# Comparison of metachronous epithelial ovarian carcinoma by next generation sequencing

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## Abstract #5545

**Introduction**: Epithelial ovarian carcinoma (EOC) is a relatively common malignancy which, by the time it becomes platinum-resistant, contains few good treatment options. Next-generation sequencing (NGS) is a promising technology with the potential to alter how this disease is managed. However, much remains unknown regarding its applicability in ovarian carcinomas. The purpose of this study is to compare metachronous epithelial ovarian carcinoma specimens arising from different sites in an attempt to better understand how to apply NGS in the management of this disease.
**Methods**: A retrospective analysis of sequencing results for 83 metachronous (defined as specimens collected greater than 28 days apart) EOC specimens was performed. In most instances, comparisons involved two different metastatic sites (n=50), while the rest involved a comparison of the primary and a subsequent metastatic specimen (n=33). All specimens had up to 47 genes analyzed using the Illumina MiSeq NGS platform. Tumors were sequenced to a depth of 1500x, enabling the detection of mutations down to 10% variant frequency in 45 of the genes analyzed. For BRCA1 and BRCA2 mutations, detection of mutations was down to 20% variant frequency.
**Results**: Metachronous paired specimens were collected from 43 to 2793 days apart (mean = 519). Mutations were detected in 23 different genes (48.9%, 23/47). Most (46.0%, 38/83) shared just one mutation, with TP53 being the most common (59.0%, 49/83). Only fourteen paired specimens had disagreement in gene results and all of these disagreed in only one gene. A change from wild type to mutated status was found in APC (I1307K, I1317K), BRAF (I463T), PIK3CA (E542K), PTPN11 (G503V), SMO (S342F), and TP53 (G245S, R248Q, R282W). Meanwhile, a reversion from mutated status to wild type was detected in APC (E1317Q, A1474T), BRCA2 (K53E, T2199N) and NOTCH1 (R1568K). Overall, 83.1% (69/83) of paired samples showed complete agreement.
**Conclusion**: This metachronous paired analysis indicates that, at least utilizing this 47-gene panel, a majority of patients with EOC showed no change in their molecular profile, regardless of where the metastatic lesion was located. Utilizing NGS argues for a more comprehensive approach to EOC therapy, and to obtain the most therapeutic options in this disease. These findings, and their clinical impact, should be validated in further studies.

## Background

Epithelial ovarian carcinoma (EOC) is a relatively common malignancy which, by the time it becomes platinum-resistant, contains few good treatment options. Next-generation sequencing (NGS) is a promising technology with the potential to alter how this disease is managed. However, much remains unknown regarding its applicability in ovarian carcinomas. The purpose of this study is to compare metachronous epithelial ovarian carcinoma specimens arising from different sites in an attempt to better understand how to apply NGS in the management of this disease.

## Methods

A retrospective analysis of sequencing results for 83 metachronous (defined as specimens collected greater than 28 days apart) EOC specimens was performed. In most instances, comparisons involved two different metastatic sites (n=50), while the rest involved a comparison of the primary and a subsequent metastatic specimen (n=33). All specimens had up to 47 genes analyzed using the Illumina MiSeq NGS platform. Tumors were sequenced to a depth of 1500x, enabling the detection of mutations down to 10% variant frequency in 45 of the genes analyzed. For BRCA1 and BRCA2 mutations, Illumina MiSeq NGS platform had a sensitivity to detect mutations or variants as low as 20% population of cells.

## Results

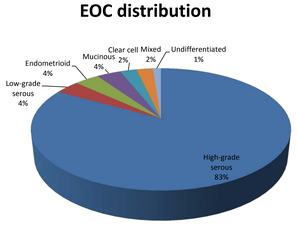


**Figure 1. Epithelial ovarian carcinoma (EOC) paired sample histologic distribution**. The figure on the right shows all histologies in our paired sample cohort. The highest number involved high-grade serous EOC (83.1%, 69/83), with low-grade serous (n=3), endometrioid (n=3), mucinous (n=3), clear cell (n=2), mixed (n=2), and undifferentiated (n=1) rounding out the others. Metachronous specimens had been collected from 43 – 2793 days apart based on the pathology reports provided. The average age at time of specimen collection was 60.9 years.

## Results (cont.)

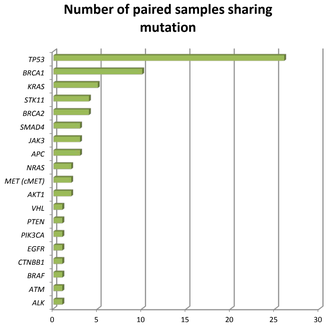


**Figure 2. Distribution of variants in EOC sample set.** Point mutations were detected in 48.9% (23/47) of the genes. Evaluating paired samples together, mutations in four of these 23 genes (i.e. *CSF1R, NOTCH1, PTPN11, SMO*) were detected in one sample but not the other. The figure on the left shows those mutations (n=19) detected in both paired specimens. Most specimens had *TP53* mutations (59.0%, 49/83) which is expected given the large number of serous carcinomas in our cohort. *BRCA1* mutations found in pairs showed 100% agreement (10/10) while *BRCA2* specimens showed agreement 66.7% (4/6) of the time.

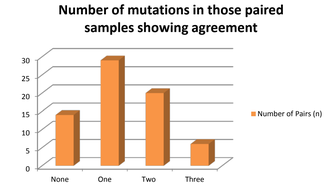


**Figure 3. Number of mutations in paired specimens showing complete agreement.** The figure on the left only shows those concordant pairs (n=69). The fourteen specimens with disagreement in one gene were excluded from this analysis. Including only this group of specimens, 174 mutations were identified in 138 specimens (1.26 mutations identified per specimen).

| Patient number | Histology | Specimen locations (i.e. first v. second site) | Shared mutation(s) in both specimens | Mutation detected in second (but not first) specimen |
|---|---|---|---|---|
| 1 | serous | colon v. pelvis, NOS | None | *PIK3CA* (E542K) |
| 2 | serous | pelvis, NOS v. abdomen, NOS | *KRAS* (G12R) | *BRAF* (I463T) |
| 3 | serous | omentum v. abdomen, NOS | None | *PTPN11* (G503V) |
| 4 | serous and clear cell | peritoneum, NOS v. peritoneum, NOS | *TP53* (195T) | *APC* (E1317Q) |
| 5 | serous | abdomen, NOS v. omentum | None | *TP53* (R248Q) |
| 6 | serous | ***fallopian tube*** v. abdomen, NOS | *TP53* (C141W) | *APC* (I1307K) |
| 7 | serous | diaphragm, NOS v. omentum | *EGFR* (A750V) | *TP53* (R282W) |
| 8 | serous | omentum v. colon, NOS | None | *TP53* (G245S) |
| 9 | serous | ***ovary*** v. peritoneum, NOS | *TP53* (S166X) | *SMO* (S342F) |

**Figure 4A**

## Results (cont.)

| Patient number | Histology | Specimen locations (i.e. first v. second site) | Shared mutation(s) in both specimens | Mutation detected in first (but not second) specimen |
|---|---|---|---|---|
| 10 | serous | omentum v. diaphragm | *KRAS* (G12C) | *NOTCH1* (R1568K) |
| 11 | serous | mesentery v. mesentery | *TP53* (G244S) | *APC* (1317Q) |
| 12 | serous | omentum v. pelvis | *PIK3CA* (P539R), *TP53* (M237I) | *APC* (A1474T) |
| 13 | serous | pleural cavity v. small Intestine | *TP53* (R196P) | *BRCA2* (T2199N) |
| 14 | serous | ***ovary*** v. bladder | *TP53* (R273L) | *BRCA2* (K53E) |

**Figure 4B**

**Figures 4A and 4B. Paired specimens with lack of concordance/agreement.** Fourteen cases, shown in the blue and red tables, had discordant results in one NGS result. None contained two or more discordant NGS results. All were serous ovarian epithelial carcinomas. The most common discordance involved *APC* (33.3%, 4/12) and *TP53* aberrations (33.3%, 4/12). The two *BRCA2* mutations were classified as variants of unknown significance (VUS). Only three discrepant cases compared primary versus metastatic disease (#6, #9, #14). The others compared different metastatic sites.

## Conclusions

- To the best of our knowledge, this is the first study where ovarian cancer paired specimens from a non-academic institution were analyzed.
- The overall low mutation rate is consistent with previous studies on EOC. A multiplatform approach may identify additional potential targets in this lethal disease. Evaluating genetic mutations alone is not sufficient.
- The high agreement rate between primary versus secondary specimens and metastatic versus metastatic site suggests taking a baseline sequencing reading may be sufficient in the majority of cases. Serial monitoring using DNA sequencing may not be necessary during late-stage disease.
- *BRCA1* results did not change in the paired specimens analyzed. However, two pairs with *BRCA2* mutations, classified as variant of unknown significance (VUS), changed from VUS to no mutation.
- Future evaluations in our lab will expand on these initial findings by evaluating larger (i.e. 592-gene) panels.

## References

1. The Cancer Genome Atlas Research Network. "Integrated genomic analyses of ovarian carcinoma". *Nature*. 474:609-615.
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3. Coleman, R.L., T.J. Herzog, *et al.* (2013). "Latest research and clinical treatment of advanced-stage epithelial ovarian cancer". *Nat Rev Clin Oncol.* 2013. 10:211-224.