour tissue analysis. *J Clin Pathol.* 2020;73:168-171.
22. Lou SK, Grenier S, Care M, et al. Validation of BRCA testing on cytologic samples of high-grade serous carcinoma. *Cancer Cytopathol.* 2021;129:907-913.
23. Cole AJ, Dwight T, Gill AJ, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. *Sci Rep.* 2016;6:26191.
24. Ahmed AA, Etemadmoghadam D, Temple J, et al. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. *J Pathol.* 2010;221:49-56.
25. Kobel K, Reuss A, du Bois A, et al. The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. *J Pathol.* 2010;222:191-198.
26. Zhang GN, Liu H, Huang JM, et al. TP53 K351N mutation-associated platinum resistance after neoadjuvant chemotherapy in patients with advanced ovarian cancer. *Gynecol Oncol.* 2014;132:752-757.

27. Zhang Y, Cao L, Nguyen D, Lu H. TP53 mutations in epithelial ovarian cancer. *Transl Cancer Res.* 2016;5:650-663.
28. Tuna M, Ju Z, Yoshihara K, Amos CI, Tanyi JL, Mills GB. Clinical relevance of TP53 hotspot mutations in high-grade serous ovarian cancers. *Br J Cancer.* 2020;122:405-412.
29. Seagle BL, Yang CP, Eng KH, et al. TP53 hot spot mutations in ovarian cancer: selective resistance to microtubule stabilizers in vitro and differential survival outcomes from the Cancer Genome Atlas. *Gynecol Oncol.* 2015;138:159-164.
30. Xu K, Yang S, Zhao Y. Prognostic significance of BRCA mutations in ovarian cancer: an updated systematic review with meta-analysis. *Oncotarget.* 2017;8:285-302.
31. Huang YW. Association of BRCA1/2 mutations with ovarian cancer prognosis: an updated meta-analysis. *Medicine (Baltimore).* 2018;97:e9380.
32. Banerjee S, Moore KN, Colombo N, et al. Maintenance olaparib for patients with newly diagnosed advanced ovarian cancer and a BRCA mutation (SOLO1/GOG 3004): 5-year follow-up of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2021;22:1721-1731.
33. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390:1949-1961.
34. Gonzalez-Martin A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med.* 2019;381:2391-2402.
35. Gross AM, Wolters PL, Dombi E, et al. Selumetinib in children with inoperable plexiform neurofibromas. *N Engl J Med.* 2020;382:1430-1442.
36. Andre F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med.* 2019;380:1929-1940.
37. de Bono J, Mateo J, Fizazi K, et al. Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med.* 2020;382:2091-2102.
38. Parrales A, Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. *Front Oncol.* 2015;5:288.
39. Wengner AM, Siemeister G, Lucking U, et al. The novel ATR inhibitor BAY 1895344 is efficacious as monotherapy and combined with DNA damage-inducing or repair-compromising therapies in preclinical cancer models. *Mol Cancer Ther.* 2020;19:26-38.
40. Wang X, Allen S, Blake JF, et al. Identification of MRTX1133, a noncovalent, potent, and selective KRAS G12D inhibitor. *J Med Chem.* 2022;65:3123-3133.

Supporting Table 1. Genes assayed in Gynecologic Cancer Panel by signaling pathway

Gene List (Gynecologic Cancer Panel, 52 SMGs)									
Signaling Pathways	**Genes**								
DNA repair	*ATM*	*ATR*	*BRCA1*	*BRCA2*	*MLH1*	*MSH6*	*POLD1*	*POLE*	*RAD51B*
Epigenetics	*ARID1A*	*ARID1B*	*ARID2*	*ARID5B*	*BAZ1A*	*CHD4*	*KMT2A*	*KMT2C*	*SMARCA4*
RTK/RAS	*BRAF*	*ERBB2*	*FGFR2*	*KRAS*	*MAP3K4*	*MAPK1*	*NF1*	*NRAS*	
Transcriptional regulation	*CBFB*	*CTCF*	*DICER1*	*ELF3*	*NFE2L2*	*SOX17*			
Cell cycle	*CCNE1*	*CDK12*	*CDKN2A*	*RB1*	*TP53*				
PI3K	*PIK3CA*	*PIK3R1*	*PPP2R1A*	*PTEN*	*STK11*				
NOTCH	*EP300*	*FBXW7*	*NOTCH3*						
Wnt	*APC*	*CTNNB1*							
Other	*ABCC9*	*CSMD3*	*CYP4X1*	*RPL22*	*SPOP*				

SMG, significantly mutated gene

Supporting Table 2. List of targeted genes and regions in the panel (52 genes, 287.43kb, 2897amplicon, 95.57% coverage)

No	Gene	Total amino acid	Covered amino acid	Target Regions
1	TP53	393	384	Whole exome
2	NF1	2839	2796	Whole exome
3	BRCA1	1863	1846	Whole exome
4	BRCA2	3418	3165	Whole exome
5	RB1	928	881	Whole exome
6	CDK12	1490	1451	Whole exome
7	CSMD3	3707	3633	Whole exome
8	NOTCH3	2321	1915	Whole exome
9	BRAF	766	39	Hotspot
10	KRAS	188	134	Hotspot
11	NRAS	189	79	Hotspot
12	CDKN2A	156	99	Whole exome
13	PTEN	403	378	Whole exome
14	PIK3CA	1068	1028	Whole exome
15	ARID1A	2285	1949	Whole exome
16	CTNNB1	781	781	Whole exome
17	PPP2R1A	589	579	Whole exome
18	DICER1	1922	1922	Whole exome
19	CHD4	1912	1874	Whole exome
20	SPOP	374	374	Whole exome
21	FBXW7	707	691	Whole exome
22	ABCC9	1549	1529	Whole exome
23	CYP4X1	509	507	Whole exome
24	MAP3K4	1608	1582	Whole exome
25	SMARCA4	1647	1542	Whole exome
26-1	POLE Pro286Arg	30	30	Hotspot
26-2	POLE Val411Leu	30	30	Hotspot
26-3	POLE Leu424Val	30	30	Hotspot
27	POLD1	504	472	Whole exome
28	PIK3R1	724	713	Whole exome

29	RPL22	128	119	Whole exome
30	ARID5B	1188	1116	Whole exome
31	CTCF	727	727	Whole exome
32	FGFR2	821	802	Whole exome
33	ERBB2	1255	1172	Whole exome
34	SOX17	414	221	Whole exome
35	MLL3	4911	4867	Whole exome
36	MLL	3969	3838	Whole exome
37	ARID2	1835	1811	Whole exome
38	ATR	2644	2583	Whole exome
39	ATM	3056	2946	Whole exome
40	APC	2843	2812	Whole exome
41	MAPK1	360	332	Whole exome
42	STK11	433	418	Whole exome
43	EP300	2414	2298	Whole exome
44	NFE2L2	605	114	Hotspot
45	ELF3	371	365	Whole exome
46	CBFB	182	173	Whole exome
47	MLH1	756	750	Whole exome
48	MSH6	1360	1310	Whole exome
49	ARID1B	2231	1914	Whole exome
50	BAZ1A	1556	1531	Whole exome
51	CCNE1	410	405	Whole exome
52	RAD51B	384	359	Whole exome

Supporting Table 3. Patient characteristics and quantity and quality of DNA retrieved from FFPE and AC

ID	Age	Primary site	Stage	FFPE (surgically resected)				AC		
				Histological diagnosis	Short-length DNA (ng/μL)	Long-length DNA (ng/μL)	RQ	Short-length DNA (ng/μL)	Long-length DNA (ng/μL)	RQ
Case 1	61	Peritoneum	III	Serous	575.6	483.6	0.84	77.4	2.7	0.03
Case 2	58	Ovary	III	Serous	157.0	84.0	0.54	73.4	22.7	0.31
Case 3	64	Peritoneum	III	Serous	21.0	3.6	0.17	9.9	1.5	0.15
Case 4	58	Peritoneum	III	Serous	164.2	29.6	0.18	2.6	0.0	0.01
Case 5	69	Peritoneum	IV	Serous	16.3	3.5	0.21	15.4	4.2	0.27
Case 6	41	Ovary	IV	Serous	332.0	98.1	0.30	1.3	0.3	0.26
Case 7	62	Fallopian tube	III	Serous	193.8	13.6	0.07	8.2	3.4	0.42
Case 8	61	Ovary	III	Clear	731.4	260.2	0.36	17.3	6.9	0.40
Case 9	68	Ovary	III	Serous	39.6	15.3	0.39	3.4	0.9	0.26
Case 10	67	Ovary	III	Serous	680.3	300.7	0.44	8.2	1.2	0.15
Case 11	63	Ovary	IV	Serous	184.2	17.3	0.09	46.0	30.5	0.66
Case 12	47	Ovary	I	Serous	2.6	0.4	0.17	43.2	19.1	0.44
Case 13	72	Peritoneum	IV	Serous	151.2	18.2	0.12	21.4	3.3	0.15
Case 14	65	Ovary	IV	Serous	206.9	40.3	0.19	3.3	1.8	0.54
Case 15	71	Fallopian tube	III	Serous	60.9	22.1	0.36	0.2	0.1	0.36
Case 16	63	Ovary	III	Serous	69.5	15.9	0.23	1.7	0.1	0.09
Case 17	70	Ovary	IV	Endometrioid	101.8	31.2	0.31	3.8	2.2	0.57
Case 18	49	Ovary	III	Serous	23.1	3.5	0.15	76.6	51.8	0.68
Case 19	66	Ovary	IV	Serous	24.9	7.8	0.32	6.1	1.6	0.26
Case 20	62	Peritoneum	III	Serous	58.1	37.4	0.64	8.1	3.5	0.43
Case 21	69	Ovary	IV	Serous	73.1	16.1	0.22	30.7	15.2	0.50
Case 22	50	Ovary	III	Serous	47.9	9.0	0.19	1.0	0.2	0.18
Case 23	78	Peritoneum	III	Serous	7.0	3.8	0.54	1.0	0.1	0.12
Case 24	66	Ovary	III	Serous	8.7	6.2	0.71	3.1	0.4	0.13
Case 25	41	Ovary	III	Serous	20.6	2.7	0.13	1.2	0.1	0.10
Case 26	61	Ovary	III	Serous	3.5	0.5	0.15	2.6	0.2	0.08
Case 27	60	Ovary	III	Serous	149.8	24.9	0.17	3.5	2.4	0.69
Case 28	66	Ovary	III	Serous	12.7	5.1	0.40	1.6	0.1	0.08

Case 29	58	Ovary	III	Serous	51.8	6.5	0.12	4.2	0.6	0.15
Case 30	60	Ovary	III	Serous	20.7	7.0	0.34	18.0	2.9	0.16
Case 31	56	Ovary	IV	Serous	12.6	2.8	0.22	27.4	10.1	0.37
Case 32	53	Ovary	III	Serous	25.9	8.0	0.31	5.6	3.5	0.63
Case 33	55	Peritoneum	III	Serous	4.6	1.6	0.34	9.2	4.8	0.52
Mean	61	ovary 23	I 1	serous 31	128.3	47.9	0.30	16.3	6.0	0.31
(range)	(41-78)	Fallopian tube 2	II 0	clear 1	(2.6-731.4)	(0.4-483.6)	(0.07-0.84)	(0.2-77.4)	(0.0-51.8)	(0.01-0.69)
		Peritoneum 8	III 23	endometrioid 1						
			IV 9							

FFPE, formalin-fixed paraffin-embedded; AC, ascitic fluid cytology; RQ, relative quantification

Supporting Table 4. Sequencing metrics of the samples

ID	Primary site	Stage	FFPE (surgically resected)					AC			
			Histological diagnosis	Mapped Reads	On Target	Mean depth	Uniformity	Mapped Reads	On Target	Mean depth	Uniformity
Case 1	Peritoneum	III	Serous	8,728,728	95%	3,103	87%	4,630,602	95%	1,611	94%
Case 2	Ovary	III	Serous	5,495,906	96%	1,924	82%	8,340,334	96%	2,985	97%
Case 3	Peritoneum	III	Serous	4,588,377	96%	1,644	90%	4,692,513	96%	1,600	85%
Case 4	Peritoneum	III	Serous	7,722,441	96%	2,732	92%	3,662,850	96%	1,244	94%
Case 5	Peritoneum	IV	Serous	3,540,816	95%	1,270	92%	6,362,682	95%	2,307	97%
Case 6	Ovary	IV	Serous	6,056,995	96%	2,141	84%	3,346,696	96%	1,213	97%
Case 7	Fallopian tube	III	Serous	2,244,059	95%	731	75%	5,198,037	95%	1,859	98%
Case 8	Ovary	III	Clear	7,726,783	97%	2,677	75%	5,499,182	96%	2,001	97%
Case 9	Ovary	III	Serous	4,546,127	96%	1,654	91%	4,320,098	96%	1,537	97%
Case 10	Ovary	III	Serous	4,528,265	96%	1,601	83%	4,895,345	95%	1,702	97%
Case 11	Ovary	IV	Serous	3,345,764	96%	1,259	93%	7,246,652	95%	2,595	97%
Case 12	Ovary	I	Serous	4,928,281	95%	1,769	95%	6,687,956	96%	2,407	96%
Case 13	Peritoneum	IV	Serous	3,426,921	96%	1,265	96%	4,719,712	96%	1,706	98%
Case 14	Ovary	IV	Serous	2,997,850	97%	1,135	92%	3,584,445	94%	1,297	98%
Case 15	Fallopian tube	III	Serous	1,882,444	95%	672	97%	527,095	90%	138	71%
Case 16	Ovary	III	Serous	2,458,674	95%	881	98%	1,071,980	97%	358	77%

Case 17	Ovary	IV	Endometrioid	2,479,786	93%	881	96%	5,716,835	95%	2,024	93%
Case 18	Ovary	III	Serous	2,093,772	95%	742	97%	8,972,704	95%	3,213	97%
Case 19	Ovary	IV	Serous	3,209,543	95%	1,145	93%	5,916,382	96%	2,174	90%
Case 20	Peritoneum	III	Serous	11,069,271	95%	3,854	94%	4,692,635	94%	1,693	96%
Case 21	Ovary	IV	Serous	4,519,379	96%	1,662	93%	4,444,347	95%	1,648	97%
Case 22	Ovary	III	Serous	3,305,500	95%	1,222	93%	3,383,572	96%	1,131	88%
Case 23	Peritoneum	III	Serous	3,579,158	95%	1,289	98%	6,312,171	97%	2,257	96%
Case 24	Ovary	III	Serous	3,083,227	95%	1,116	97%	4,021,092	96%	1,439	93%
Case 25	Ovary	III	Serous	3,244,475	94%	1,147	97%	8,619,058	95%	3,010	98%
Case 26	Ovary	III	Serous	5,611,236	95%	2,004	96%	4,455,807	96%	1,587	96%
Case 27	Ovary	III	Serous	2,785,976	97%	1,054	92%	4,223,304	94%	1,530	98%
Case 28	Ovary	III	Serous	3,199,639	95%	1,158	95%	3,952,924	95%	1,400	97%
Case 29	Ovary	III	Serous	5,715,832	96%	2,064	97%	3,757,546	97%	1,357	95%
Case 30	Ovary	III	Serous	5,273,470	95%	1,872	96%	4,589,338	96%	1,642	96%
Case 31	Ovary	IV	Serous	7,150,237	96%	2,577	97%	6,881,591	95%	2,477	97%
Case 32	Ovary	III	Serous	7,430,605	94%	2,615	96%	7,105,740	96%	2,608	98%
Case 33	Peritoneum	III	Serous	3,744,258	95%	1,277	88%	3,372,121	95%	1,101	80%
Mean	ovary 23	I 1	serous 31	4,597,388	95%	1,641	92%	5,006,162	95%	1,783	94%
(range)	Fallopian tube 2	II 0	clear 1	(1,882,444-11,069,271)	(93-97%)	(672-3,854)	(75-98%)	(527,095-8,972,704)	(90-97%)	(138-3,213)	(71-98%)
	Peritoneum 8	III 23	endometrioid 1								
		IV 9									

FFPE, formalin-fixed paraffin-embedded; AC, ascitic fluid cytology

Supporting Table 5. Actionable mutations discovered in FFPE and/or AC

ID	Gene	Mutation	FFPE	AC	Cancer Type	Drugs
Case3	*NF1*	splice site	-	+	Neurofibroma	Selumetinib
					All Solid Tumors	Cobimetinib
						Trametinib
Case8	*KRAS*	p.Gly12Asp	+	+	Histiocytosis	Cobimetinib
					All solid tumors	Cobimetinib
						Binimetinib
						Trametinib
	ARID1A	p.Gln2037fs	+	+	All Solid Tumors	PLX2853
						Tazemetostat
Case11	*BRCA1*	p.Glu1210fs	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib
					Pancreatic Adenocarcinoma	Olaparib
Case17	*KRAS*	p.Gly12Val	+	+	Histiocytosis	Cobimetinib
					All Solid Tumors	Cobimetinib
						Binimetinib
						Trametinib
Case19	*PIK3CA*	p.Glu545Gln	+	+	Breast Cancer	Alpelisib+Fulvestrant
Case21	*BRCA2*	p.Val1068fs	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib
					Pancreatic Adenocarcinoma	Olaparib
						Rucaparib
Case26	*PIK3CA*	p.Gln546Lys	+	+	Breast Cancer	Alpelisib+Fulvestrant

Case26	***ARID1A***	**p.Gln1494Ter**	**+**	**+**	**All solid tumors**	**PLX2853**
						Tazemetostat
Case27	***CDK12***	**p.Ser170fs**	**+**	**+**	**Prostate Cancer**	**Olaparib**
					All Solid Tumors	**Pembrolizumab**
						Nivolumab
						Cemiplimab
Case30	***BRCA1***	**p.Gly1759del**	**+**	**+**	**Ovarian Cancer**	**Niraparib**
						Olaparib
						Rucaparib
					Prostate Cancer	**Olaparib**
						Rucaparib
					Breast Cancer	**Olaparib**
						Talazoparib
					Pancreatic Adenocarcinoma	**Olaparib**
Case30	***CDKN2A***	**splice site**	**+**	**+**	**All Solid Tumors**	**Abemaciclib**
						Palbociclib
						Ribociclib
Case32	***BRCA1***	**p.Ala1620fs**	**+**	**+**	**Ovarian Cancer**	**Niraparib**
						Olaparib
						Rucaparib
					Prostate Cancer	**Olaparib**
						Rucaparib
					Breast Cancer	**Olaparib**
						Talazoparib
					Pancreatic Adenocarcinoma	**Olaparib**

FFPE, formalin-fixed paraffin-embedded; AC, ascitic fluid cytology

Supporting Table 6. FDA-approved drug matched mutations discovered in FFPE and/or AC

ID	Gene	Mutation	FFPE	AC	Cancer Type	Drugs
Case3	*NF1*	splice site	-	+	Neurofibroma	Selumetinib
Case11	*BRCA1*	p.Glu1210fs	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib
Case19	*PIK3CA*	p.Glu545Gln	+	+	Breast Cancer	Alpelisib+Fulvestrant
Case21	*BRCA2*	p.Val1068fs	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib
Case26	*PIK3CA*	p.Gln546Lys	+	+	Breast Cancer	Alpelisib+Fulvestrant
Case27	*CDK12*	p.Ser170fs	+	+	Prostate Cancer	Olaparib
Case30	*BRCA1*	p.Gly1759del	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib
Case32	*BRCA1*	p.Ala1620fs	+	+	Ovarian Cancer	Niraparib
						Olaparib
						Rucaparib
					Prostate Cancer	Olaparib
						Rucaparib
					Breast Cancer	Olaparib
						Talazoparib

FDA, U.S. Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; AC, ascitic

fluid cytology