Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

Precision Medicine and Imaging

Clinical Cancer Research

Immunogenomic Analyses of Advanced Serous Ovarian Cancer Reveal Immune Score is a Strong Prognostic Factor and an Indicator of Chemosensitivity

Dapeng Hao[1], Jie Liu[1], Meng Chen[2], JingJing Li[1], Li Wang[3], Xiaobo Li[4], Qi Zhao[1], and Li-jun Di[1]

Abstract

Purpose: Ovarian cancer is one of the first human cancers for which *in situ* immune response was reported to be important for the clinical outcome. To elucidate the mechanistic relationship between immune repertoire and cancer genotype in ovarian cancer, the development of a well-defined immune score for ovarian cancer is required.

Experimental Design: From a collection of 2,203 patient samples of advanced ovarian cancer from public available resources, we evaluated the prognostic values for a compendium of immune marker genes and proposed an immune score. The relationships between immune score, tumor-infiltrating immune cells, cancer genotypes, and their impact on patient outcome were characterized.

Results: Loss of chemokine and IFNγ pathway genes is frequent in ovarian cancer and is significantly associated with low immune score and poor outcome. Chemotherapy can increase the immune score of tumors by inducing the expression of IFNγ inducible chemokines. High immune score is significantly associated with BRCA1/2 mutation status and the response to chemotherapy. Multivariate analysis revealed that immune score is a strong predictor of patient survival and the response to immunotherapy.

Conclusions: Our results reveal the drivers of the immune repertoire of advanced ovarian cancer and demonstrate the importance of immune score as an independent prognostic signature and a potent indicator of intratumoral immune status. *Clin Cancer Res; 24(15); 3560–71. ©2018 AACR.*

Introduction

Since the first piece of evidence for the infiltration of cytotoxic T cells in epithelial ovarian cancer (EOC) was reported in 1991 (1), many studies have confirmed a spontaneous antitumor immune response in EOC patients (2). EOC is one of the first human cancers for which tumor-infiltrating lymphocytes (TIL) was found to be associated with improved patient survival and delayed recurrence of disease (3). Recent studies further confirmed the prognostic value of TILs using multiple EOC cohorts (4). In light of the promising effect of immunotherapy in the initial studies of EOC (5, 6), especially in patients that are resistant to the conventional therapy, ongoing clinical trials are designed to evaluate the effect of immune-checkpoint inhibitors in EOC patients (7). However, pioneering studies have shown that only a minority of patients is responsive to immunotherapy, and thus the identification of these patients has become critical. A common feature of the patients responsive to immunotherapy is the high activity of preexisting antitumor immunity (8). A high density of TILs, as an indicator of *in situ* immune activity, is reported to be important in the setting of the immune-checkpoint blockade (8, 9). Thus, deeply understanding the determinants of *in situ* immune activity in EOC would assist the administration of immunotherapy in this disease.

Currently, there is no consensus signature to estimate the immune activity in EOC and to stratify patients accordingly. Molecular subtyping has been proposed and identified an immunoreactive subtype for high-grade serous ovarian cancer (HGSC), but it provides limited prognostic information (10). However, although numerous prognostic signatures have been developed to stratify EOC patients (11), they are not indicative of the antitumor immune activity. In colorectal cancer, a criterion of *in situ* immune activity, designated "immunoscore" has been demonstrated to be the strongest prognostic factor that may serve as a new component of cancer classification (12, 13), indicating that assessment of immune microenvironment via a standard criterion provides a potent prognostic tool. An immune score has many advantages over traditional prognostic signatures in EOC. First, it helps to stratify patients into those suitable for checkpoint blockade and those would benefit more from conventional therapies. Second, a well-defined immune score would facilitate the comparison of different immune modulators and the exploration of the mutual influence between chemotherapy and tumor immune

[1]Faculty of Health Sciences, University of Macau, Macau, China. [2]Department of Respiratory Medicine, The Fourth Affiliated Hospital of Harbin Medical University. [3]Metabolomics Core, Faculty of Health Sciences, University of Macau, Macau, China. [4]Department of Pathology, Harbin Medical University, Harbin, 150081, China.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Li-jun Di, University of Macau, Avenida da Universidade taipa, Macau, China. Phone: 853-88224497; Fax: 853-88222314; E-mail: lijundi@umac.mo

doi: 10.1158/1078-0432.CCR-17-3862

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

Translational Relevance

This study characterized the immune microenvironments of advanced ovarian cancer from an immunogenomic perspective. We developed immune score to assess the immune status in advanced ovarian tumors, which would be important for up-front stratification of patients in clinical trials of immunotherapy strategies. This study demonstrated that chemokine and IFNγ pathway genes, whose expression could be stimulated by chemotherapeutic agents, are drivers of immune repertoire in ovarian cancer, suggesting that chemotherapy may benefit patients with a low density of infiltrating immune cells. This study highlights the importance of the assessment of immune microenvironment via immune score in the setting of immunotherapy strategies.

microenvironment (14, 15). More importantly, it facilitates the genomic analyses of genotype–immunophenotype relationships that are now imperative for an improved understanding of the immunogenomic profile of EOC.

Here, we aim to establish an immune score for advanced (late-stage and high-grade) serous ovarian cancer to investigate the relationship between cancer genotype and immune activity. We hypothesize that an immune score that is highly reflective of preexisting antitumor immunity should be a strong prognostic signature in EOC. Starting from a large-scale investigation for the prognostic value of immune marker genes in 16 cohorts of advanced serous ovarian cancer patients, we developed an immune score that can be used as a potent indicator of the antitumor activity of naturally infiltrating immune cells and a strong prognostic marker of EOC patients. Based on the immune score, we investigated the genotype–immunophenotype relationships and identified the genomic determinants of immune infiltration in ovarian cancer. We further demonstrated that immune score predicted the response to chemotherapy, regardless of DNA repair defects such as *BRCA1/2* mutations. We also showed that neoadjuvant chemotherapy significantly increased the immune score of tumors, suggesting that chemotherapeutic agents can stimulate an immune signaling profile in cancer cells.

Materials and Methods

Cohort datasets of ovarian cancer

Sixteen public cohort datasets with transcriptome data of high-grade, late-stage serous ovarian cancer were obtained through database searches of PubMed, ArrayExpress, TCGA, and GEO (Table 1), and processed as described in our previous study (16). In this study, we further added a recently released cohort dataset of 80 primary ovarian cancers (17). All the datasets have at least 40 transcriptome-profiled primary samples of advanced serous ovarian cancer (grade 2 or 3 and FIGO stage III or IV) with overall survival time-to-event data. We restricted our analysis to the primary, advanced serous ovarian tumors with survival data. To avoid systematic bias across datasets, we analyzed each dataset independently and then integrated using meta-analysis for the evaluation of an overall prognostic value of genes. The response to chemotherapy in ovarian cancer cohorts was determined by corresponding previous studies (10, 17). Chemotherapy status was defined as sensitive if there is no evidence of disease progression within 6 months of the end of primary treatment, and was defined as resistant if there is evidence of disease progression within 6 months from the end of primary treatment.

Immune cell marker genes

A compendium of 782 marker genes related to 28 tumor-infiltrating immune cell types was obtained from the previous study (18). These genes are representative of specific immune cells and are not expressed in cancer cells or in normal tissues. Gene set enrichment analysis (GSEA) using these marker genes has been proved to be effective to evaluate the infiltration of immune cells (18).

Immune score

The prognostic evaluation of immune marker genes and antigen-presenting genes was determined by Cox proportional hazards regression analysis in the 16 cohort datasets of ovarian cancer. An overall hazard ratio (HR) and the variance of HR were estimated by meta-analysis using fixed-effects model. In total, 76 genes were identified to be significantly prognostic of favorable outcome (Supplementary Table S1; Cox proportional hazard model, $P < 0.02$, FDR < 0.1). For each of the 76 prognostic genes, the HR and the standard estimates (SE) of HR were used to

Table 1. Cohort datasets of high-grade, late-stage serous ovarian cancer

Datasets	Platform	No. of samples	Late stage, high-grade[a]	Ref (PMID)
E.MTAB.386	HumanRef-8 v2	129	128	22348002
GSE13876	GPL7759	157	130	19192944
GSE14764	U133A	80	63	19294737
GSE17260	GPL6480	110	84	20300634
GSE18520	U133 Plus 2.0	53	53	19962670
GSE26193	U133 Plus 2.0	107	61	22101765
GSE26712	U133A	185	185	18593951
GSE30161	U133 Plus 2.0	58	44	22348014
GSE32062	GPL6480	260	260	22241791
GSE32063	GPL6480	40	40	22241791
GSE49997	GPL2986	204	165	22497737
GSE9891	U133 Plus 2.0	267	214	18698038
Dressman et al.	U133A	117	108	17290060
TCGA	HG-U133A	557	495	21720365
GSE51088[b]	GPL7264	123	93	24368280
OV.AU	RNAseq	80	80	26017449

[a]FIGO stage III or IV.
[b]Metastases and borderline tumors excluded.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

determine the weight of a marker gene in immune score. Then, the immune score of a sample is given by:

$$S = \sum_{i=1}^{76} \frac{1 - \mathrm{HR}_i}{\mathrm{SE}(\mathrm{HR}_i)} \times x_i,$$

where x_i is the normalized expression of gene i in that sample. The Z-score normalized immune score was used in our analyses.

Genomic data of TCGA

Level 3 data of TCGA in this study, including clinical information, gene expression data (U133A array and RNAseq data), somatic mutation data (MAF file), and copy number variation (CNV) data were downloaded from Broad GDAC FIREHOSE (http://gdac.broadinstitute.org). Neo-antigens of ovarian cancer were downloaded from the database of The Cancer Immunome Atlas (TCIA; ref. 18). Copy number gain or loss of genes, which was determined by GISTIC 2.0 software, were documented as "1, 2" and "−1, −2," respectively. GISTIC values (−2, −1, 0, 1, 2) of genes were used to estimate the association between CNV and immune score using linear regression model. Samples with somatic mutation of BRCA1/2 were obtained from the MAF file of TCGA ovarian cancer, whereas samples with germline mutations of BRCA1/2 were obtained from the previous publication of TCGA group (10).

For pan-cancer survival analysis, RNAseq data of 9,264 tumor samples across 24 cancer types were downloaded from GEO (GSE62944). This dataset provides the expression values of genes processed using the same pipeline (19). Log2 transformed fragment per kilobase per million (FPKM) was used for analysis.

Quantitative real-time PCR

OVCAR-3 cells were obtained from ATCC on 23/05/2015 and were checked free of mycoplasma contamination by PCR and culture. OVCAR-3 cells were treated with vehicle, cisplatin (20 μmol/L) for 36 hours or IFNγ (100 IU; P5664, Beyotime) for 12 hours. Total RNA was extracted using Trizol (Invitrogen; Life Technologies) according to the manufacturer's instructions. RNA was reverse-transcribed into cDNA using PrimeScript RT Master Mix Kit (RR036Q; Takara). Quantitative real-time PCR (qRT-PCR) was performed using the Real-Time PCR Detection System (Applied Biosystems) with a FastStart Universal SYBR Green Master (Rox; Sigma) and a standard temperature protocol. Expression levels of target mRNA were normalized relative to levels of 18S RNA.

Statistical analysis

Standard statistical tests including Student T test, Wilcoxon rank sum test, Fisher exact test, log-rank test, and Cox proportional hazard regression were used for the analysis of clinical data and genomics data. Meta-analysis was performed using R package "metaphor." GSEA was performed using Bioconductor package "clusterProfiler." Differential expression was determined using eBayes function of "Limma" package. All the analyses were performed in R 3.3.1.

Results

Large-scale meta-analysis reveals the prognostic value of intratumoral immune gene expression

To develop a prognostic scoring system indicative of immune infiltration, a compendium of 782 marker genes related to 28 tumor-infiltrating immune cell types was analyzed (18). These genes are representative of specific immune cell types and not expressed in cancer cells or normal tissues. The prognostic value of these genes, as well as the antigen-presenting genes, was evaluated in 16 publicly available cohorts of more than 2,200 primary tumors of advanced serous ovarian cancer (Table 1). We then used a meta-analysis to leverage the 16 datasets for an overall prognostic evaluation for each immune marker genes. In total, 69 marker genes and seven antigen-presenting genes were identified as favorable prognostic factors (Cox proportional hazard model, $P < 0.02$, FDR < 0.1). The number of favorable prognostic immune marker genes was significantly higher than expected by chance (empirical $P < 0.0001$ by random perturbations). The 76 favorable prognostic genes and their corresponding immune subpopulations are shown in a network (Fig. 1A). The majority of them are from immune subpopulations of adaptive immunity, including Th1, Th2, activated B cell or T cell, effector memory T cell, and γδ T cell. Several markers of subpopulations from innate immunity, such as NK cell, NK T cell, macrophage, MDSC, CD56 dim, and neutrophil, are also included in the network.

The fact that a large fraction of marker genes of not only T cell but also other immune subpopulations are prognostic suggests a complicated tumor microenvironment as a reflection of host immunity. We thus developed an immune score based on the combined expression of these genes by taking their contributions to risk prediction into account. High immune score reflects an overall high expression of favorable prognostic genes. In TCGA dataset, we observed a significant correlation between our immune score and the fraction of immune cell infiltration estimated by an independent leukocyte-specific methylation signature (Pearson $r = 0.55$; Supplementary Fig. S1A; ref. 20). We also observed a strong correlation between immune score and the cytolytic activity of infiltrating T cells (Pearson $r = 0.81$; Supplementary Fig. S1B; ref. 21). Therefore, the immune score based on the expression of all the favorable prognostic marker genes reflects the intratumoral immune activity in ovarian cancer.

To further chart the immune pattern underlying the immune score, we assessed the infiltration of the immunome of 28 subpopulations using single-sample gene set enrichment analysis (ssGSEA) as previously described (18), and computed their correlation with the immune score (Fig. 1B; Supplementary Table S2). Based on the score of ssGSEA, the prognostic value of different immune subpopulations was also evaluated within each dataset and was integrated using meta-analysis for an estimation of overall prognostic effect. The infiltration of most immune subpopulations was positively correlated to immune score and was associated with favorable prognosis in ovarian cancer datasets, especially the activated $CD8^+$ T cell and activated $CD4^+$ T cell (Supplementary Fig. S2A). On the contrary, the plasmacytoid dendritic cell was negatively associated with immune score in many datasets, and was likely to be associated with a poor prognosis (Supplementary Fig. S2B). The significantly favorable prognostic immune cells, ordered by their HR, are effector cells (activated $CD4^+$ T, activated $CD8^+$ T, activated B, effector memory $CD4^+$, and effector memory $CD8^+$), CD56 bright natural killer cells, T follicular helper cells and type 1 T helper cells. In general, the infiltration of subpopulations related to adaptive immunity has a stronger correlation with immune score and is more likely to be favorable prognostic factor than subpopulations related to innate immunity (Fig. 1B). In fact, all the immune

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

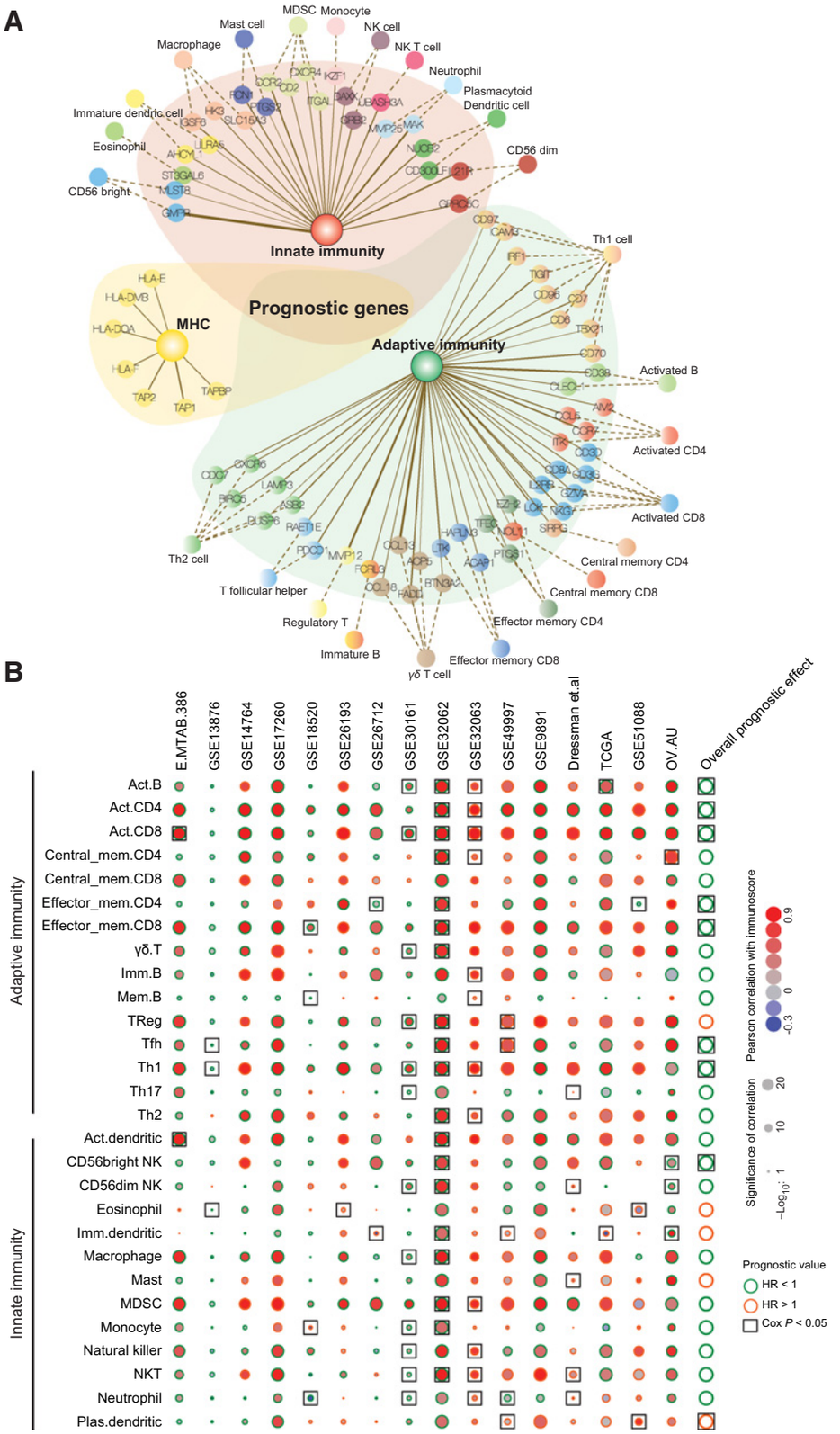

Figure 1.
Prognostic value of immune marker genes and immune cells. **A,** Prognostic immune marker genes and their corresponding cell types. Markers (second layer) associated to the same cell type (outermost layer) share the color of the node and are connected by dotted lines. The width of solid lines is proportional to the prognostic significance. **B,** Bubble plot of correlation between immune score and infiltrating immune subpopulations across 16 cohort datasets of advanced ovarian cancer (sorted alphabetically for adaptive and innate immunity, respectively). The size of the circles gives the significance of the correlation, whereas the color indicates the Pearson correlation coefficient. The color of border indicates favorable (green; HR < 1) or poor prognosis (orange; HR > 1). The rectangle indicates $P < 0.05$ determined by univariate Cox proportional hazard model. The overall prognostic effect is estimated by meta-analysis using fixed-effects model.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

subpopulations of adaptive immunity except Treg cells are associated with favorable outcome of ovarian cancer.

Loss of chemokine and IFNγ pathway genes are associated with low immune score

We hypothesized that the immunophenotype could be shaped by the genotypes of tumor. To test this, we looked for associations between gene alteration and the immune score in TCGA dataset using a regression-based method. However, we did not observe a significant association of immune score with the mutation of any gene; neither did we observe a significant correlation with the mutation burden or the burden of neo-antigens (Supplementary Fig. S3). However, we found numerous genes for which the copy number status was significantly associated with immune score ($n = 3800$ at FDR < 0.05), which was consistent with the fact that ovarian cancer is a C-class tumor characterized by recurrent copy number alterations (22). We plotted the significance of associations between immune score and copy number status across the genome, which revealed several highly enriched chromosome regions (Fig. 2A). On chromosomes 1, 2 and 20, genes are likely to be amplified and the amplification is associated with lower immune score (negative correlation), whereas on chromosome 4 and 9, genes are likely to have copy number deletion and the deletion is associated with lower immune score (positive correlation). Chromosome 4 and 9 are of particular interest, because these two regions peaked at the loci encoding chemokines and IFNγ pathway genes. For example, chemokines including *CXCL9*, *CXCL10*, and *CXCL11*, which elicit chemoattraction for T cells and NK cells, are all located in 4q21-23. The expression of these chemokines, which could be induced by IFNγ, is essential for immune infiltration (23). Recent studies have shown that the loss of IFNγ pathway genes is a mechanism of repressing antitumor immunity and the resistance to CTLA-4 blockade (24). Here we suggest that the loss of chemokine genes should also be considered as an alternative mechanism since advanced ovarian cancer is characterized by the deletion of chromosome arm 4q (10).

Using qPCR and public available data (25), we confirmed that IFNγ could induce the expression of *CXCL9*, *CXCL10*, and *CXCL11* in ovarian cancer cells and ovarian epithelial cells (Fig. 2B and C). Based on the copy number alteration of IFNγ pathway genes and chemokines, we found that ovarian tumors could be divided into three groups: group 1 with copy number loss of both IFNγ pathway genes and chemokines, group 2 with copy number loss of either IFNγ pathway genes or chemokines, and group 3 without copy number loss of most of these genes (Fig. 2D). Consistently, we found that the group 1 showed the lowest immune score whereas the group 3 showed the highest immune score (Fig. 2E). The association between the loss of IFNγ pathway genes and immune score was also observed in TCGA melanoma dataset where the loss of these genes has been confirmed as a mechanism of immune resistance (Supplementary Fig. S4; ref. 24). In fact, a large fraction of chemokines and IFNγ pathway genes are favorable prognostic factors in ovarian cancer according to our meta-analysis of the 16 cohort datasets (Fig. 2D), suggesting that these genes are required for intratumoral immune activity. As expected, the expression of *IRF-1* in tumors with the loss of IFNγ pathway genes was significantly lower than other tumors (Supplementary Fig. S4D). Consequently, loss of IFNγ pathway genes dampened the correlation between IFNγ and immune score across ovarian tumors (Fig. 2F). These results suggest that the loss of chemokines and IFNγ pathway genes impairs the regulation on immune infiltration and may be associated with immune resistance in EOC.

Chemotherapy induces chemokine expression and increases immune score

Intratumor microenvironment can be harshly changed by neoadjuvant chemotherapy (26–28). We therefore compared 25 pairs of post- versus pre-chemotherapy ovarian tumors with each pair of tumors obtained from the same patient from a previous study (29), which revealed 825 upregulated and 917 downregulated genes (FDR < 0.05). The downregulated genes were highly enriched in cell cycle related functions, whereas the upregulated genes were mostly enriched for immunoreactive functions (Fig. 3A), suggesting that chemotherapy has stimulated the infiltration of immunoreactive cells. The marker genes of adaptive immunity subpopulations were highly enriched in post-chemotherapeutic ovarian tumors, including CD8A, CD8B, GZMA, and PRF1 among the top upregulated genes (Fig. 3B). Consistently, we observed significantly increased immune score and cytolytic activity in post-chemotherapy tumors compared with their paired pre-chemotherapy tumors (Fig. 3C). Moreover, tumors having a strong pre-existing immune activity are also likely to a have a relatively stronger immune activity after chemotherapy than other tumors.

We next investigated whether chemotherapy agents could enhance the capability of tumor cells responding to host immunity. The expression of chemokines and antigen-presenting genes are important for the recruitment of lymphocytes (30–32). In the 16 cohort datasets, we observed strong correlations between the expression of these genes and the immune score in ovarian cancer (Supplementary Fig. S5). Using a publicly available dataset (33), we found that carboplatin could engender the overexpression of chemokine and antigen-presenting genes in EOC cells (Fig. 3D). We also tested this in another EOC cell line (OVCAR-3), and observed similar overexpression of chemokine and antigen-presenting genes after treatment of cisplatin for 36 hours (Fig. 3E). These results suggest that tumor cells treated with chemotherapy agents have an enhanced ability to foster the infiltration of immunoreactive cells.

We hypothesized that the clinical response to chemotherapy could be dependent on the anti-tumor immune response. Because there are few genomic data for post-therapeutic tumors, we tested the association between the response to chemotherapy and pre-existing immune activity. We classified patients into the high immune score group and the low immune score group in two of our collected datasets with available information about the response to chemotherapy (10, 17). Consistent with the hypothesis, we found that patients in the high immune score group have a better response to chemotherapy than patients in the low immune score group (Fig. 3F). In total, we found 98 chemo-sensitive patients and 47 chemo-resistant patients in the high immune score group, and 90 chemo-sensitive patients and 70 chemo-resistant patients in the low immune score group in the two datasets (Fisher exact test, $P < 0.05$). In addition, chemo-sensitive tumors were enriched of activated $CD4^+$ T cells compared to chemo-resistant tumors (Supplementary Fig. S6).

Immune score is predictive of clinical outcome independent of BRCA mutation

Patients with a high immune score displayed better overall survival (OS) than patients with a low immune score in most of

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

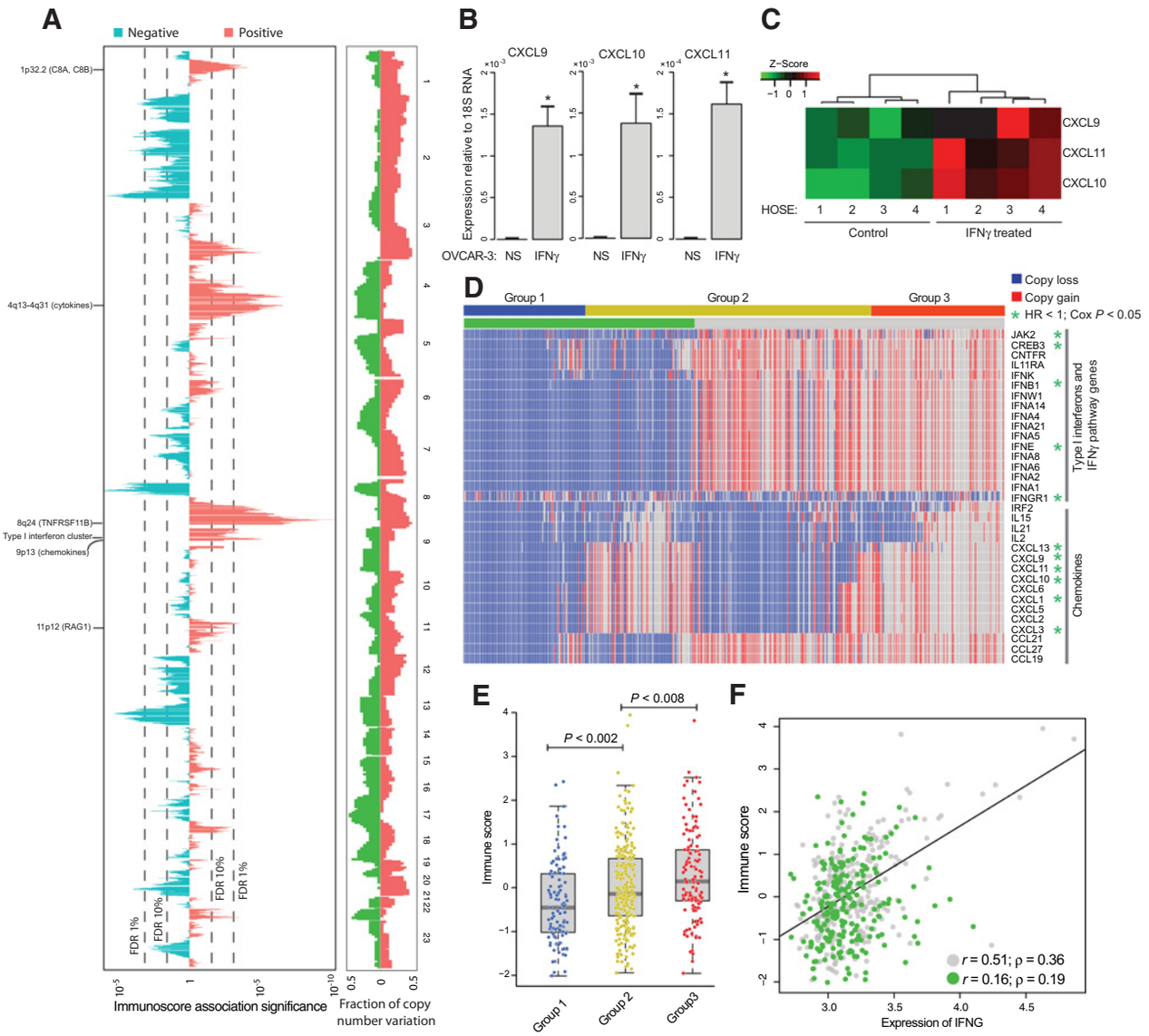

Figure 2.

Genomic associations with immune score. **A,** The significance of association between immune score and copy number variation for all genic loci (cyan for negative correlation and red for positive correlation). Dotted lines represent significance cutoffs of 1% and 10% FDRs. Chromosome regions significant at the 1% FDR and associated with potential driver genes are shown in the right side. **B,** QPCR result showing the elevated expression of chemokines after 12 hours treatment of IFNγ (100 IU/mL) in OVCAR-3 cells. **C,** Heatmap showing the expression of chemokines with or without treatment of IFNγ (500 IU/mL, 6 hours) in HOSE cells. $N = 4$ replicates are used in each group. **D,** Oncoplot of the copy number status of IFNγ pathway genes/type I interferons and chemokines. Copy number loss or gain was determined by GISTIC 2.0 and defined as score < 0 or >0, respectively. Green bar and grey bar indicate the samples with or without copy number loss of IFNγ pathway genes and type I interferons, respectively. **E,** Association between immune score and copy number status of IFNγ pathway genes and chemokines. Samples of ovarian cancer are divided into three groups as shown in **D**, including those with loss of chemokines and IFNγ pathway genes (group 1), those with loss of chemokines or IFNγ pathway genes (group 2) and those with no copy loss of them (group 3). Significance is determined by Wilcoxon rank sum test. **F,** The correlation between immune score and IFNG (encoding gene of IFNγ). Samples of ovarian cancer are divided into two groups with or without copy number loss of IFNγ pathway genes, as shown by the green and grey bar in **D**. Pearson correlation (r) and Spearman correlation (ρ) are calculated.

collected EOC datasets (Fig. 4A), as estimated by multivariate Cox proportional hazards model controlled for clinical factors including age, tumor stage, and grade. Meta-analysis result suggested a significant favorable prognostic value in EOC patients (high immune score vs. low immune score, HR = 0.71 [0.64, 0.80], $P = 8.3E{-}9$]. Using pan-cancer RNA-seq data, we further examined the association of immune score with OS in 18 solid cancers, of which two were significant in addition to ovarian cancer: skin cutaneous melanoma (SKCM) and uterine corpus endometrial carcinoma (UCEC; Fig. 4B). Meta-analysis revealed a significant

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

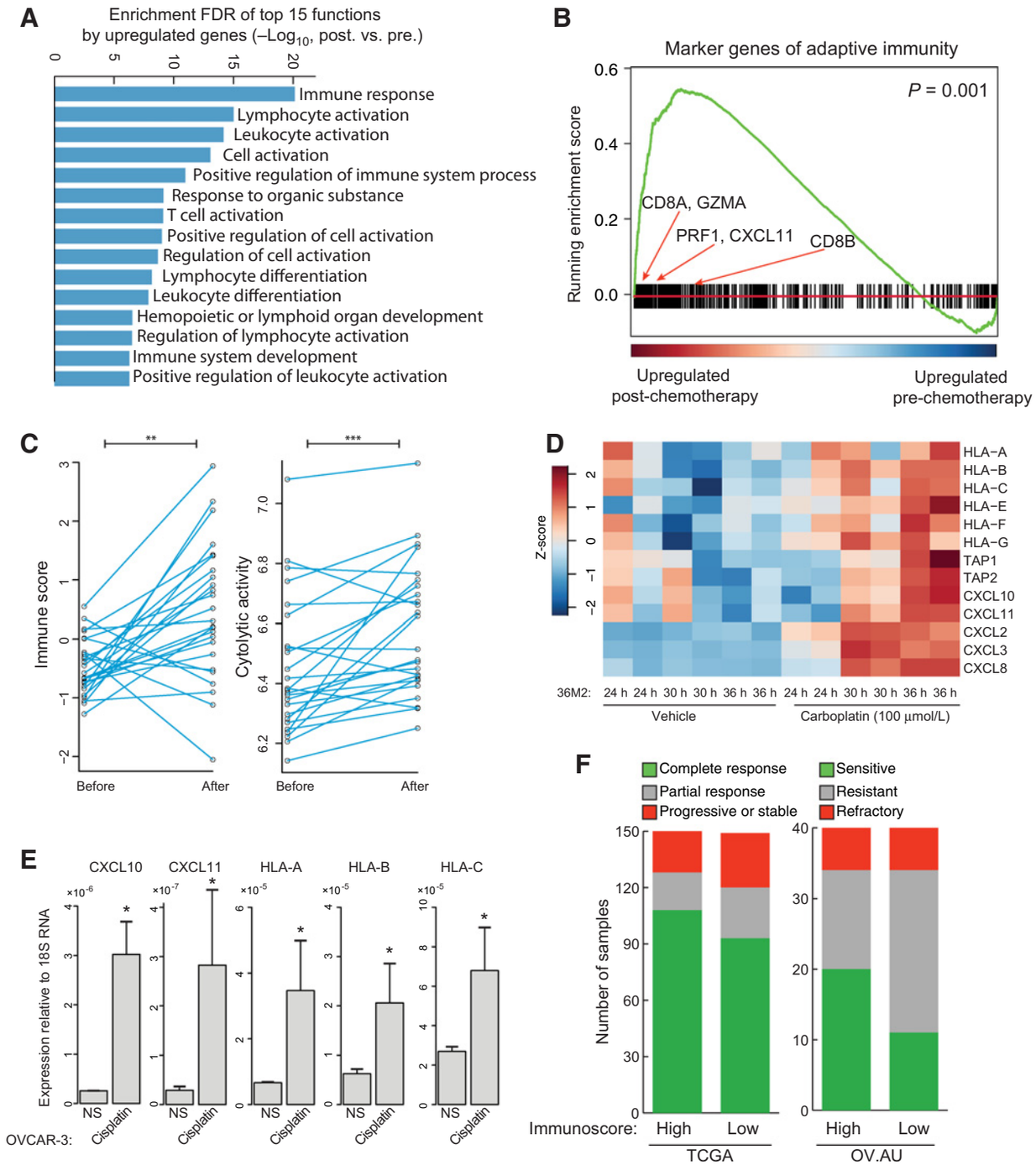

Figure 3.

Chemotherapy stimulates immune signaling in ovarian cancer. **A,** Top 15 enriched GO biological processes by the upregulated genes in post- vs. pre-chemotherapy tumors. Enrichment analysis was performed using DAVID bioinformatics resources 6.7. **B,** GSEA of 431 marker genes of adaptive immunity reveals the association between immune response and chemotherapy. **C,** Pairwise comparison of immune score and cytolytic activity (log2 transformed) in tumors post- and pre-chemotherapy. Significance is determined by pairwise *T* test. **D,** Heatmap showing the expression of chemokines and antigen-presenting genes with or without treatment of carboplatin in ovarian cancer cells. **E,** QPCR result showing the elevated expression of chemokines and antigen-presenting genes after 36 hours treatment of cisplatin (20 μmol/L) in OVCAR-3 cells. Significance is determined by Student t test. **F,** Association between immune score and the response to chemotherapy in ovarian cancer patients.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

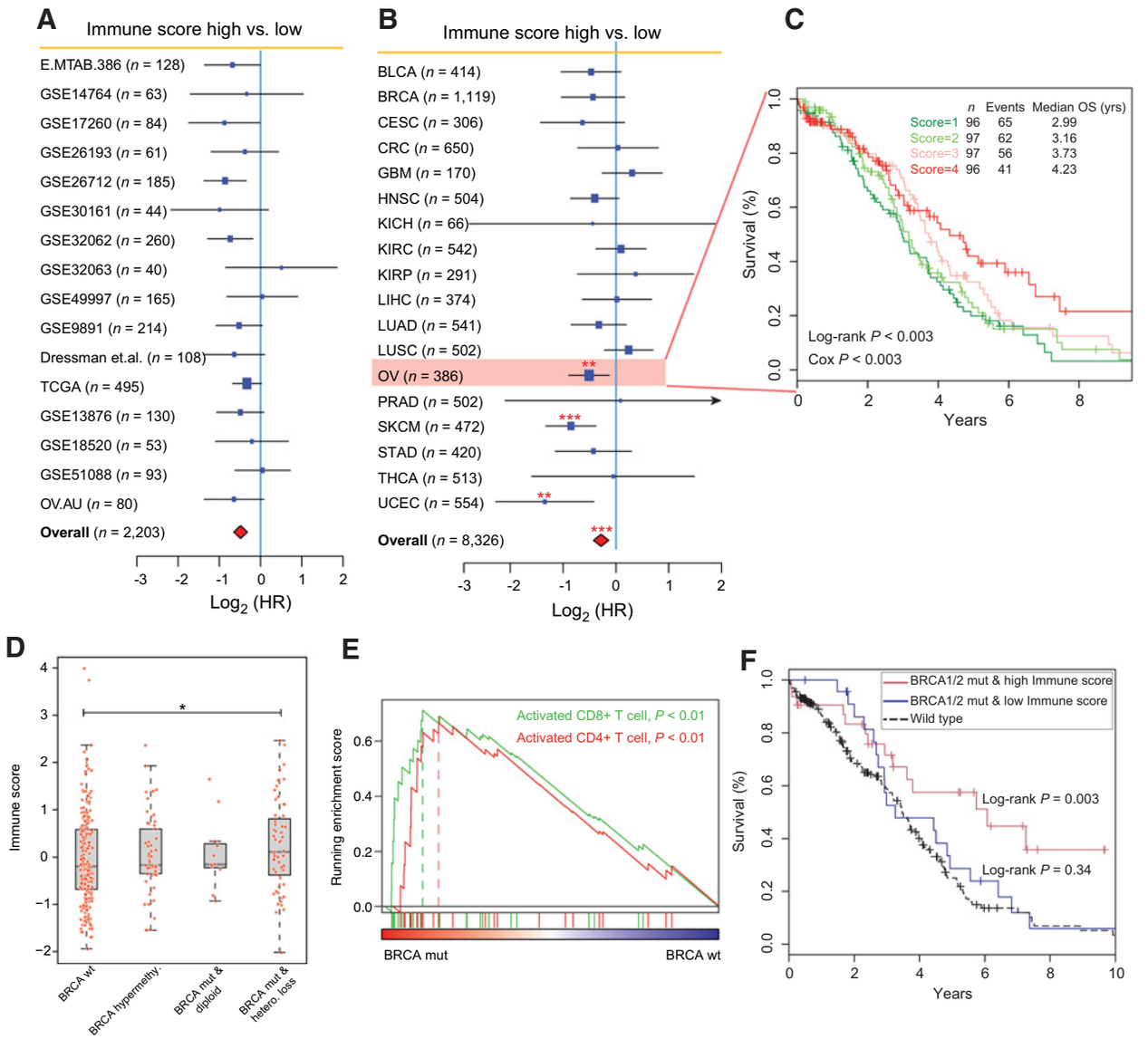

Figure 4.

Prognostic evaluation of immune score. **A,** Forest plot visualizing HRs of multivariate survival analyses of immune score (high vs. low by median) in all cohort datasets. Significance is determined by Cox proportional hazard model. The red diamond shows the fixed-effects meta-analysis summary of HRs over 16 datasets (HR = 0.71; 95% CI, 0.64–0.80, $P = 8.3e{-}09$). **B,** Pan-cancer multivariate survival analyses of immune score in solid cancer types. The red diamond shows the fixed-effects meta-analysis summary of HRs over 18 cancer types (HR = 0.82; 95% CI, 0.74–0.91, $P = 0.0001$). **C,** Kaplan–Meier survival curves of TCGA ovarian cancer patients with different immune scores. **D,** Association between immune score and BRCA1/2 status. Significance is determined by Wilcoxon rank sum test. **E,** GSEA reveals the association of BRCA1/2 mutation status with the infiltrating $CD8^+$ and $CD4^+$ T cells. **F,** Kaplan–Meier survival curves of TCGA ovarian cancer patients, divided by BRCA1/2 mutation status and immune score.

pan-cancer effect of immune score (high immune score vs. low immune score, HR = 0.82 [0.74, 0.91], $P = 1.3E{-}4$). To further explore the prognostic value of immune score, we divided the patients into four equal groups according to the immune score of samples. We observed that the OS of patients was determined by immune score, with a median OS of 2.98, 3.16, 3.73, and 4.32 years for the four groups of patients with increasing immune score (log-rank test, $P < 0.003$).

Recently, BRCA1/2 mutations have been shown to have a significant impact on TILs (34). Mutations of *BRCA1/2* occur in a substantial fraction of advanced ovarian cancer samples (21%) and are predictive of favorable outcome due to an improved response to chemotherapy (10). We analyzed the difference of immune score between BRCA wild-type tumors and tumors with BRCA deficiency. The immune score was not significantly different in tumors with BRCA hypermethylation compared to wild-type tumors. However, it showed a significant increase in tumors with BRCA mutations accompanied with heterozygous deletion of BRCA (Fig. 4D). Tumors with BRCA mutation were significantly enriched for infiltration of activated $CD8^+$ and $CD4^+$ T cells

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

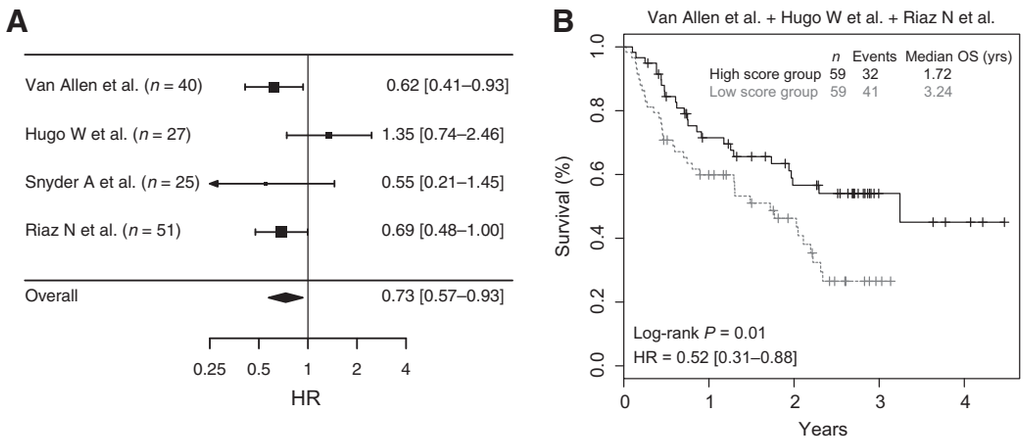

Figure 5.

Prognostic value of immune score in immunotherapy datasets. **A,** Forest plot visualizing HRs of immune score in immunotherapy datasets. The diamond shows the fixed-effects meta-analysis summary of HRs over the four datasets ($P = 0.01$). The last column shows the HR of immune score determined by univariate Cox proportional hazards regression analysis. **B,** Kaplan–Meier survival curves of melanoma patients received checkpoint blockade therapy. Patients are divided into high immune score group and low immune score group.

(Fig. 4E). Survival analysis of groups, defined by BRCA mutation and immune score, showed that patients with *BRCA1/2* mutation and high immune score displayed prolonged OS than patients with wild-type *BRCA1/2*, whereas patients with *BRCA1/2* mutation but low immune score displayed no significant survival difference than wild-type patients (Fig. 4F; Supplementary Fig. S7). These results confirm that *BRCA1/2* mutation is a determinant of tumor infiltrate, and is more likely to be predictive of clinical outcome when it has successfully stimulated the immune infiltration.

Immune score predicts response to immunotherapy

The identification of predictive markers is crucial for immunotherapy with checkpoint blockade as only a minority of patients is responsive to the therapy. We demonstrated that, in melanoma, the ovarian cancer derived immune score was prognostic (Fig. 4B) and that loss of IFNγ pathway genes resulted in significantly decreased immune score (Supplementary Fig. S4), suggesting that the immune score also captured the preexisting antitumor immune response in melanoma. Because preexisting immune activity is a powerful marker in the setting of checkpoint blockade (8), we reasoned that the immune score might also be predictive of the response to immunotherapy. We first tested the prognostic value of immune score in the four immunotherapy datasets with transcriptome data released (9, 35–37). These include three melanoma datasets received anti-CTLA-4 or anti-PD-1 therapy and an urothelial cancer datasets received anti-PD-L1 therapy. A meta-analysis of the four datasets shows that the immune score is significantly associated with favorable survival in immunotherapy datasets (Fig. 5A; HR = 0.73 [0.57–0.93], $P = 0.01$). Significantly prolonged survival was observed for high vs. low immune score patients in the combined melanoma cohort (Fig. 5B; median OS 3.24 years vs. 1.72 years, log-rank $P = 0.01$). Totally, we found 28 (41.2%) responders of immunotherapy in the high score group of the four datasets, and only 19 (26.8%) responders in the low score group ($P = 0.077$ by Fisher exact test). Notably, the immune score enabled the classification of responders and nonresponders with a predictive power comparable with the expression of the target of immunotherapy (Supplementary Fig. S8).

Discussion

Using large-scale datasets, we systematically evaluated the prognostic values of immune infiltration in advanced EOC patients and proposed an immune score to investigate the genomic determinants of *in situ* immune activity and its correlation with the response to chemotherapy. Association with immune score identified the loss of chemokine genes and IFNγ pathway genes that are significantly correlated with low immune infiltration and poor outcome of patients. The immune score is predictive of outcome and the response to chemotherapy and immunotherapy, suggesting that it provides an appropriate tool for pretreatment stratification of patients and for posttrial evaluation.

The inhibition of immune infiltration has been increasingly identified as an important mechanism for tumor to evade from host immunity (38). TILs are often suppressed by selective neoantigen exposure (21), immune suppressive cytokines, and the deletion of IFNγ pathway genes (24). PD-1 blockage has been found to enhance T-cell migration by elevating IFNγ inducible chemokines in the tumor (39), indicating that the loss of chemokines and IFNγ pathway genes would impair the response to immunotherapy. However, large-scale genomic analysis has identified that the chromosome region 4q and 9p, which contain chemokine genes and IFNγ pathway genes respectively, are among the most significant regions with recurrent copy number loss in EOC, with a loss frequency of 67% and 66%, respectively (10). Nevertheless, in most cases, it is likely to be the heterozygous loss, which means the expression of these genes could still be induced by appropriate stimulations. We found that platinum agents could induce the expression of chemokine genes and antigen-presenting genes (Fig. 3D and E), and boosted the immune infiltration in post-chemotherapy tumors. Although conflicting data exist regarding the immunostimulatory versus immunorepressive capability of platinum agents (40, 41), we found that they can increase anti-tumor immunity via

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

upregulation of MHC genes and T-cell-attractant chemokines in ovarian cancer, as evidenced by the increased cytolytic activity of post-chemotherapy tumors (Fig. 3C). These results are consistent with the reported interplay between immunotherapy and chemotherapy (42, 43), and suggest an opportunity for designing combinatorial approaches for EOC patients whose tumors have a heterozygous loss or normal copies of chemokine genes.

The importance of spontaneous antitumor immunity was highlighted by the comprehensive associations between TILs and patient's survival in human solid cancers (44, 45). For example, in colorectal cancer, it was recently reported that the poor survival of patients with microsatellite stability tumor is likely due to the low density of TILs (13). In EOC, numerous studies have reported the prognostic value of TILs (4). However, there is currently no consensus on how to best characterize the immune infiltrates for prognostic evaluation. Using a compendium of prognostic immune marker genes, we developed the immune score as a reflection of the complexity of tumor immunogenicity in order to provide the strongest prognostic value for EOC. We chose a compendium of immune marker genes over individual marker gene (i.e., *CD8A*) or the cytolytic activity (estimated using expression of *GZMA* and *PRF1*) (21, 33), due to the fact that these commonly used marker genes, including *CD8A*, *GZMA*, and *PRF1*, are not strongest prognostic factors among the marker genes that we have identified (Fig. 1A).

The strong prognostic value of immune score and its association with TILs indicate that EOC could response to immunotherapy. Pilot studies have shown that immunotherapies that capitalize on pre-existing antitumor immune response are more likely to be successful (8). Therefore, reflective factors of tumor immunogenicity, such as neoantigen load, the expression of MHC class I genes (18), and the normalization of intratumor blood vessels (23), are predictive for the response to immunotherapy. Notably, impressive clinical response to immunotherapy has been seen for tumors with exceptionally high mutation loads in melanoma and non–small cell lung cancer (46, 47). However, EOC has a relatively low mutation load, and consequently low abundance of neoantigens in this disease (10). As a result, we failed to observe a significant correlation between mutation load and TILs in terms of CD8A expression or the cytolytic activity (Pearson correlation test, $P > 0.05$). Therefore, novel signatures of preexisting antitumor immune response need to be developed. We assumed that an immune signature that is most prognostic in EOC could be most reflective for preexisting antitumor immunity. The immune score is designed based on this assumption and indeed it is predictive of the outcome after immunotherapy (Fig. 5). Although we do not have a dataset yet to verify the immune score for EOC patients, the similarity of the genotype–immunophenotype relationship between melanoma and EOC, suggesting a similar intratumor immune microenvironment between the two cancer types.

Patients with EOC have experienced little improvement with regard to the treatment and outcome during the past decades (48). Most patients diagnosed with advanced ovarian cancer received multiple cycles of platinum-based chemotherapy (49). So far, the major determinant of clinical outcome for these patients is still the response to chemotherapy, which highly depends on whether the tumors have DNA repair defects, including especially the *BRCA1/2* mutations (10). However, here we found that both the response to chemotherapy and the survival benefit of *BRCA1/2* mutations are significantly associated with tumor immunogenicity. High immune score is associated with improved response to chemotherapy and a higher frequency of *BRCA1/2* mutations in EOC. In addition, patients with *BRCA1/2* mutations and low immune score are less likely to have survival benefit comparing to the patients with high immune score (Fig. 4F). There results indicate that assessment of tumor immunity via immune score provides an independent indicator of chemo-sensitivity. Moreover, the increased immune score after chemotherapy is consistent with the recent finding that chemotherapy enhances host immune response in metastatic ovarian tumors (28). These findings open up opportunities for new combinatorial therapies, because chemotherapy can stimulate host immune response that is associated with the effectiveness of immunotherapy, and in return, the immune response also improves chemo-sensitivity.

There are limitations in this study. First, the remarkable intratumor or intrapatient heterogeneous immune microenvironment of advanced serous ovarian cancer has not been considered. It has been recently demonstrated that the immune infiltration of different lesions of the same tumor can be highly distinct in ovarian cancer (50). Importantly, the tumor heterogeneity determines the effect of chemotherapy (51), as well as the effect of checkpoint blockade immunotherapy (52). Second, the immune score uses a serious of immune marker genes and thus is unspecific about the constitution of specific immune subpopulations. In addition, prospective studies should be performed to further evaluate the data before our findings could be more broadly applied. Nevertheless, it is a valuable signature because we systematically assessed its association with immune infiltrates and evaluated its prognostic value by the comprehensive collection of ovarian cancer cohorts. Our critical findings are consistent with previous studies. The immune score should help to further define the prognosis of EOC patients, to facilitate the comparison of the results from various immune modulators and to stratify patients who will benefit more from suggested combinatorial therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Hao, X. Li
Development of methodology: D. Hao, Q. Zhao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Chen, J. Li
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Hao
Writing, review, and/or revision of the manuscript: D. Hao, L. Wang, L.-j. Di
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Liu, L. Wang
Study supervision: L.-j. Di

Acknowledgments

L.-j. Di is supported by the Science and Technology Development Fund (FDCT) of Macao SAR (FDCT 025/2014/A1 and FDCT 088/2014/A2), the Multi-Year Research Grant from the University of Macau (MYRG2015-00037-FHS, & MYRG2015-00167-FHS) and National Natural Science Foundation of China (Grant No. 81772980). L. Wang is supported by the Multi-Year Research Grant from the University of Macau (MYRG2016-00251-FHS). D. Hao was supported by National Natural Science Foundation of China (Grant No. 31701153).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 29, 2017; revised March 7, 2018; accepted April 9, 2018; published first April 16, 2018.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

Reference

1. Ioannides CG, Freedman RS, Platsoucas CD, Rashed S, Kim YP. Cytotoxic T cell clones isolated from ovarian tumor-infiltrating lymphocytes recognize multiple antigenic epitopes on autologous tumor cells. J Immunol 1991;146:1700–7.
2. Schlienger K, Chu CS, Woo EY, Rivers PM, Toll AJ, Hudson B, et al. TRANCE- and CD40 ligand-matured dendritic cells reveal MHC class I-restricted T cells specific for autologous tumor in late-stage ovarian cancer patients. Clin Cancer Res 2003;9:1517–27.
3. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203–13.
4. Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol 2012;124:192–8.
5. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol 2015;33: 4015–22.
6. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. PNAS 2003;100:4712–7.
7. Hamanishi J, Mandai M, Konishi I. Immune checkpoint inhibition in ovarian cancer. Int Immunol 2016;28:339–48.
8. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
9. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015;350:207–11.
10. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609–15.
11. Waldron L, Haibe-Kains B, Culhane AC, Riester M, Ding J, Wang XV, et al. Comparative meta-analysis of prognostic gene signatures for late-stage ovarian cancer. J Natl Cancer Inst 2014;106.
12. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the `Immunoscore' in the classification of malignant tumours. J Pathol 2014;232:199–209.
13. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016;44:698–711.
14. Lake RA, Robinson BW. Immunotherapy and chemotherapy–a practical partnership. Nat Rev Cancer 2005;5:397–405.
15. Wan S, Pestka S, Jubin RG, Lyu YL, Tsai YC, Liu LF. Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells. PLoS One 2012;7:e32542.
16. Hao D, Li J, Jia S, Meng Y, Wang L, Zhang C, et al. Integrated analysis reveals tubal and ovarian originated serous ovarian cancer and predicts differential therapeutic responses. Clin Cancer Res 2017.
17. Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature 2015;521:489–94.
18. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. Cell reports 2017;18:248–62.
19. Rahman M, Jackson LK, Johnson WE, Li DY, Bild AH, Piccolo SR. Alternative preprocessing of RNA-sequencing data in the cancer genome atlas leads to improved analysis results. Bioinformatics 2015;31: 3666–72.
20. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 2012;30:413–21.
21. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 2015;160:48–61.
22. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nat Genet 2013;45:1127–33.
23. Tian L, Goldstein A, Wang H, Ching Lo H, Sun Kim I, Welte T, et al. Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. Nature 2017;544:250–4.
24. Gao J, Shi LZ, Zhao H, Chen J, Xiong L, He Q, et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell 2016;167:397–404 e9.
25. Abiko K, Matsumura N, Hamanishi J, Horikawa N, Murakami R, Yamaguchi K, et al. IFN-gamma from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. Br J Cancer 2015;112:1501–9.
26. Peng J, Hamanishi J, Matsumura N, Abiko K, Murat K, Baba T, et al. Chemotherapy induces programmed cell death-ligand 1 overexpression via the nuclear factor-kappaB to foster an immunosuppressive tumor microenvironment in ovarian cancer. Cancer Res 2015;75:5034–45.
27. Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res 2005;11:6713–21.
28. Bohm S, Montfort A, Pearce OM, Topping J, Chakravarty P, Everitt GL, et al. Neoadjuvant chemotherapy modulates the immune microenvironment in metastases of tubo-ovarian high-grade serous carcinoma. Clin Cancer Res 2016;22:3025–36.
29. Ahmed AA, Mills AD, Ibrahim AE, Temple J, Blenkiron C, Vias M, et al. The extracellular matrix protein TGFBI induces microtubule stabilization and sensitizes ovarian cancers to paclitaxel. Cancer Cell 2007;12:514–27.
30. Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med 2011;208:1949–62.
31. Chew V, Chen J, Lee D, Loh E, Lee J, Lim KH, et al. Chemokine-driven lymphocyte infiltration: an early intratumoural event determining long-term survival in resectable hepatocellular carcinoma. Gut 2012;61:427–38.
32. Marelli-Berg FM, Frasca L, Weng L, Lombardi G, Lechler RI. Antigen recognition influences transendothelial migration of CD4+ T cells. J Immunol 1999;162:696–703.
33. Konstantinopoulos PA, Fountzilas E, Pillay K, Zerbini LF, Libermann TA, Cannistra SA, et al. Carboplatin-induced gene expression changes in vitro are prognostic of survival in epithelial ovarian cancer. BMC Med Genet 2008;1:59–70.
34. Clarke B, Tinker AV, Lee CH, Subramanian S, van de Rijn M, Turbin D, et al. Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. Mod Pathol 2009;22: 393–402.
35. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell 2016;165:35–44.
36. Snyder A, Nathanson T, Funt SA, Ahuja A, Buros Novik J, Hellmann MD, et al. Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: an exploratory multi-omic analysis. PLoS Med 2017;14:e1002309.
37. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 2017;171:934–49 e15.
38. Bowtell DD, Bohm S, Ahmed AA, Aspuria PJ, Bast RC Jr., Beral V, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer 2015;15:668–79.
39. Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res 2012;72:5209–18.
40. Lesterhuis WJ, Punt CJ, Hato SV, Eleveld-Trancikova D, Jansen BJ, Nierkens S, et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. J Clin Invest 2011;121:3100–8.
41. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 2011;1:54–67.
42. Galluzzi L, Zitvogel L, Kroemer G. Immunological mechanisms underneath the efficacy of cancer therapy. Cancer Immunol Res 2016;4:895–902.
43. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015;28:690–714.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

44. Miller K.Estrogen and DNA damage: the silent source of breast cancer? J Natl Cancer Inst 2003;95:100–2.
45. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med 2006;354:270–82.
46. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.
47. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189–99.
48. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: Cancer J Clin 2010;60:277–300.
49. Pignata S, Cannella L, Leopardo D, Pisano C, Bruni GS, Facchini G. Chemotherapy in epithelial ovarian cancer. Cancer Lett 2011;303:73–83.
50. Jimenez-Sanchez A, Memon D, Pourpe S, Veeraraghavan H, Li Y, Vargas HA, et al. Heterogeneous tumor-immune microenvironments among differentially growing metastases in an ovarian cancer patient. Cell 2017;170:927–38 e20.
51. Schwarz RF, Ng CK, Cooke SL, Newman S, Temple J, Piskorz AM, et al. Spatial and temporal heterogeneity in high-grade serous ovarian cancer: a phylogenetic analysis. PLoS Med 2015;12:e1001789.
52. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9.

Published OnlineFirst April 16, 2018; DOI: 10.1158/1078-0432.CCR-17-3862

Clinical Cancer Research

Immunogenomic Analyses of Advanced Serous Ovarian Cancer Reveal Immune Score is a Strong Prognostic Factor and an Indicator of Chemosensitivity

Dapeng Hao, Jie Liu, Meng Chen, et al.

Clin Cancer Res 2018;24:3560-3571. Published OnlineFirst April 16, 2018.