Combination of gene set signatures correlates with response to nivolumab in platinum-resistant ovarian cancer

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Abstract

Background Based on our previous phase II clinical trial of anti-programmed death-1 (PD-1) antibody nivolumab for platinum-resistant ovarian cancer (n=19, UMIN000005714), we aimed to identify the therapeutic response biomarkers to nivolumab in ovarian cancer.

Methods Tumor gene expressions were evaluated by proliferative, mesenchymal, differentiated, and immunoreactive gene signatures derived from high-grade serous carcinomas in The Cancer Genome Atlas and a signature established prior to ovarian clear cell carcinoma. Gene sets were scored using the single-sample gene set enrichment analysis, and resulting scores were used to assess the correlation between each gene set and the clinical response to nivolumab therapy. Statistical analyses were performed to identify pathways differentially expressed by either the complete response (CR) or progressive disease (PD) groups.

Results The clear cell gene signature significantly had higher score in the CR group, and the proliferative gene signature had significantly higher score in the PD group where nivolumab was not effective (respective p-values 0.005 and 0.026). Combinations of gene signatures improved correlation with response, where a projection of immunoreactive, proliferative, and clear cell signatures differentiated clinical response.

Conclusion Ovarian cancer-specific gene signature and related pathway scores provide a preliminary indicator for ovarian cancer prior to receiving anti-PD-1 antibody therapy.

Introduction

Ovarian cancer, the leading cause of death among gynecological malignancies, includes high-grade serous (75%), endometrioid (10%), clear cell (10%), and mucinous (3%) carcinomas in histopathological subtyping¹. The standard first-line chemotherapy of ovarian cancer employs a combination of taxane and platinum agents. Clear cell carcinoma, with an increased prevalence in East Asian populations, is resistant to chemotherapy^{2,3}. Several cancer types have been significantly studied to identify transcriptome profiles in bulk tumor tissue that correlate with response to chemotherapy. We have previously shown that the correlation between tumor microenvironment and subtype-specific transcriptome profiles, resulting in the “Classification of Ovarian Cancer” (CLOVAR) gene expression signatures⁴, could be indicative of antitumor and prognostic response to individual small molecule chemotherapeutic agents in ovarian cancer⁵. Particularly, the ovarian mesenchymal subtype has the worst prognosis but is responsive to taxane^{6,7}.

Gene expression continues to be a central focal point as cancer therapy strategies broaden to incorporate the emerging use of immune checkpoint inhibitors that target the immunosuppressive resistance mechanisms of tumors. The transcriptomes of both the intra-tumoral and stromal immune-tumor interface are possibly associated with the immune context of the cancer with prognostic (survival),

predictive (response), and mechanistic (differential transcript) understanding, as recently reviewed by Galon and Bruni ⁸.

In a recent study, Liu et al. reported the results of a phase II study combining the immune checkpoint anti-programmed death-1 (PD)-1 inhibitor nivolumab and bevacizumab in relapsed ovarian cancer. In this study, patients were stratified according to tumor resistance or sensitivity to platinum ⁹, and the findings showed that platinum-sensitive patients responded better than platinum-resistant patients. Concurrently, we previously conducted a phase II study comprising 19 patients regarding nivolumab exclusively for platinum-resistant recurrent ovarian cancer. In this study, two patients, including one patient with clear cell carcinoma, demonstrated a complete response (CR) to the therapy, contributing to an overall response rate of 15% and a disease control rate of 45% ¹⁰. Although tumor expression of the programmed death-ligand 1 (PD-L1) is predictive of the therapeutic response in certain tumor types such as melanoma and non-small cell lung cancer ^{11–15}, we found that PD-L1 expression alone was insufficiently predictive of response in the case of ovarian cancer ^{10,16}.

Despite reports that immune checkpoint inhibitor therapy is currently demonstrating remarkable and durable response in a number of solid and hematological cancers ^{17–21}, its response rates are still far from optimal ⁹. As reviewed by Cogdill et al., the appropriate dosage of chemotherapeutic agents administered in immune checkpoint inhibitor therapy remains unclear for patients who positively respond to this type of therapy. Additionally, there is an urgent need to develop predictive biomarkers ²². Identifying the predictive biomarkers in immune checkpoint inhibitor therapy is remarkable, specifically for ovarian cancer, where regulatory approval for clinical therapy by immune checkpoint inhibitors has not yet been obtained in any country ²³.

Although individual gene expression can be used to determine consistent expression-outcome correlations, it is also possible to consider tumor- and immune-contextualized functional groups of gene expression and to subsequently identify their correlations between functional groups and specific outcomes ^{24,25}. This is important considering that it is difficult to solely analyze ovarian cancer by individual transcript levels ^{10,16} and that heterogeneous bulk tissue RNA analysis is still the most practical strategy for clinical settings. Furthermore, given that the combination of small molecule and anti-PD-1 antibody therapy regimens has been expected to be effective as a treatment strategy, the availability of a prognostic and predictive biomarker suggesting response or resistance to ovarian cancer immunotherapy based on tumor and immune expression is highly beneficial for routine clinical practice.

In this study, we report the development and results of a systematic method to leverage gene expression measured in platinum-resistant ovarian tumors by recasting individual gene transcript levels into functionally related groups that can subsequently be complementarily combined to predict a response to immune checkpoint inhibitor therapy. Despite limited sample size, we find that combinations of clear cell-specific ²⁶, CLOVAR immunoreactive, differentiated, mesenchymal, and proliferative ⁴ gene set signatures rationally map the expression-response/resistance space for the samples available. We also identified

clear cell carcinoma signature and immune-related pathways differentially expressed in CR and progressive disease (PD) subgroups. In addition to providing an interpretable and actionable result, the methodology is sufficiently straightforward that it can easily be applied to future studies with larger cohorts and can improve decision-making and subsequent response rates to immune checkpoint inhibitor therapy for this challenging subclass of gynecological malignancies.

Materials And Methods

Patient samples

Twenty patients with platinum-resistant recurrent ovarian cancer were enrolled in a clinical trial of nivolumab, anti-PD-1 antibody, at Kyoto University from 2011 to 2015 (UMIN000005714)¹⁰. The study was approved by the Kyoto University Graduate School and Faculty of Medicine, Kyoto University Hospital Ethics Committee (approval number: G531), in which donors provided written informed consent in accordance with institutional and national guidelines. Patient tumor samples were obtained by surgical dissection and stored as paraffin-fixed formalin-embedded (FFPE) tissue following recommendations of best practices for FFPE-based gene expression measurement²⁷.

One patient experienced thyroiditis-induced fever and tachycardia after receiving the first dose of the nivolumab trial and discontinued further treatment. This patient was excluded from the overall response analysis and data analyses, resulting in 19 patients being available for statistical investigations.

Clinical response was measured at 8 weeks following the onset of anti-PD-1 therapy (Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria²⁸). Histological subtyping was performed. Other characteristics including dose group, adverse events, and cancer antigen 125 levels during the course of treatment have been comprehensively presented in the literature¹⁰.

Gene expression measurement

Total RNA was extracted from the FFPE tumor samples by a DNA/RNA FFPE Kit (Qiagen, Valencia, CA, USA). Ninety nanograms of RNA were analyzed using a GeneChip Human Transcriptome Array 2.0 microarray in accordance with the protocol of the SensationPlus FFPE Amplification and WT Labeling Kit (Affymetrix/Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analyses

Normalization of gene expression was performed using the single space transformation robust multi-array average method available in Expression Console v1.41. Normalized expression was quality checked following recommendations for microarray processing²⁹ and subsequently input for single-sample gene set enrichment analysis (ssGSEA)³⁰. In summary, ssGSEA converts the raw expression values of a single sample into ranks based on expression value, and, for each gene set (signature) in a collection of signatures, outputs a score reflecting the association between the constituent genes of the signature and

their ranks among all the genes in a sample. The use of multi-gene groups is advantageous in counteracting fluctuation in expression and misinterpretation of direct expression value ranks.

In the first expression analysis, we subjected patient samples to analysis by ssGSEA constrained to the application of five oncological gene signatures. Four well-studied signatures corresponding to CLOVAR immunoreactive, proliferative, mesenchymal, and differentiated gene expression subtypes in high grade serous carcinoma ⁴, and a clear cell carcinoma-specific signature ²⁶ constituted the gene sets tested. All gene sets are in the public domain.

Values output by ssGSEA were normalized to the range 0 ~ 1 inclusive (affine transformation), per oncological signature. Significance of differential ssGSEA pathway scores amongst the CR / partial response (PR) / stable disease (SD) / PD groups was detected using a one-way analysis of variance (ANOVA) test. Rejection of the null hypothesis that all groups were equal for a given signature was determined when the probability of such (p-value) was less than a cutoff of 0.05. As a secondary investigation, we normalized the oncological signatures by the “z-scale” transformation (subtraction from the mean and subsequent division by the standard deviation), followed by affine scaling per patient sample.

In a second gene expression analysis, the samroc method ³¹ for detecting significance between pairs of groups was used to identify differential pathways. Gene sets based on the publicly available Molecular Signatures Database version 6.1 (<https://software.broadinstitute.org/gsea/msigdb/>), containing 21425 pathways, were used. CR-other and PD-other analyses and CR + PR versus SD + PD comparison were initially performed, which was necessary due to the study sample size available. The latter comparison also confirmed the sensitivity of the CR-other result as a function of the sample size. Raw probability values of null hypotheses that groups were equal (p-values) were compensated for multiple hypothesis testing (of the 21425 pathways) using the “p.adjust” function built into the R statistical environment (<http://www.r-project.org>), resulting in false discovery rate p-values. A cutoff of p less than 0.05 was considered significant.

Weights in fitting signature scores versus clinical response

Weights for a linear combination of the five ovarian cancer-specific signatures were obtained by solving a least-squares fitting equation $\mathbf{a} \cdot \mathbf{X} = \mathbf{b}$ available from the “linalg.lstsq” solver built into the NumPy matrix processing library (www.scipy.org), an extension of the Python programming language. The matrix $\mathbf{X}$ contained the raw 0 ~ 1 ssGSEA scores as described above and therefore was of shape 19×5. The vector $\mathbf{b}$ (of length 19) was set to follow the RECIST 1.1 criteria for changes in tumor volume, and therefore, quantities in $\mathbf{b}$ corresponding to tumor growth evaluation criteria were PD = 0.2, SD = 0, PR = - 0.3, and CR = - 1.0.

Results

Correlation between gene signature groups and clinical response

Nineteen patients, histologically comprising 14 with high-grade serous carcinomas (73%), three with endometrioid carcinomas (16%), and two with clear cell carcinomas (11%), were analyzed. These ratios were concordant with prior publication ¹. There were two patients with CR, one with PR, six with SD, and 10 with PD; the response rate was consistent with that of an independent study ¹⁶. One patient exceptionally demonstrated a complete elimination of the primary target lesion but experienced a para-aortic metastasis. Therefore, she was provided a special designation of SD (CR) while being allocated in the PR group for signature-response analyses. The pair of complete responders had cancer antigen 125 levels that exponentially decayed over the time course of treatment.

Differential gene expression analysis between tumors of the response groups by ANOVA revealed that the pair of patients with CR had significantly increased clear cell gene signature scores (Fig. 1 upper left, $p = 0.005$). Besides one PD patient whose expression yielded a high clear cell score, scores of the PD patients were systemically reduced. Conversely, tumors from the PD patients had significantly higher CLOVAR proliferative gene signature scores compared to those from the other groups (Fig. 1 upper right, $p = 0.026$). There was no overlap in the proliferative signature score values or confidence intervals between the CR and PD groups. Considering these results, the clear cell and proliferative gene signatures are suggestive of positive or negative response to anti-PD-1 antibody treatment in ovarian cancer.

Although not statistically significant at a cutoff value of $p = 0.05$, clear trends were observed in the scores of the other signatures tested. These included the increased immunoreactive signature scores in the patients with CR and PR (Fig. 1, bottom left), the higher differentiation signature scores in the patients with CR (Fig. 1 bottom center, $p = 0.054$), and the increased mesenchymal signature scores in the patients with PD (Fig. 1, bottom right).

For reference, we also performed signature significance testing by stratifying samples into their clear cell, serous, and endometrial histopathological subtypes. A figure analogous to Fig. 1 is provided as Supplementary Fig. S1. Besides the anticipated result that the clear cell samples had higher clear cell signature scores than the other samples, there were no significant correlations. One patient with a serous carcinoma also exhibited hallmarks of the clear cell subtype when a sample of the tumor was viewed under the microscope; this patient had an increased clear cell signature score (Supplementary Fig. S1).

Mutual complementarity effects of gene signature groups

Unsupervised hierarchical clustering of patients and signatures was performed (Fig. 2). After additional annotation with clinical response, a reasonable trend between the signature groups and their response was obtained. Although the immunoreactive, differentiated, and mesenchymal gene signatures did not yield probability values that passed the $p = 0.05$ threshold to be considered significant (Fig. 1), Fig. 2 suggests that the combination of both significant and statistically nonsignificant gene signature scores provides complementary points of evidence and improved decision-making support over the evidence

suggested by any individual signature score. For example, although the patients with PD dominantly had increased scores in the proliferative gene signature, consideration of the proliferative and mesenchymal signatures in parallel provided more complete coverage of the PD patients than either signature alone. Consideration of the combined clear cell, differentiated, and immunoreactive signatures better suggests a response (non-PD) than any isolated signature value. Similar heatmaps with patients grouped by clinical response or by an additional per-patient normalization prior to clustering are available in Supplementary Fig. S2 and Supplementary Fig. S3, respectively.

Based on the trends of Figs. 1, 2, Supplementary Fig. S2, and Supplementary Fig. S3, we attempted to quantify the weights for each signature score that could subsequently be combined to forecast response to nivolumab. By solving a least-squares fit of the score-response data, we derived the following response predictor with a functional form:

$$RESP = -0.439 \times CLE + 0.013 \times DIFF - 0.364 \times IMR + 0.308 \times MES + 0.350 \times PRO$$

where a negative response score indicates a positive response to treatment (reduction in tumor volume) and a positive score indicates a lack of response. Further analyses confirmed that the weights were invariant to any uniform scaling of the response group categories. This formula could be applied as is in a clinical setting when tumor bulk RNA expression is measured and ssGSEA value is computed from the result. A new fitting of weight constants (**a**) can be updated upon acquisition of new data simply by extending the patient-signature matrix (**X**) and the vector of patient responses (**b**).

A visual interpretation of a patient's ssGSEA scores is helpful to both the physician and the patient. Given the signs and magnitudes of the weights computed in the formula above, analyses were subsequently performed to project a combination of the three gene signature scores as a point in a three-dimensional coordinate space, with coloring of points by clinical response. As demonstrated in Fig. 3, a projection using the combination of immunoreactive, proliferative, and clear cell signatures provides a quick visual guide to the prediction of antitumor response via spatial clustering. Supplementary Fig. S4 is an animation demonstrating a 360-degree view of this three-dimensional projection.

For further analysis, ssGSEA values of each individual signatures (Fig. 1) were evaluated by histogram, and their pairwise correlations were computed by Pearson's product moment correlation (Supplementary Fig. S5). We observed from the individual signature score distributions that the clear cell gene signature is distinctively bimodal, whereas the CLOVAR subtype signatures follow more of a Gaussian or skewed distribution. With respect to the pairwise signature correlation, a negative correlation ($r = -0.69$) was observed between the proliferative and clear cell gene signatures, which is an anticipated finding but critically serves as a necessary negative control for the investigation. A positive correlation between the differentiated and clear cell signatures ($r = 0.65$) supports the view that clear cell carcinoma is a well-differentiated subtype, as previously reported³². When restricted to upregulated genes reported as in the literature, the differential-clear cell signature correlation increases ($r = 0.75$).

Differential pathways in response groups

A secondary ssGSEA analysis was performed to search for additional gene signatures and pathways that correlated with patient responses. As the CR and PD patients were significantly different in ANOVA analyses based on the CLOVAR and clear cell signatures (Fig. 1), CR-vs-other and PD-vs-other samroc analyses were performed, where in this case the significantly larger collection of pathways contained in the Molecular Signatures Database was used (see Materials and Methods section).

Using the collection of genes that had higher average expression in the CR patients than in the other response groups, samroc analysis corroborated the effectiveness of the clear cell-specific signature (Supplementary Table S1). In the downregulated genes of the CR group, pathways associated with positive regulation of stem cell differentiation and epithelial-to-mesenchymal transition were detected as significant (Supplementary Table S2), thus inferring the suppression of these well-known tumor growth processes.

From the tissues of the PD patients, samroc analysis of upregulated genes identified not only the serous-specific proliferative gene signature but also gene sets characterizing pathways for positive regulation of extracellular matrix organization and mesenchymal cell proliferation (Supplementary Table S3). The PD group also demonstrated downregulation of pro-inflammatory cytokines, including tumor necrosis factor binding, regulation of interleukin synthesis, cytokine secretion and inflammatory response, and B-cell mediated immunity (Supplementary Table S4).

We repeated the analyses by combining the CR group and the partial responder and querying how the samroc detection of pathways would be affected. Although some new pathways emerged as significant only in the CR + PR upregulated gene group, there was a reasonable overlap within the original CR-only analysis, including the clear cell signature (Supplementary Fig. S6 and Supplementary Table S5). Analysis of the downregulated genes in the CR + PR group returned pathways having more overlap with the CR-only group than the number of new pathways distinct to only the CR + PR group. Those commonly downregulated pathways once again included regulation of stem cell differentiation, mesenchymal-to-epithelial transition, and regulation of cell-cell adhesion mediated by cadherin (Supplementary Fig. S7 and Supplementary Table S6).

Discussion

Ovarian cancer has proven to be a challenging cancer type to treat, as it has multiple subtypes that confer differing responses to therapy. The platinum-taxane regimen was conceived with the hypothesis that it could cover the variety of subtypes, although resistance to the regimen is common. We have previously shown that the mesenchymal subtype was sensitive to taxane but not to platinum, whereas the proliferative subtype was sensitive to platinum⁵. The introduction of targeted agents such as bevacizumab has shown improved response in proliferative subtype patients³³ and platinum-resistant mesenchymal subtype patients. The role of immune checkpoint inhibitors such as nivolumab in the subtype-therapy-response dynamic and ways to effectively select appropriate patients receiving these inhibitors remain unclear.

Some studies, including ours, have investigated how the transcriptome could be leveraged for the extraction of information that contributes to such a dynamic and holistic understanding of ovarian cancer. Considering that nivolumab targets the PD-(L)1 interface, it is instinctive that studies report the correlation between PD-L1 (B7-H1) or PD-L2 (B7-H2) expression and clinical response. Although this strategy has proven effective for melanoma, it is not applicable to ovarian cancer. A recent phase II study with a large sample size found that higher PD-L1 expression was correlated with higher response, although response rates were still only 7–10% in two cohorts³⁴. Another recent pembrolizumab KEYNOTE study (with a sample size similar to that of our study herein) reported an objective response rate of 11.5%¹⁶. Hence, in the case of ovarian cancer, we can deduce that it requires more than the straightforward measurement of B7 ligands to effectively prescribe PD-1 checkpoint inhibitor therapy.

In this study, we aimed to establish a systematic and reusable method to capture a systems-level biological insight from a limited number of ovarian tumor transcriptomes, considering relative scores of histopathological subtype-specific gene set associations (CLOVAR) and gene sets from more general biological phenomena, in a two-step manner. Individual gene sets demonstrated informative trends. These subsequently could be combined into an interpretable linear combination that can contribute to the holistic subtype-therapy-response dynamic, specifically in the context of nivolumab, for patients with platinum-resistant ovarian cancer. The obtained linear combination readily and significantly explained the outcomes of the PD group in the proliferative and mesenchymal subtypes, which have poor prognoses. These two subtypes are currently being addressed with the use of bevacizumab³³. In contrary, the formula indicated that response to nivolumab could be expected through increased immunoreactivity and clear cell signature scores. This latter fact is particularly interesting, as it covers the subtypes in which bevacizumab is ineffective.

Due to a number of factors beyond our control, the study had a limitation in that it had a small sample size, and our findings must be contextualized as a prospect. Nonetheless, in combination with recent reports of ovarian cancer therapy response studies, immune checkpoint inhibitor therapy and the subtype-treatment-response dynamic can be enriched by our results.

Recently, Moore et al. presented the first results of the phase 3 IMagyn050/GOG3015/ENGOT-OV39 trial. In this trial, the addition of atezolizumab to bevacizumab and platinum-taxane chemotherapy failed to significantly improve progression-free survival (PFS) in patients with newly diagnosed advanced ovarian cancer. However, results from exploratory PFS analyses demonstrated a trend favoring atezolizumab in a subgroup of patients with 5% or more PD-L1-expressive immune cells. Here, the median PFS with the atezolizumab regimen was not obtained, as compared to the median of 20.2 months in the bevacizumab/chemotherapy arm (hazard ratio [HR], 0.64; 95% confidence interval [CI], 0.43–0.96; significant). Furthermore, in histological subgroup analyses, patients with clear cell carcinoma showed favorable PFS (HR, 0.64; 95% CI, 0.33–1.24; not significant) in the atezolizumab arm (n = 29) compared to the placebo arm (n = 22), which could warrant further evaluation due to small sample size³⁵. These findings of clinical benefit via atezolizumab in the immune cell PD-L1-high subgroup and in the patients

with clear cell carcinoma are consistent with our results that response to nivolumab could be expected through elevated immunoreactivity and clear cell phenotype.

Additional studies on the general strategy of treatment by combinations of small molecule and immune checkpoint inhibitor antibodies are underway. For example, an investigation in recurrent platinum-resistant ovarian cancer therapy by combining the recent PARP inhibitor niraparib with pembrolizumab has recently been reported ³⁶. A major effort for advanced ovarian cancer tumor therapy using targeted small molecule inhibitors with a candidate anti-PD-1 antibody is also ongoing (NCT03737643, ClinicalTrials.gov). This study evaluates the use of durvalumab treatment in combination with chemotherapy and bevacizumab, followed by maintenance durvalumab, bevacizumab, and olaparib treatment ³⁷. When conducted with appropriate biobanking techniques, these and similar studies could immediately apply the methodologies we have proposed in this report and eventually produce novel biomarkers for improved clinical decision-making.

The transcriptome presents a real-time and dynamic view of a tumor. As costs for measuring the transcriptome in bulk tumor tissue and at the stroma-tumor interface continue to decrease, the costs will eventually decrease within the range of those by which insurers will cover, and it will be possible to analyze patients at the individual gene and biological pathway level. Furthermore, this can be combined with analyses of DNA sequencing data such as tumor mutation burden or microsatellite instability analysis. This is highly needed in the study of ovarian cancer to populate the data space of the subtype-therapy-response dynamic, which will help in the updating of biomarkers and the improvement of treatment response rates.

In conclusion, the paired gene signature and pathway analyses have provided starting ground for developing reliable predictors of clinical response to immune checkpoint inhibitor therapy in ovarian cancer. The collection of signature scores holistically correlated with clinical response to anti-PD-1 antibody therapy, and the mathematical model developed could be implemented for immediate application in routine clinical work. Additional studies with large sample sizes will contribute to the fine-tuning of the subtype-therapy-response dynamic undetermined in this study.

Declarations

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Author Contributions

Conception, JH, RM, JB, NM, TB, KA, KY, KY, MT, IK, and MM; Acquisition of data, RM, JH, and YH; Data analyses, RM, JB, and JH; Funding acquisition, JH; Investigation, JH, NM, TB, KA, KY, YH, KY, MT, IK, and MM; Methodology, RM, JB, and JH; Project administration, JH and MM; Supervision, MM; Validation, JB; Visualization, RM, JH, and JB; Writing – original draft, RM, JH, and JB. All authors have read and agreed to the published version of the manuscript.

Competing interests

JH reports research grants from Ono during the conduct of the study and from Daiichi Sankyo, MSD, and Sumitomo Dainippon outside of the submitted work. JB reports a research grant from Daiichi Sankyo unrelated to this work and a potential conflict of interest as a consultant for the pharmaceutical industry. The other authors have no conflict of interest to declare.

Availability of data and materials

The datasets generated analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Kyoto University Graduate School and Faculty of Medicine, Kyoto University Hospital Ethics Committee (approval number: G531), in which donors provided written informed consent in accordance with institutional and national guidelines.

Consent for publication

Not applicable.

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Figures

Figure 1

Significance testing of ovarian signature scores stratified by clinical response Using paraffin-fixed formalin-embedded-derived gene expression, single-sample gene set enrichment analysis (ssGSEA) was applied with gene signature sets tailored to ovarian cancer, resulting in per-signature scores normalized to the range 0~1 (ssGSEA score). Statistical significance by one-way analysis of variance is achieved for the clear cell signature in complete responders to anti-programmed death-1 antibody therapy and for the proliferative signature in patients who exhibited progressive disease. The PR* group comprises one patient classified as PR by standard Response Evaluation Criteria in Solid Tumours protocols and one exceptional patient whose primary target lesion was completely eliminated but experienced a metastasis.

Figure 2

Figure 2

Complementarity of gene signatures in predicting clinical response Independent unsupervised clustering of patients and gene signature groups, including an annotation track mapping patients to their clinical response to nivolumab therapy. An additional annotation track of histopathological subtype is provided. Score values were normalized by centering on the mean for each signature, and average linkage was used to create the dendrogram. Combinations of signature groups reinforce prediction of clinical response to therapy. The patient whose primary ovarian carcinoma was eliminated but experienced a new metastatic lesion is indicated by striped response color and a special designation of SD (CR).

Figure 3

Three-dimensional projections using clear cell, proliferative, and immunoreactive gene signature single-sample gene set enrichment analysis (ssGSEA) scores. Expansion from two-dimensional to three-dimensional projection improves demarcation and understanding with respect to favorable and poor responses. A 360-degree viewpoint animation of the project is available as supplementary data. Notation: Cle, ssGSEA score of clear cell gene signature; Pro, ssGSEA score of proliferative gene signature; Imr, ssGSEA score of immunoreactive gene signature. Point colors are green, red, blue, and black for complete response, partial response, stable disease, and progressive disease, respectively.

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