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REVIEW ARTICLE
Prognostic immunologic signatures in epithelial ovarian cancer

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Epithelial Ovarian Cancer (EOC) is a deadly gynecologic malignancy in which patients frequently develop recurrent disease following initial platinum-taxane chemotherapy. Analogous to many other cancer subtypes, EOC clinical trials have centered upon immunotherapeutic approaches, most notably programmed cell death 1 (PD-1) inhibitors. While response rates to these immunotherapies in EOC patients have been low, evidence suggests that ovarian tumors are immunogenic and that immune-related genomic profiles can serve as prognostic markers. This review will discuss recent advances in the development of immune-based prognostic signatures in EOC that predict patient clinical outcomes, as well as emphasize specific research areas that need to be addressed to drive this field forward.

INTRODUCTION

Epithelial Ovarian Cancer (EOC) is the most lethal of all gynecologic cancers, due to the fact that patients are frequently diagnosed at a late stage and disease recurrence eventually develops following frontline platinum-taxane based chemotherapy [1]. Advances in the development of targeted approaches have paved the way for therapies such as the anti-angiogenic drug bevacizumab and the poly (ADP ribose) polymerase (PARP) inhibitor olaparib to be used in routine clinical care. However, these targeted therapies have only modestly extended patient progression-free survival (PFS), and have not significantly improved EOC patient overall survival (OS) [2, 3]. In recent years, immunotherapy has represented one of the most promising targeted approaches across all cancer subtypes, and currently many EOC clinical trials are focused on immunotherapeutic regimens that inhibit the programmed cell death 1 (PD-1) axis. However, early trial results have demonstrated low response rates to PD-1-based monotherapies, ranging from 10 to 15% [4]. Therefore, in an effort to improve response rates there has been a plethora of EOC clinical trials initiated that involve inhibiting PD-1 in combination with other standard of care and experimental targeted approaches [5].

Despite the fact that EOC patient response rates to currently investigated immunotherapies remain underwhelming, studies have demonstrated that ovarian tumors are immunogenic and can induce anti-tumor immune responses. In addition, it has been widely reported that intratumoral CD3 + and CD8 + T cells are favorable to patient prognosis [6, 7]. Therefore, while patients' response rates to clinically investigated immunotherapies remain modest, evidence is emerging that immune-based factors have the ability to serve as prognostic markers for patient clinical outcomes [8]. Recently, the use of genomic information available from public datasets coupled with immunogenomic bioinformatic tools and machine learning has allowed for an explosion of research aimed at the development of novel prognostic immunogenomic EOC signatures. Furthermore, other translational approaches have been employed to determine if tissue-based or circulating immunologic signatures can predict specific outcomes such as chemotherapy or immunotherapy response.

This review will center upon immunogenomic signatures of patient prognosis that have been identified through computational analysis of publically available datasets, while also touching upon original profiling studies that have identified intratumoral immunologic signatures indicative of survival, chemoresistant disease, and immunotherapy response, as well as circulating signatures. Finally, research areas that are still needed in order to develop a prognostic EOC immune based biomarker with clinical utility will be highlighted.

Immunogenomic prognostic signatures developed from bioinformatic intratumoral immune composition scoring

Undoubtedly, computational approaches have dominated the field of immunogenomic prognostic signature development for ovarian cancer. Bioinformatic tools such as single-sample gene set enrichment (ssGSEA), ESTIMATE, and CIBERSORT, have made it possible to estimate total infiltration of immune cell subsets from heterogeneous tumoral genomic data. These advances have led to a plethora of EOC based bioinformatic studies that have utilized the publicly available The Cancer Genome Atlas (TCGA) datasets to develop genomic signatures based upon immune infiltration. Wu et al employed CIBERSORT to determine immune cell composition and develop tumor microenvironment (TME) scores in data sets obtained from TCGA and International Cancer Genome Consortium. Differential expression analysis revealed 329 differentially expressed genes (DEGs) in patients with a high versus low TME score [9]. Gene ontology analysis confirmed that the majority of these DEGs were involved in immune-related processes. A total of 48 DEGs were found to be associated with OS, with only seven genes validating in additional publicly available datasets. Two genes emerged with the highest significance: *GPB1*, a GTPase

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involved in cell cycle control [10] and the transcription factor [11] *ETV7*. In vitro small interfering (siRNA) knockdown of *GPB1* and *ETV7* in A2780 cells resulted in greater proliferation, migration, and colony formation. While these in vitro studies further demonstrate the protective effects of these genes, future directions should include targeting these genes in the context of an immune TME [9].

An additional study by Lu et al. created immune and non-immune expression groups based on TME features and immune cell infiltration from TCGA data and confirmed the prognostic significance of these groupings using two independent validation cohorts [12]. The immune cohort group was defined by higher PD-1 signaling, immune cell infiltration, microsatellite instability, increased tumor mutational burden (TMB), and higher neoantigen levels. Moreover, this active immune cohort was stratified by immune activation or cancer associated fibroblast (CAF) expression, in which the CAF sub-cohort was defined by immunosuppressive features such as transforming growth factor-beta (TGF-β) signaling, and enrichment in epithelial-to-mesenchymal transition (EMT) and M2 macrophages. Furthermore, the group sought to replicate the activated immune cell type in vitro by performing a knockdown of *DIRAS3*, a tumor suppressor that was under expressed in the immune activation sub-cohort. The down-regulation of *DIRAS3* in the EOC cell line SKOV3 promoted cell death when treated with paclitaxel compared to control cells. Moreover, the knockdown of *DIRAS3* resulted in an upregulation of programmed death ligand-1 (PD-L1) and STAT1 phosphorylation, suggesting that IFNγ signaling was upregulated [12]. Together, this in vitro data suggests that the downregulation of *DIRAS3* in SKOV3 cells models an immune activated EOC subtype, however these results should be further studied in an immuno-competent preclinical mouse model.

Similarly, Wei et al utilized ssGSEA to divide EOC patients into three immune cell subtypes of high, medium, and low immunity classifications [13]. Interestingly, they found that TMB levels were not significantly different between each cohort, but *BRCA1* mutation was significantly associated with the high immunity subgroup. The high immunity subgroup also exhibited elevated levels of tumor infiltrating lymphocytes (TILs) and the following immune checkpoint molecules: PD-1, LAG-3, TIM-3, CTLA-4, PD-L1, PD-L2, CD80, and CD86. Furthermore, T and B cell receptor signaling, NF-Kβ signaling, Th17 cell differentiation, IL-17, and TNF signaling were all upregulated in the high immunity subgroup. Intriguingly, the medium immunity sub-group was significantly associated with worse prognosis, while no significant differences were detected between the low and high immunity cohorts. Nonetheless, immune gene set scores survival analysis revealed that high expression of immune checkpoint molecules, MHC-1, antigen-presenting cells co-inhibition, T-cell co-inhibition, Th1 cells, Th2 cells, and T regulatory cells (T_{regs}) were all associated with a significantly improved OS, however hazard ratios for these outcomes were not reported [13].

Furthermore, Ding et al utilized the ESTIMATE algorithm to stratify patients based upon calculated gene expression of immune and stromal cell subsets, with a total of 294 DEGs revealed between patients with a low and high immune and stromal score [14]. Functional enrichment exposed that these DEGs were related to pathways involving focal adhesion, human papillomavirus infection, PI3K-Akt signaling, proteoglycans, and cytokine-cytokine receptor interaction. LASSO and COX regression analysis further identified that 34 of the 294 immune-related DEGs were significantly associated with improved patient OS and a nine-gene signature was created involving protective (*UBD, GBP2, CXCL11, CXCL13, D4S234E*) and risky (*VSIG4, CXC3R1, C5AR1, TFP12*) genes [14]. Examining the nine-gene signature individually with immune cell infiltration, they found that *CXC3R1* correlated with B cell levels, *GBP2* correlated with CD8 + T cells and DCs, *CXCL13* correlated to CD4 + T cells, and *VSIG4* correlated with macrophages and neutrophils [14]. The prognostic significance of this signature was validated in an additional GSE cohort and a time-dependent receiver operating characteristic (ROC) analysis was also performed in both datasets, which further demonstrated the predictive accuracy of the nine-gene signature.

Khadirnaikar et al. identified five prognostic genes *(C1QTNF3, CD246, ADA, C6, and CASP8)* through COX regression analysis that were most associated with worse EOC patient survival from a GSE dataset, and used these genes to develop an "immune prognostic score" (IPS) [15]. Interestingly, none of these five prognostic genes correlated with one another, suggesting that their prognostic value is independent. Additionally, they found that *C1QTNF3, CD246, ADA, and CASP8* were all elevated in cancer compared to heathy controls, while *C6* was under expressed. Gene ontology analysis revealed that these specific genes were associated with immune system processes, and GSEA analysis showed that these genes were negatively associated with INFγ levels and positively associated with EMT, hypoxia, and KRAS signaling, suggesting that this prognostic score indicates a less active immune TME and increased tumorigenic properties [15]. These findings were strengthened through the validation signature in a TCGA ovarian cancer cohort, which revealed a similar significant relationship to OS, although the hazard ratio was decreased compared to the training cohort.

There have also been several bioinformatic studies that have stratified patients into high and low risk immune cohorts, in which the low risk immune cohort is associated with improved survival [16–19]. Shen et al utilized ssGSEA and developed a 129-gene immunogenomic prognostic signature, in which the majority of the genes were cytokines, cytokine receptors, and were involved in antimicrobial processes [16]. This immune-based signature successfully categorized patients based upon high and low risk immune groupings. This signature was validated in five separate ovarian cancer cohorts, in which the signature performance improved and exhibited higher hazard ratios compared to the training cohort. Corroborating this finding, a study by Zhang et al identified a 20-gene immune related paired signature, that divided patients into high and low risk prognostic cohorts in which the immune prognostic genes were similarly made up of cytokines, cytokine receptors, and antimicrobials [17]. Functional pathway analysis revealed that the high-risk group was enriched for EMT and TGFβ, as well as immunosuppressive M2 tumor associated macrophages, while the low-risk group exhibited higher CD8 + T cell expression [17]. The prognostic predictability of this 20-gene signature was validated in a meta validation cohort; however, the hazard ratio was markedly decreased compared to the test cohort.

A study performed by Yan et al. stratified EOC patients into high and low risk groupings based on a five-gene prognostic signature, including *CXCL11, S1PR4, TNFRSF17, FPR1, and DHRS95*, which successfully predicted patient OS. Interestingly, no difference in tumor mutational burden (TMB) was observed between high and low risk immune patients, but a significant association with chemosensitivity in the low risk group was detected. They also found that immune cell subset composition differed in high- and low-risk groups, with more monocytes and M2 immunosuppressive macrophages and fewer CD8+ T cells and M1 macrophages in the high-risk group compared to the low-risk group [18]. This immune score was further validated in an RNA-sequencing and two microarray datasets, and an ROC analysis was performed to assess the score's predictive survival accuracy. Finally, Zhang and colleagues developed a 17-gene immune related gene pair prognostic signature that split patients into high- and low-risk immune groupings. Toll-like receptor and chemokine signaling pathways were significantly associated with a lower risk immune score [19]. Similarly, in the study performed by Yan et al, ROC analysis was also performed on both the training and validation cohorts, greatly strengthening the signature's predictive accuracy

for OS [18]. Overall, these studies highlight that patients in high-risk immune groups exhibit a decrease in immune activating cell subsets and an increase in immunosuppressive immune cell subsets.

Pan-cancer immunologic signatures developed with ovarian cancer datasets

Ovarian cancer TCGA cohort data has also been included in larger analyses that have sought to develop a pan-cancer immunogenomic signature. Jones et al developed an immune prognostic signature based on gene clusters related to TME function, TILs, T cell trafficking, and M2 tumor associated macrophages [20]. This genomic signature was then tested on the high-grade serous ovarian cancer (HGSOC) TCGA cohort and validation HGSOC cohorts from the Cleveland Clinic and Mayo Clinic. The ratio of T cell trafficking to M2 macrophages was most significantly associated with improved OS. The immune signatures identified were also significantly associated with *BRCA1/2* mutation status and collagen, type II, alpha 1 (*COL2A1*) expression, which interestingly is associated with TGF-β/Smad signaling [21]. A pan-cancer analysis was also performed by Chifman et al of over 5000 microarray dataset samples of breast, colon, lung, ovarian, and prostate tumor specimens [22]. A nine-gene immune-based prognostic metagene signature was composed of various enrichments of immune cell-subtype gene clusters of T and NK cells, lymphatic B cells, plasma B cells, monocytes, DCs, MHC-Class II family, neutrophils, and IFNγ signaling and was found to be significantly associated with improved OS in all cancer subtypes studied, with the highest significance detected in breast and skin cancer cohorts [22].

Specialized immunogenomic signatures
There has been a handful of studies examining specific immunogenomic signatures such as transcription factors (TF), micro (mi)-RNA, and long-non-coding (lnc)-RNA. A study by Li et al developed a 17 TF gene signature from TCGA and GEO databases that could predict OS in serous ovarian cancer patients [23]. A pathway analysis of the gene signature revealed enrichment in chronic inflammatory response, positive upregulation of VEGF, T cell mediated cytotoxicity regulation, TNF biosynthesis, MyD88-dependant toll-like receptor signaling, coagulation regulation, and lymphocyte migration. Furthermore, when using the TF signature to designate patients with a high and low immune risk score, the low immune risk score group exhibited improved survival [23]. An additional investigation corroborated that TFs are integral to immune response and prognosis in EOC, as it was found that a survival associated 21-gene immunogenomic signature was significantly positively correlated with levels of four TFs (CIITA, BAFT, VDR, and CBX2), highlighting the important critical interactions between TFs and immune response genes in EOC [24].

Moreover, Ray et al developed a combined miRNA and mRNA signature utilizing TCGA ovarian and breast cancer cohort datasets and found that the combinational approach of both signatures was superior at predicting prognosis compared to either alone [25]. In addition, when incorporating the miRNA signature, M1 macrophages emerged as the immune-cell subtype most associated with improved prognosis [25]. A study by Guo et al found that two protective lncRNAs (RP11-284N8.3.1 and AC104699.1.1) both independently predicted survival in EOC patients. Gene ontology analysis revealed that both these lncRNAs play a prominent role in immune system activation [26]. Finally, Korsunsky et al performed a miRNA array in EOC patient tumors and found that levels of miRNA-197, miRNA-22, miRNA-22#, miRNA-28, miRNA-339-5p, miR-340#, miRN-328-5p, miR-629, miR-661, miRNA-98 are linked to heightened infiltration of T cells, NK cells, cytotoxic TILs, and macrophages, suggesting that miRNA markers could potentially serve as immune prognostic markers as

well [27]. Taken as a whole, these studies demonstrate that specialized immunogenomic signatures can serve as prognostic biomarkers in EOC. While the implementation of a multi-omics biomarker appears to improve prognostic predictability, this approach is not without challenges. A major concern is the difficulties that come with integrating different omics datasets, as measures derived from different methods may not correlate well with one another. Furthermore, cost is a significant limitation, as is the availability of adequate patient samples collected in an appropriate manner that allow for efficacious testing of potential multi-omics markers [28].

Immunologic signatures associated with disease progression and survival
Bioinformatic based approaches have also been used to capture immunologic changes within an ovarian tumor as disease progresses. An investigation by Chang et al compared expression of immune-based genes in stage I, II, III, and IV patient tumors and performed gene ontology assessment, which revealed five key deregulated processes as EOC tumors progress: B cell activation and differentiation, T helper cell mediated immunity, antigen receptor mediated signaling, regulation of leukocyte chemotaxis and extravasation, and macrophage activation [29]. Moreover, a study by Liu et al examined specific immune cell subset composition across EOC tumor grades and discovered a significantly decreased level of CD4 + resting memory T cells in grade 3 tumors versus grade 1 or 2, while resting DCs were significantly higher in grade 2 tumors [30]. Furthermore, CD8 + T cells and M1 macrophages were strongly correlated with other immune cell populations, and patients with higher M1 macrophage and CD4 + memory activated T cells exhibited improved survival [30].

There have been a few studies that have looked at immunologic signatures based upon the stratification of PFS and OS. Our group recently performed an immune modeling analysis incorporating RNA sequencing and machine learning to generate an immunologic prognostic signature in HGSOC patient tissue [31]. This analysis defined a multidimensional immune "biomarker" characterized by increased expression of CTLA-4, LAG-3, and T_{regs}, which was associated with improved patient PFS. Furthermore, individual analyte performance revealed that LAG-3 and the immune activating co-receptor ICOS were significantly higher in patients with improved outcomes, a result that was also corroborated by TCGA data. In addition, infiltration of immune cell subsets was not significantly associated with PFS, suggesting that the observed upregulation of these factors was not caused by a general increase in immune infiltrate [31]. Siamakpour-Reihani et al also performed an immunogenomic analysis that revealed IL6R expression as being significantly associated with improved OS and validated their findings in TCGA and GSE datasets [32]. Finally, Mairinger et al performed a Nanostring Pan Cancer immune profiling panel in HGSOC patient tissue and discovered that high expression of SLAM7, CXCL9, HSD11B1, COLEC12 were significantly associated with improved OS. HSD11B1 was also associated with improved PFS, which was corroborated by TCGA data [33].

Another immune profiling study by Rådestad et al performed a risk factor analysis based on prognostic immune related proteins in EOC ascites and tumors [34]. Utilizing ELISA and flow cytometry based approaches, they developed an eight-immune related risk factor signature consisting of high levels of soluble IFNα2, macrophage inflammatory protein (MIP)-1α, and MIP-1β in ascites; high percentage of TIM-3+PD-1-LAG-3-CD8 + T cells in ascites; low percentage of CD4γδ T cells in the ascites and tumor; tumoral CD8 + co-inhibitory negative T cells; and high percentage of tumor CD127 + TIM-3 + CD8 + T cells. Patients with 5-8 of these immune risk factors, compared to only 0-4 exhibited a significantly shorter OS [34]. Overall, these studies exemplify that immune

signatures are related to patient outcomes in EOC and possess prognostic utility. However, additional studies are needed to integrate results from these disparate studies and identify simplified, clinically useful biomarker candidates. Future directions to address this problem include measuring these candidates in a larger prospective cohort so that the predictive value of these putative markers can be compared within the same study cohort. Moreover, it will be imperative to take into account the practicality of measuring these biomarkers in the least invasive and cost-effective manner for the patient.

Signatures of immunotherapy response

Immune profiling has given way to the discovery of novel factors that could potentially be developed as immunotherapies and may aide in identifying patients who would respond favorably to immunotherapeutic interventions. In recent years, machine learning tools, such as the tumor immune dysfunction algorithm (TIDE) has allowed researchers to predict how well patients would respond to PD-1 checkpoint blockade, utilizing TCGA ovarian cancer datasets. Liu et al found that patients who had an immune signature enriched in CD4 + resting memory cells were predicted to be significantly more responsive to PD-1 based immunotherapy compared to a cluster of patients that exhibited enrichment in resting DCs, M1 macrophages, activated NK cells, plasma cells, CD8 + T cells, and T_{regs} [30]. Interestingly, clincopathological parameters within both groups were similar. Similarly, Li et al utilized the TIDE algorithm to test if their identified TF-based prognostic signature would characterize patient immunotherapy response, and discovered that patients categorized as low risk by the TF-based signature were predicted to be more responsive to PD-1 based therapeutics [23].

In EOC, there has only been one prospective study that utilized immunogenomic profiling methods to uncover prognostic markers of combined immunotherapy and PARP response. The study, which utilized highly-multiplexed single-cell imaging and single-cell spatial analysis from patient tumors enrolled in an anti-PD-1 and PARP Phase I/II clinical trial (NCT02657889), identified that a "mutational signature 3" indicative of homologous recombination DNA repair deficiency and a positive immune score represented by interferon-primed exhausted T cells within the TME were associated with improved response to combinatorial blockade [35]. In addition, single-cell spatial analysis revealed that interactions between CD8 + T cells and PD-L1 + tumor cells and macrophages could serve as mechanistic determinants of combined PARP and PD-1 therapeutic response [35]. Overall, while it is valuable to employ machine based algorithms to initially test immune prognostic based signatures, there is a critical deficit in prospective immunogenomic profiling studies, which will be essential in order to establish whether immune-based prognostic signatures can serve as biomarkers for EOC immunotherapy response.

Immunogenomic markers of chemotherapy response

In other cancers, such as breast and colon, analyses have been performed that have revealed differences in immune-based genes that correlate with varying chemotherapy response [8]. However, immune signatures of chemotherapy response are just beginning to be explored in EOC. In mouse models, frontline chemotherapy can induce intratumoral T cell infiltration, antigen presentation, and expression of PD-L1 [5]. Furthermore, an in vivo study by Vankerckhoven et al. found that when comparing carboplatin, paclitaxel, pegylated liposomal doxorubicin, gemcitabine, carboplatin/paclitaxel combined, and carboplatin/gemcitabine combined, that carboplatin/paclitaxel in combination most successfully promoted an active immune TME by producing the highest interferon-gamma (INFγ) serum levels and the strongest decrease in immunosuppressive immune-cell subsets within the ascites [36]. Interestingly, the combination of carboplatin and gemcitabine seemed to promote an immunosuppressive TME, as an increase in T_{regs} was detected, as well as an increase of serum macrophage-inflammatory-protein-10β, suggesting that chemotherapies exert differential effects on the ovarian TME [36].

Specific immune based signatures are also related to chemoresistant disease in EOC. Koti et al. performed a broad genomic analysis that revealed STAT1, widely regarded as a tumor suppressor and member of the IFNγ signaling pathway [37] was significantly differentially expressed between chemosensitve and chemoresistant EOC tumors, and that high intratumoral expression of STAT1 is associated with an increased patient PFS [38]. These results were further validated at the protein level in an expanded cohort of chemoresistant and chemosensitive patients [38]. Furthermore, a computational study led by Hao and colleagues uncovered that a high immune score in patient tumors is characterized by high expression of INFγ pathway genes and that this score is associated with an improved chemotherapy response [39]. A study by Mairinger et al found 11 immune-related DEGs attributed to platinum response. ATG10, BMI1, FCF1, PDGFC, HSD11B1 were overexpressed and TNFRSF9, COLEC12, TCF7 were under expressed in platinum sensitive tumors, while EWSR1, CD274, and KLRCI were overexpressed in platinum resistant tumors [33]. Signaling pathways for NK-mediated cell toxicity, T and B cell receptor signaling, and leukocyte migration were enriched in platinum resistant patients, which was attributed to a high proportion of T_{regs}, as indicated by enhanced levels of CD3, CD4, and CD25 [33]. Finally, Weberpals et al reported that PD-L1 gene expression and tumor inflammation scores were higher in patient samples with a good response to chemotherapy, and also reported specific gene mutations were associated with response [40].

A few studies have sought to determine immunologic effects of neoadjuvant chemotherapy (NACT) in EOC. Jimenez-Sanchez et al performed immunogenomic analysis on site-matched EOC tumors pre- and post-NACT and found an increase in natural killer (NK) cell infiltration, with no significant differences in CD8 + cytotoxic T cells observed [41]. This observation was validated in an in vivo EOC mouse model, which showed that NK cell infiltration was enhanced following cisplatin treatment, suggesting that NK cells are the primary immune cell-subset affected by NACT. Furthermore, through T-cell receptor (TCR) sequencing, the group found that TCR oligoclonal expansion, frequently used as a marker of T cell activation following neoantigen recognition, was significantly higher in patient tumors post-NACT [41]. The second study by Brunekreeft et al performed flow cytometry and immunohisto-chemical analysis of a cohort of pre- and post-NACT treated tumors and found no significance difference in the number of TILs in each group [42]. They also detected low levels of major-histocompatibility complex-1 (MHC-1) in post-NACT tumors. However, an important caveat of this study was that matched pre- and post-NACT samples were not used, which may have significantly altered the group's findings. Finally, Mesnage et al employed immunohistochemistry to examine tumor infiltrating lymphocytes (TILs) and PD-L1 positivity in tissue sections from pre-treatment and post-treatment NACT patients, identifying increased TILs and PD-L1 levels following NACT [43] Interestingly, the finding that TILs were increased post-NACT is discrepant with the study by Brunekreeft et al, which is potentially a result of the lack of matched samples utilized in the Brunekreeft study, or differential techniques utilized. One study sought to understand the relationship of chemotherapy-induced immunologic changes and clinical outcomes. Bohm et al. showed that effector T cells are activated and T_{regs} are reduced in omental metastases following NACT, particularly in good responders [44]. Despite these findings, there is overall a deficiency in studies investigating immunologic effects of chemotherapy in ovarian cancer, particularly in relationship to clinical outcomes. There has yet to be a comprehensive investigation of immune-based genes, pathways, and cell subsets in pre- and post-NACT matched patient ovarian cancer tumors.

Table 1. Summary of EOC prognostic immunologic signatures.

Signature identified	Cohort	Methods	Predictive for
48-gene signature: *GPB1, ETV7* demonstrating highest significance [9].	TCGA	CIBERSORT	OS
PD-1 pathway, immune cell infiltration, microsatellite instability, TMB, and neoantigen enrichment [12].	TCGA	ssGSEA	OS
Immune checkpoint molecules (PD-1, LAG-3, TIM-3, CTLA-4, PD-L1, PD-L2, CD80, CD86), MHC-1, antigen-presenting cells co-inhibition, T-cell co-inhibition, Th1 cells, Th2 cells, and T_regs [13].	TCGA	ssGSEA, ESTIMATE, and CIBERSORT	OS
Nine gene signature: (protective *UBD, GBP2,CXCL11,CXCL13,D4S234E*) and (risky:*VSIG4,CXC3R1,C5AR1,TFP12*) genes [14].	TCGA	ESTIMATE	OS
Five-gene signature: *(C1QTNF3,CD246, ADA,C6,*and *CASP8* [15].	GSE9899	GSEA	OS
129-gene signature with significant enrichment in cytokine, cytokine receptor, and antimicrobial processes [16].	TCGA, GSE26712, GSE32062, GSE14764, GSE23554, GSE26712, GSE3149, GSE18520, GSE19829, GSE26193, GSE30161, GSE63885, GSE9891, GSE17260, GSE32062, GSE53963,GSE73614, GSE13876, and GSE9997	ssGSEA	OS
20 immune-related gene pair signature with enrichment in cytokine, cytokine receptor, antimicrobial processes, antigen processing and presentation, BCR signaling, TCR signaling, NK cell cytotoxicity [17].	GSE26712 and GSE32062	CIBERSORT	OS
Five gene signature: *CXCL11, S1PR4, TNFRSF17, FPR1, and DHRS95* [18].	TCGA	ssGSEA	OS
17 immune-related gene pair signature enriched for caspase and interferon receptor families [19].	GSE14764, GSE26712, and TCGA	InnateDB database and LASSO regression	OS
Cytotoxic lymphocyte immune signature, T-cell trafficking (TCT), and TCT to M2 macrophage ratio [20].	TCGA	RNA-seq cluster analysis	OS
Nine immune-based metagene signature; T and NK cells, lymphatic B cells, plasma B cells, monocytes, DCs, MHC-Class II family, neutrophils,and IFNy signaling [22].	GSE18520, GSE26193, GSE26712, GSE27943, and GSE6008	Extracting microarray gene expression patterns and identifying co-expressed genes (EPIG)	OS
17 TF-based signature enriched for chronic inflammatory response, VEGF upregulation, T-cell mediated cytotoxicity, TNF biosynthesis, MyD88-dependant-toll-like receptor signaling, coagulation regulation, and lymphocyte migration [23].	GSE9899 and TCGA	LASSO COX Regression and TIDE Algorithm	OS and Immunotherapy Response
21-gene signature; enriched most significantly for inflammatory response pathways [24].	TCGA	LASSO COX Regression and CIBERSORT	1-, 3-, and 5-year OS
miRNA and mRNA signature indicative of M1 Macrophage infiltration [25].	TCGA	CIBERSORT	OS
lncRNAs: RP11-284N8.3.1 and AC104699.1.1 [26].	TCGA	COX Regression	OS
miRNA signature: miRNA-197,miRNA-22, miRNA-22#, miRNA-28, miRNA-339-5p, miR-340#, miRN-328-5p, miR-629, miR-661, miRNA-98 [27].	20 advanced EOC patient primary tissue (TCGA validation)	Applied Biosystems OpenArray MicroRNA Array and COX Regression	OS

Table 1. continued

Signature identified	Cohort	Methods	Predictive for
Immunofunctiome deregulation signature: B cell activation and differentiation, T helper cell mediated immunity, antigen receptor mediated signaling, regulation of leukocyte chemotaxis and extravasation, and macrophage activation [29].	GSE3149, GSE9891, GSE14767, GSE15622, GSE18520, GSE19829, GSE23554, GSE26193, GSE26712, GSE27651, GSE30161, GSE51373, GSE63885, and GSE65986	Gene set regularity (GSR) modeling	Tumor Stage
CD4 + resting memory cells, M1 macrophages [30].	TCGA	CIBERSORT, TIDE algorithm, and Pharmaceutical Sensitivity Genomics in Cancer (GDSC)	Tumor Grade, OS, and Immunotherapy Response
Multidimensional biomarker: LAG-3, CTLA-4, and T$_{regs}$. Individual analyte performance: LAG-3 and ICOS [31].	26 HGSOC treatment naïve patient tumors (TCGA validation)	Cofactor Genomics ImmunoPrism® Assay	PFS and OS
IL6R [32]	51 HGSOC treatment naïve patient tumors (TCGA, GSE14764, GSE26712, GSE18520, and GSE19161 validation)	Affymetrix HG-U133A GeneChip Arrays	OS
KLCR1 TCF7 HSD11B1 COLEC12 PDGFC FCF1 BMI1 TNFRSF9 ATG10 EWSR1 (Chemotherapy Response). HSD11B1 (PFS). SLAM7 CXCL9 HSD11B1 COLEC12 (OS) [33].	23 HGSOC treatment naïve patient tumors (TCGA validation)	Nanostring nCounter®PanCancer Immune Profiling Panel	PFS, OS, and Chemotherapy ResponseMairinger)
Risk factor signature: high levels of soluble IFNα2, MIP-1α, MIP-1β in ascites, high % TIM-3 + (PD-1-LAG-3-) CD8 + T cells in ascites, low %CD4γβ T cells in the ascites and tumor, tumoral CD8 + co-inhibitory negative T cells, high % tumor CD127 + TIM-3 + CD8 + T cells [34].	35 treatment naïve EOC patient tumors, ascites, and blood	ELISA and flow cytometry	OS
Mutational signature 3 (homologous recombination DNA repair deficiency)and interferon-primed exhausted T cells [35].	Anti-PD-1 and PARP clinical trial (NCT02657889)	Highly-multiplexed single-cell imaging and single-cell spatial analysis	Immunotherapy Response
STAT-1 [38]	34 advanced serous ovarian cancer treatment naïve tumors	Nanostring nCounter® Human Inflammation Panel (immunohistochemistry validation)	Chemotherapy Response and PFS
INFγ inducible chemokines [39].	TCGA, GSE13876, GSE14764, GSE17260, GSE18520, GSE26193, GSE26712, GSE30161, GSE32063, GSE32062, GSE49997, GSE51088, and ArrayExpress.	Gene set enrichment analysis (GSEA)	Chemotherapy Response
BRCA2 mutation status, PD-L1, and EMSY. [40]	39 HGSOC patient tumors	RNA Sequencing and Nanostring nCounter®	Chemotherapy Response
NK cell infiltration, oligoclonal T cell expansion [41].	40 site matched pre and post NACT patient tumors	RNA and TCR sequencing, ssGSEA, and ESIMATE algorithm	Chemotherapy Response
Decreased MHC-1 expression [42].	20 unmatched pre and post NACT patient tumors	Flow cytometry and immunofluorescence	Chemotherapy Response
TILs and PD-L1 expression [43].	83 matched pre and post NACT patient tumors	Immunohistochemistry	Chemotherapy Response
Increased effector T cell activation, PD-1, PD-L1, and CTLA-4 expresion. Decrease in T$_{regs,}$ TNF, IL8 and IL6 expression[44].	54 matched HGSOC patient pre and post NACT omental biopsies and blood draws	Immunohistochemistry, flow cytometry, RNA sequencing, and electrochemiluminescence.	Chemotherapy Response
CXCL4, CCL20, CXCL1, CXCL19 and CXCL10 [47].	40 advanced stage patient serum samples	R&D Systems Proteome Profiler Human Cytokine Array, and LEGENDplex Human Proinflammatory Chemokine Panel	PFS and OS

Circulating immunological signatures

The development of peripheral immune markers is integral when optimizing the utility of a prognostic signature for EOC patients.

While tumor sampling is readily used for immune-based biomarker assessment, obtaining on-treatment and post-treatment specimens is challenging, as it is costly and invasive

for patients to undergo additional, unnecessary biopsies [45]. Moreover, diagnostic biopsies can be associated with adverse complications such as infection or bleeding [46]. In other cancer subtypes, such as melanoma and NSCLC, there has been a push in recent years to investigate immune-based circulating markers. Numerous studies have examined specific peripheral immune receptors and factors and have determined a correlation of these circulating immune signatures with clinical response [45] However, in EOC, there has only been one study that has performed immune-based profiling in circulating blood [47]. Mlynska and colleagues found that higher treatment-naïve, serum levels of the chemokines CXCL4, CCL20, CXCL1 are significantly associated with a shortened PFS and OS. Furthermore, they established that increased circulating levels of CXCL19 and CXCL10 can discriminate which patients with detectable levels of intratumoral immune infiltration will experience a shorter PFS [47]. While these results are illuminating, they are not comprehensive, as only a select panel of cytokines and chemokines were profiled. Therefore, an emphasis should be placed on examining a full range of circulating immunologic signatures in EOC, as the non-invasive nature of these signatures would possess the ultimate clinical utility.

DISCUSSION

Overall, bioinformatic approaches have accelerated research that seeks to identify immunologic prognostic signatures in EOC and have demonstrated proof-of-principle that immune-based factors are indicative of patient prognosis in EOC. A summary of these studies can be seen in Table 1. Despite the plethora of these in silico investigations, there has been a lack of in vitro mechanistic studies and studies in immunocompetent mouse models that validate these findings. Moreover, translational prospective studies are needed in order to investigate if immunologic signatures are prognostic indicators of chemotherapy or immunotherapy response, as a complete understanding of how the immune microenvironment responds to these therapies and how these changes relate to clinical outcomes is lacking. In addition to traditional tumor-based markers, peripheral factors represent an integral part of the development of a multidimensional immune signature related to prognosis and therapy response, given the ease of accessibility to patient serum and plasma. While tumor sampling during the initial tumor reducing surgery can be readily used for immune biomarker assessment, obtaining on-treatment and post-treatment tissue specimens is unrealistic. In cancers such as melanoma and NSCLC there have been numerous studies that have examined specific peripheral immune receptors and factors associated with clinical response [45]. However, comprehensive studies have yet to be performed in ovarian cancer that seek to identify secreted immune markers indicative of survival and response to clinical regimens. Taken as a whole, translational immune profiling research will facilitate the identification of immunogenomic signatures and scientifically rational biomarkers that not only predict patient survival and response to EOC therapeutic regimens, but also inform novel therapeutic strategies that target the tumor-host immune microenvironment.

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AUTHOR CONTRIBUTIONS
NER and JRR conceptually developed this manuscript. NER and MW performed literature reviews. NER wrote the manuscript in its entirety, and JRR edited it.

COMPETING INTERESTS
The authors declare no competing interests.

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