Prognostic gene expression signature for high-grade serous ovarian cancer

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Background: Median overall survival (OS) for women with high-grade serous ovarian cancer (HGSOC) is ~4 years, yet survival varies widely between patients. There are no well-established, gene expression signatures associated with prognosis. The aim of this study was to develop a robust prognostic signature for OS in patients with HGSOC.

Methods: Expression of 513 genes, selected from a meta-analysis of 1455 tumours and other candidates, was measured using NanoString technology from formalin-fixed paraffin-embedded tumour tissue collected from 3769 women with HGSOC from multiple studies. Elastic net regularization for survival analysis was applied to develop a prognostic model for 5-year OS, trained on 2702 tumours from 15 studies and evaluated on an independent set of 1067 tumours from six studies.

Results: Expression levels of 276 genes were associated with OS (false discovery rate < 0.05) in covariate-adjusted single-gene analyses. The top five genes were TAP1, ZFHX4, CXCL9, FBN1 and PTGER3 (P < 0.001). The best performing prognostic signature included 101 genes enriched in pathways with treatment implications. Each gain of one standard deviation in the gene expression score conferred a greater than twofold increase in risk of death [hazard ratio (HR) 2.35, 95% confidence interval (CI) 2.02–2.71; P < 0.001]. Median survival [HR (95% CI)] by gene expression score quintile was 9.5 (8.3 to 10.7), 5.4 (4.6–7.0), 3.8 (3.3–4.6), 3.2 (2.9–3.7) and 2.3 (2.1–2.6) years.

Conclusion: The OTTA-SPOT (Ovarian Tumor Tissue Analysis consortium - Stratified Prognosis of Ovarian Tumours) gene expression signature may improve risk stratification in clinical trials by identifying patients who are least likely to achieve 5-year survival. The identified novel genes associated with the outcome may also yield opportunities for the development of targeted therapeutic approaches.

Key words: formalin-fixed paraffin-embedded, gene expression, high-grade serous ovarian cancer, overall survival, prognosis

INTRODUCTION

Epithelial ovarian cancer (EOC) causes ~125 000 deaths globally every year, and long-term survival rates have changed little in the past three decades. Approximately 70% of women with EOC are diagnosed with advanced-stage disease (stages III/IV), and fewer than 50% will survive more than 5 years. There are five major EOC histotypes: high-grade serous, low-grade serous, endometrioid, clear cell and mucinous. High-grade serous ovarian cancer (HGSOC) comprises about two-thirds of cases, is responsible for most deaths and is characterized by profound genomic and clinical heterogeneity.

The most informative prognostic factors for HGSOC are International Federation of Gynecology and Obstetrics (FIGO) stage, residual disease following debulking surgery, BRCA1 or BRCA2 germline mutation and tumour-infiltrating lymphocyte scores. Patients with HGSOC who carry a loss-of-function germline mutation in BRCA1 or BRCA2 have an increased sensitivity to platinum-based chemotherapy and PARP inhibitor treatment and a medium-term survival advantage. However, the frequent development of drug-resistant disease limits the effectiveness of current therapies.

Gene expression data have been used to define four tumour molecular subtypes of HGSOC (C1/mesenchymal, C2/immune, C4/differentiated and C5/proliferative).
Using transcriptome-wide data from fresh frozen tissues, The Cancer Genome Atlas (TCGA) project used 215 tumours to identify an overall survival (OS) expression signature of 193 genes that has been validated on three other HGSOC gene expression datasets.12 Despite these findings, gene expression biomarkers have not been implemented clinically owing to several important shortcomings. The majority of the individual markers comprising the 193 gene signature were not statistically significant across all studies, suggesting that the signature may not be robust. The sample sizes in other discovery efforts have been too small for robust statistical inference.12 In addition, previous studies used fresh frozen samples, resulting in logistic and cost barriers to examining large clinically relevant datasets, and translation to the clinical setting.

The aim of this study was to identify a robust and clinically relevant prognostic HGSOC profile that can be applied to formalin-fixed paraffin-embedded (FFPE) tumour tissue.

PATIENTS AND METHODS

Twenty studies provided pretreatment FFPE tumour samples from 4071 women diagnosed with HGSOC (supplementary Table S1, available at Annals of Oncology online). All HGSOC cases with available tissue were included. During this period, patients with HGSOC were treated with chemotherapy (carboplatin and paclitaxel) after primary debulking surgery. Study protocols were approved by the respective Institutional Review Board/Ethics Approval Committee for each site (supplementary Table S1, available at Annals of Oncology online).

A schematic of the overall study design is shown in Figure 1. There were four main components: gene selection, gene expression assay, development of prognostic gene signature in a training set and validation of prognostic signature in an independent validation set.

Gene selection

Candidate prognostic genes were identified by carrying out an individual participant meta-analysis of six transcriptome-wide microarray studies,11–16 which included tumour samples from 1455 participants. Association of gene expression with OS was evaluated by Cox proportional hazards regression adjusted for molecular subtype (supplementary Table S2, available at Annals of Oncology online). In total, 200 genes from the meta-analysis, most achieving a permutation-based false discovery rate (FDR)17 of <0.05, and an additional 313 candidate genes based on the literature and unpublished data were selected (supplementary Tables S3 and S4, and supplementary Figure S1, available at Annals of Oncology online; for more details see supplementary Material, available at Annals of Oncology online). Five genes, RPL19, ACTB, PGK1, SDHA and POLR1B, were included as house-keeping genes for normalization.

Gene expression assay in tumour samples from study participants

FFPE tumour samples were processed with the NanoString nCounter technology at three different locations: Vancouver, Los Angeles and Melbourne. A control set of 48 FFPE tumour samples was run at each location and the average intraclass correlation coefficient was 0.987. Approximately 2% of the samples were run in duplicate and the average Spearman’s correlation coefficient $r_s$ was 0.995. Single-patient classification methods were used with reference samples to control for batch effects.18 The data in this publication have been deposited in NCBI’s Gene Expression Omnibus19; GEO Series accession number GSE132342 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132342). A total of 3329 samples passed quality control of which 3769 had survival data and assessable gene expression for 513 genes. Data can be found in NCBI GEO: Accession numbers GSE132342 and GPL26748.

Overall survival analysis of individual genes

Samples that contributed to the meta-analysis dataset ($n = 211$) were removed from subsequent selected analyses to enforce independence of study samples between the gene selection and final survival analysis. Time-to-event analyses were carried out for OS with right censoring at 10 years and left truncation of prevalent cases. Associations between log-transformed normalized gene expression and survival time were tested using likelihood ratio tests with Cox proportional hazards models adjusted for age, race and stage, and stratified by study. Patients with missing race or stage information were assigned to ‘unknown’ categories. Age was modelled using a B-spline with a knot at the median age, which yielded a better fit than using knots at quartiles or categorical variables. Stage was dichotomized into early (FIGO stage I/II) and advanced (FIGO stage III/IV). Genes were scaled to have a standard deviation of one, so hazard ratios (HRs) correspond to a change of one standard deviation. A Benjamini–Hochberg FDR of <0.05 was used to identify notable associations. Because the expression of genes can be correlated, an analysis of correlated genes was performed using data from TCGA. Advanced stage ovarian cancer usually has disease spread throughout the abdomen, and therefore sensitivity analyses were performed to assess effects of the anatomical location of tumour samples included in the study by removing observations corresponding to samples known to be extraovarian ($n = 437$).

Prognostic signature development and validation

Studies were initially randomized to training set ($N = 14$) and validation set ($N = 6$). The TRI study was randomized to the validation set, but, because 107 samples were part of the meta-analysis data used for gene selection, the study was split, so those 107 samples were included in the model training dataset. Thus 2702 samples from 15 studies were used for model training and 1067 samples from 6 studies were used for validation (supplementary Table S1, available at Annals of Oncology online). In the training set, four
**Figure 1. Schematic of study design.**

*The TRI study was split across the training and validation sets due to 107 samples overlapping with the meta-analysis. GWAS, genome-wide association studies; HGSOC, high-grade serous ovarian cancer.*
modelling approaches (stepwise regression, elastic net regularized regression, boosting and random survival forests) were applied to construct competing gene expression–based biomarkers. Each was evaluated in the training data using 10-fold cross-validation for its prognostic value for OS at 2 and 5 years of follow-up using an area under the curve (AUC) measure derived from receiver operator characteristic analysis (see supplementary Material, available at Annals of Oncology online, for additional details). The best performing method, elastic net regularized regression, was applied to the full training set to determine the final gene signature and scoring method, which was then evaluated using the independent testing set. All models were constrained to include age and stage, where age was modelled as categorical based on quartiles of the training dataset with groups aged <53, 53–59, 60–66, and ≥67. Stage was modelled as described earlier for the OS individual gene analysis.

RESULTS

Association of expression of individual genes with OS in HGSOC

In a gene-by-gene analysis of the full dataset adjusted for age, race and stage, and stratified by study, 276 of the 513 selected genes were associated with OS (FDR < 0.05). Of these, 138 were selected from the meta-analysis of six published microarray studies (supplementary Table S2, available at Annals of Oncology online)11–16 and 144 from candidate gene approaches (supplementary Tables S5 and S6, available at Annals of Oncology online). HRs for one standard deviation change in gene expression ranged from 0.84 to 1.19, with multiple genes exhibiting associations at very stringent significance levels (e.g. 19 genes with \( P < 1 \times 10^{-8} \); supplementary Tables S5 and S6, available at Annals of Oncology online). The five most significant genes were TAP1, ZFHX4, CXCL9, FBN1 and PTGER3 (Table 1). We did not find extensive evidence of high co-expression between these five genes and genes measured in TCGA project (supplementary Table S7, available at Annals of Oncology online). In sensitivity analyses we found that excluding samples from omentum and other extra-ovarian sites did not substantially affect the results (supplementary Tables S8 and S9, available at Annals of Oncology online).

Development of a novel prognostic gene signature

The four predictive modelling approaches that were evaluated in the training data using 10-fold cross-validation yielded median AUCs that ranged from 0.69 to 0.73 for 2-year OS and 0.69 to 0.74 for 5-year survival (supplementary Figure S2, available at Annals of Oncology online) with better prediction of 5-year OS than of 2-year OS. The elastic net approach yielded the highest median AUC for both 2- and 5-year OS and was selected for final development of the signature. Using the model on the full training dataset resulted in a prognostic signature of 101 genes in addition to age and stage (supplementary Table S10, available at Annals of Oncology online). Of these, 66 genes were associated with OS (FDR < 0.05) in the single gene models. There was no obvious subset of signature genes that performed as well or nearly as well as the full 101 gene signature (supplementary Figure S3, available at Annals of Oncology online).

Performance of the signature including age and stage was AUC 0.69 [95% confidence interval (CI) 0.65–0.73] and AUC 0.75 (95% CI 0.72–0.78) for 2- and 5-year OS, respectively (Figures 2 and 3; supplementary Figure S4, available at Annals of Oncology online). This was substantially better than age and stage alone with AUC 0.61 (95% CI 0.57–0.65) and AUC 0.62 (95% CI 0.59–0.67) for 2- and 5-year OS, respectively, particularly for the 5-year OS outcome with non-overlapping 95% CI. One standard deviation change in the gene expression score was associated with an HR of 2.35 [(95% CI 2.02–2.71); \( P = 5.1 \times 10^{-31} \)], and median survival [HR (CI)] varied substantially across quintiles of the gene expression score [9.5 (8.3 to 10.3), 5.4 (4.6–7.0), 3.8 (3.3–4.6), 3.2 (2.9–3.7) and 2.3 (2.1–2.6) years, respectively, from smallest to largest quintile (Q1–Q5); Table 2].

For a subset of cases, there was clinical and experimental data for known prognostic factors. All samples had molecular subtype classification (Talhouk et al.20); residual disease was known for 1771 cases, primary chemotherapy treatment for 687, germline BRCA mutation status for 904 and nuclear CD8 tumour-infiltrating lymphocyte counts6 for 1111 (supplementary Table S11, available at Annals of Oncology online). When examined by quintile of gene expression score there were differences, as expected, for each of the known prognostic factors, including age and stage that were included in the model (Table 3). However, in sensitivity analyses, applying the signature to specific

| Table 1. Hazard ratios and 95% CIs for top five prognostic genes in covariate-adjusted single-gene analyses |
|-----------------|-----------------|-----------------|
| **Gene**       | **HR (95% CI)** | **Selection**   | **Correlated gene**  |
| TAP1           | 0.84 (0.80–0.87) | \( 8.3 \times 10^{-18} \) | Meta |
| ZFHX4          | 1.19 (1.14–1.25) | \( 1.4 \times 10^{-15} \) | Meta |
| CXCL9          | 0.85 (0.82–0.88) | \( 1.8 \times 10^{-15} \) | Meta and candidate |
| FBN1           | 1.18 (1.13–1.24) | \( 4.2 \times 10^{-14} \) | Candidate |
| PTGER3         | 1.18 (1.13–1.24) | \( 1.2 \times 10^{-13} \) | Meta |

CI, confidence interval; HR, hazard ratio.

a Most correlated gene according to Spearman’s rank correlation coefficient, \( r_s \), computed in The Cancer Genome Atlas (TCGA) Ovarian Serous Cystadenocarcinoma RNA-seq dataset.

b SPARC was included in this project and was less significant.
patient groups, a robustness of stratification was demonstrated, suggesting that the prognostic power of the signature is not explained by the individual factors, residual disease, treatment, BRCA status or CD8 score (Figure 3 and supplementary Figures S5–S7, available at *Annals of Oncology* online). The signature score showed modest differences by molecular subtype (supplementary Figure S8, available at *Annals of Oncology* online), and adjusting for molecular subtype in the Cox analysis resulted in only minor changes to the HR estimates for signature quintiles (Table 2). The signature was shown to be prognostic within a homogenous group of 316 stage IIIC cases with no residual disease, within early stage cases (FIGO IA and IB) and within patients whose samples were collected from the omentum (supplementary Figures S9 and S10, available at *Annals of Oncology* online). Within patients with ovarian cancer who are initially sensitive to chemotherapy, expression may be strong indicators of immune system, but the top two pathways were PI-3K (phosphoinositide 3-kinase) cascade and GPCR (G protein–coupled receptor) ligand binding. Four other pathways were related to the cell cycle and mitosis, with the remaining enriched for fibroblast growth factor receptor (FGFR) and epidermal growth factor receptor (ERBB) signalling, and one pathway related to homologous combination repair.

**DISCUSSION**

In a large-scale study of patients with HGSOC, we identified a 101-gene expression signature able to predict clinically relevant differences in OS. Using methods that are both economical and applicable to standard clinical sampling techniques, we showed that the signature performs substantially better than age and stage alone for prognosis of both 2- and 5-year OS. The number of patients and samples included in this study is an order of magnitude greater than previous comparable studies of gene expression and OS in patients with HGSOC.12,21,22 Thus, we have been able to more precisely quantify the prognostic value of gene expression.

We report definitive associations between OS and expression of 276 genes. Of the five most significant genes (TAP1, ZFHX4, CXCL9, FBN1 and PTGER3), four have been previously reported to be associated with survival in HGSOC. The top prognostic gene, TAP1, is involved in the antigen-presenting pathway. Expression was reduced in metastatic HGSOC, positively associated with OS,23 as observed here, and linked to tumour regression in response to treatment.24 Further, hypomethylation of TAP1 was associated with improved time to disease recurrence.25 CXCL9 is a chemokine that mediates the recruitment of T cells to solid tumours.26 High expression of intratumoural CXCL9 was associated with higher OS23 and higher lymphocytic infiltration, which is also a robust prognostic factor in HGSOC.28,31,32 and a feature of the immunoreactive HGSOC molecular subtype.13 CXCL9 has also been proposed as a therapeutic target due to evidence that it inhibits angiogenesis and promotes antitumour adaptive immunity.29,30 Strikingly, the signature was able to further refine prognostic groups within patients with high tumour-infiltrating lymphocyte counts suggesting that CXCL9 and TAP1 expression may be strong indicators of immune competency in HGSOC.

**FBN1** is an extracellular matrix protein previously found to be a biomarker associated with early recurrence in patients with ovarian cancer who are initially sensitive to chemotherapy and strongly correlated with desmoplasia in HGSOC. The prostaglandin E2 receptor PTGER3 is expressed in ovarian tumour cells and is associated with relapse-free survival.33 By contrast, ZFHX4 does not have previous associations with HGSOC.

Associations between the expression of specific genes in tumour tissues and OS in patients with HGSOC may suggest new drug targets and lead to insights into biological variation in treatment response. For example, cases in the Q5 quintile with the poorest outcome had increased expression of IGF2, FGFR1 and MYC, a possible argument for the use of...
IGFR1, FGFR, bromodomain (MYC) or a combination of PARP and CDK4/6 inhibitors (MYC). More immediately, the signature may help clinicians identify patients most in need of intervention, such as patients that could potentially benefit from neoadjuvant chemotherapy (NACT). Alternatively, in clinical trials it could be used to stratify randomization by patients’ risk, thereby reducing heterogeneity within subgroups and increasing heterogeneity between subgroups. The signature will be incorporated into future prospective clinical trials to determine if it can predict response to specific treatments.

Measurement of the signature required standard FFPE tissue used in routine histopathology. In addition, data preprocessing and normalization were conducted on an individual level, thus translatable to a general patient population. That is, 5-year OS prognosis of future patients

Table 2. Hazard ratios and 95% CIs for quintiles of the gene expression signature score in validation data

| Quintile | N  | Deaths | Median survivala | HR (95% CI) | Adjusted for age and stage | Adjusted for molecular subtype age and stage |
|----------|----|--------|------------------|-------------|-------------------------|------------------------------------------|
|          |    |        |                  |             |                         |                                          |
| Q1       | 214| 81     | 9.47 (8.32 to 11) | 0.44 (0.33–0.58) | 0.34 (0.22–0.55) | 0.37 (0.23–0.59)                          |
| Q2       | 213| 117    | 5.38 (4.63–6.97)  | 0.73 (0.57–0.93) | 0.71 (0.55–0.91) | 0.74 (0.58–0.96)                          |
| Q3       | 213| 145    | 3.80 (3.34–4.60)  |              |                         |                                          |
| Q4       | 213| 158    | 3.23 (2.85–3.68)  | 0.156 (1.24–1.97) | 1.56 (1.24–1.97) | 1.56 (1.24–1.97)                          |
| Q5       | 214| 179    | 2.27 (2.09–2.62)  | 2.23 (1.76–2.78) | 2.11 (1.67–2.67) | 2.07 (1.63–2.61)                          |

CI, confidence interval; HR, hazard ratio.
a Median survival (95% CI) in years for patients in the validation set.
Table 3. Clinical data for the 3769 patients that passed quality control and the percentage of patients in each quintile of the gene expression score

|                      | Total | Q1 | Q2 | Q3 | Q4 | Q5 | P value |
|----------------------|-------|----|----|----|----|----|---------|
| N                    | 3769  | 754| 753| 754| 753| 754|         |
| Median survival (years) | 4.1   | 9.5| 5.4| 3.8| 3.2| 2.3| <1 x 10^-5 |
| % 5-year survival     | 41    | 75 | 57 | 39 | 25 | 10 |         |
| Age median            | 63    | 58 | 57 | 61 | 64 | 70 |         |
| Age range            | 25–89 | 39–78| 25–86| 36–82| 27–89| 39–86|         |
| Age quartile Q1      | 894   | 30.8| 31.3| 20.0| 13.4| 4.5|         |
| Age quartile Q2      | 838   | 21.5| 20.0| 22.9| 21.2| 14.3|         |
| Age quartile Q3      | 961   | 16.0| 20.2| 21.4| 23.6| 18.7|         |
| Age quartile Q4      | 1076  | 3.5 | 10.4| 16.4| 21.3| 38.5|         |
| FIGO stage I/II      | 607   | 97.4| 2.6 | 0.0 | 0.0 | 0.0 | <1 x 10^-5 |
| FIGO stage III/IV    | 3067  | 2.3 | 23.0| 24.1| 24.4| 24.6|         |
| Primary chemo1 1     | 136   | 16.2| 22.1| 23.5| 19.1| 19.1| 0.163   |
| Primary chemo2 2     | 190   | 16.3| 20.0| 21.6| 22.1| 20.0|         |
| Primary chemo3 3     | 361   | 11.1| 16.9| 22.4| 20.5| 29.1|         |
| Residual disease: No | 614   | 32.4| 22.1| 17.8| 15.5| 12.2| <1 x 10^-5 |
| Residual disease: Yes| 1157  | 6.0 | 19.2| 24.1| 24.5| 26.2|         |
| Germline BRCA1 mutation | 130  | 23.8| 31.5| 26.2| 11.5| 6.9 | 2.22 x 10^-7 |
| Germline BRCA2 mutation | 71   | 28.2| 26.8| 18.3| 18.3| 8.5 |         |
| Germline no BRCA1/2 mutation | 663 | 19.6| 16.7| 18.7| 20.7| 24.3|         |
| CD8 TIL score 0      | 192   | 19.8| 14.6| 12.5| 21.4| 31.8|         |
| CD8 TIL score 1–2    | 186   | 18.3| 14.0| 18.8| 21.5| 27.4|         |
| CD8 TIL score 3–19   | 515   | 19.8| 24.1| 20.8| 17.9| 17.5|         |
| CD8 TIL score >19    | 218   | 34.4| 31.2| 16.5| 11.5| 6.4 |         |
| Molecular subtype C1.MES | 1105 | 5.4 | 10.4| 20.7| 27.4| 36.0| <1 x 10^-5 |
| Molecular subtype C2.IMM | 907  | 23.2| 28.8| 21.2| 16.2| 10.7|         |
| Molecular subtype C4.DIF | 1144 | 32.6| 25.5| 17.9| 12.8| 11.2|         |
| Molecular subtype C5.PRO | 613  | 18.1| 14.0| 20.7| 25.8| 21.4|         |
| FIGO stage IA and IB | 111   | 96.4| 3.6 | 0.0 | 0.0 | 0.0 | <1 x 10^-5 |
| FIGO stage IIIC No   | 1979  | 3.5 | 23.7| 24.4| 24.1| 24.6| <1 x 10^-5 |
| FIGO stage IIIC residual disease: No | 316   | 6.3 | 31.0| 24.4| 20.9| 17.4| 6.24 x 10^-4 |
| FIGO stage IIIC residual disease: Yes | 846   | 2.6 | 21.5| 25.3| 24.6| 26.0|         |

Q1 is the quintile with the best survival and Q5 the worst survival. Samples with missing data are reported in supplementary Table S11, available at Annals of Oncology online. P values for BRCA1/2 mutation status were calculated for BRCA1 or BRCA2 mutation versus no mutation.

FIGO, International Federation of Gynecology and Obstetrics; TIL, tumour-infiltrating lymphocyte.

* Treatment: 1 = known to have received first-line chemotherapy treatment of ≥4 cycles of IV carboplatin AUC 5 or 6 and paclitaxel 135 or 175 mg/m² every 3 weeks. 2 = known to have received first-line chemotherapy treatment of ≥4 cycles of IV carboplatin and paclitaxel three times weekly but at doses presumed to be carboplatin AUC 5 or 6 and paclitaxel 135 or 175 mg/m². 3 = all remaining cases with chemother regimens that do not fit criteria 1 or 2 and include unknown or no chemotherapy.

can be evaluated against the patient population reported here by (i) following the same steps described here for generating the normalized gene expression data, (ii) computing an individual signature score and (iii) assigning an HR based on the score or comparing it with the reported quintiles (supplementary Material, available at Annals of Oncology online). NanoString gene expression is highly reproducible as seen by our quality control metrics (supplementary Material, available at Annals of Oncology online) and the FDA approval of the Prosigna test for breast cancer.

The question of heterogeneity by ancestry or ethnicity was beyond the scope of this study but should be pursued in future research. Another important question is whether molecular subtype can improve biomarker performance. A substantial proportion of signature genes were identified by the subtype-adjusted meta-analysis, suggesting that the strong performance of the signature is not solely attributable to differences among molecular subtypes. In addition, all of the individual genes used in the molecular subtype classification were included in development of the signature.

Although the cases received chemotherapy, the FFPE samples used in this study were chemo-naive, as few patients had NACT during the calendar period in which these samples were collected. Because the signature appears to be prognostic in omentum samples, future studies may assess the value in NACT patients, using pretreatment omental biopsies or post-treatment tumour samples. Future work will also address if the signature can predict platinum-refractory patients.

We have developed a robust prognostic signature for HGSOC that can be used to stratify patients and identify those in need of alternative treatments. Gene set enrichment analysis applied to the signature indicates an important role for the immune system in OS and supports further investigation of immune therapy in ovarian cancer. More generally, the identification here of high-confidence prognostic genes may lead to new hypotheses for targeted treatments.

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DISCLOSURE

BYK served on Invitae Corporation’s Advisory Board from 2017 to 2018. IAM has acted on the Advisory Boards for AstraZeneca, Clovis Oncology, Tesaro, Carrick Therapeutics and Takeda. His institution receives funding from AstraZeneca. RG is on the Advisory Boards for AstraZeneca, Tesaro, Clovis and Immunogen and does consultancy work for SOTIO. She has received support to attend conferences from AstraZeneca, Roche and Tesaro. Her institution has received research funding from Boehringer Ingelheim and Lilly/Ignyta and she is the national co-ordinating investigator for the UK for trials sponsored by AstraZeneca and Tesaro and site principal investigator for trials sponsored by AstraZeneca, Tesaro, Immunogen, Pfizer, Lilly and Clovis.
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REFERENCES

1. Vaughan S, Coward JI, Bast Jr RC, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Not Rev Cancer. 2011;11: 719–725.
2. Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68:284–296.
3. 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.
4. du Bois A, Reuss A, Pujade-Lauraine E, et al. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multi-center trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OGV) and the Groupe d’Investigateurs Nationaux Pour les Etudes des Cancers de l’Ovaire (GINECO). Cancer. 2009;115:1234–1244.
5. Bolton KL, Chenevix-Trench G, Goh C, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307:382–390.
6. Candido-dos-Reis FJ, Song H, Goode EL, et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. Clin Cancer Res. 2015;21:652–657.
7. Goode EL, Block MS, Kalli KR, et al. Dose-response association of CD8+ tumor-infiltrating lymphocytes and survival time in high-grade serous ovarian cancer. JAMA Oncol. 2017;3:e173290.
8. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203–213.
9. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet. 2017;18:1274–1284.
10. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379:2495–2505.
11. Tothill RW, Tinker AV, George J, et al. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res. 2008;14:5198–5208.
12. Cancer Genome Atlas Research. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–615.
13. Bonome T, Levine DA, Shih J, et al. A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. Cancer Res. 2008;68:5478–5486.
14. Karlan BY, Derig J, Walsh C, et al. POSTN/TGFBI-associated stromal signature predicts poor prognosis in serous epithelial ovarian cancer. Gynecol Oncol. 2014;132:334–342.
15. Konceny GE, Haluska P, Janicek F, et al. A phase II, multicenter, randomized, double-blind, placebo-controlled trial of ganitumab or placebo in combination with carboplatin/paclitaxel as front-line therapy for optimally debulked primary ovarian cancer: the TRIIO14 trial. J Clin Oncol. 2014;32:5529.
16. Konceny GE, Wang C, Hamidi H, et al. Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. J Natl Cancer Inst. 2014;106:duj249.
17. Millstein J, Volkson D. Computationally efficient permutation-based confidence interval estimation for tail-area FDR. Front Genet. 2013;4:179.
18. Talhouk A, Kommoss S, Mackenzie R, et al. Single-patient molecular testing with NanoString nCounter data using a reference-based strategy for batch effect correction. PLoS One. 2016;11:e0153844.
19. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002;30:207–210.
20. Talhouk A, George J, Wang C, et al. Development and validation of the gene-expression Predictor of high-grade-serous Ovarian carcinoma molecular subTYPE (PrOTYPE). Clin Cancer Res. 2020. https://doi.org/10.1158/1078-0432.CCR-20-0103.
21. Jin C, Xue Y, Li Y, et al. A 2-protein signature predicting clinical outcome in high-grade serous ovarian cancer. Int J Gynecol Cancer. 2018;28:51–58.
22. Mankoo PK, Shen R, Schultz N, et al. Time to recurrence and survival in serous ovarian tumors predicted from integrated genomic profiles. PLoS One. 2011;6:e24709.
23. Nymoen DA, Hetland Falkenthal TE, Holth A, et al. Expression and clinical role of chemoresponsive-associated genes in ovarian serous carcinoma. Gynecol Oncol. 2015;139:30–39.
24. Jimenez-Sanchez A, Memon D, Poupe S, et al. Heterogeneous tumor-immune microenvironments among differentially growing metastases in an ovarian cancer patient. Cell. 2017;170:927–938.e20.
25. Wang C, Cicek MS, Charbonneau B, et al. Tumor hypomethylation at 6p21.3 associates with longer time to recurrence of high-grade serous epithelial ovarian cancer. Cancer Res. 2014;74:3084–3091.
26. Gorbachev AV, Kobayashi H, Kudo D, et al. CXCL9 chemokine ligand 9/monokine induced by IFN-gamma production by tumor cells is critical for T cell-mediated suppression of cutaneous tumors. J Immunol. 2007;178:2278–2286.
27. Bronger H, Singer J, Windmuller C, et al. CXCL9 and CXCL10 predict survival and are regulated by cyclooxygenase inhibition in advanced serous ovarian cancer. Br J Cancer. 2016;115:553–563.
28. Ovarian Tissue Tissue Analysis (OTTA) Consortium, Goode EL, Block MS, et al. Dose-response association of CD8+ tumor-infiltrating lymphocytes and survival time in high-grade serous ovarian cancer. JAMA Oncol. 2017;3:e173290.
29. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation — a target for novel cancer therapy. Cancer Treat Rev. 2018;63:40–47.
30. Xiao P, Guo Y, Zhang H, et al. Myeloid-restricted ablation of Shp2 restrains melanoma growth by amplifying the reciprocal promotion of CXCL9 and IFN-gamma production in tumor microenvironment. Oncogene. 2018;37:5088–5100.
31. Zhang R, Tian L, Chen LJ, et al. Combination of MIG (CXCL9) chemokine gene therapy with low-dose cisplatin improves therapeutic efficacy against murine carcinoma. Gene Ther. 2006;13:1263–1271.
32. Zhang W, Ota T, Shridhar V, et al. Network-based survival analysis re- veals subnetwork signatures for predicting outcomes of ovarian cancer treatment. PLoS Comput Biol. 2013;9:e1002975.
33. Reintratz S, Finknagel F, Adhikary T, et al. A transcriptome-based global map of signaling pathways in the ovarian cancer microenvi- ronment associated with clinical outcome. Genome Biol. 2016;17:108.
34. Konceny GE. Combining PARP and CDK4/6 inhibitors in MYC driven ovarian cancer. EbioMedicine. 2019;43:9–10.
