**Development and validation of an immune related, prognostic signature and nomogram in ovarian cancer**

Xiao-Ping Liu1, Shaojie Wu1, HongJie Shi2,3, Yang Li3, Guanyi Wang2,3, Chen Chen2,3, Gang Li2,3, Qing Gong4, Lijuan Gan5, Yingying Hu6\*, Shuang Zhou7\*, Sheng Li1,2,3\*

1. Department of Urology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China.
2. Cancer Precision Diagnosis and Treatment and Translational Medicine Hubei Engineering Research Center, Wuhan 430071, China.
3. Department of Biological Repositories, Zhongnan Hospital of Wuhan University, Wuhan 430071, China.
4. Department of Obstetrics and Gynecology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China.
5. Department of Gynecological Oncology, Zhongnan Hospital of Wuhan University, Hubei Key Laboratory of Tumor Biological Behaviors, Hubei Cancer Clinical Study Center, Wuhan, 430071, China.
6. Department of Obstetrics and Gynecology, The fourth affiliated hospital, Zhejiang University School of Medicine, Yiwu, China.
7. Radiology Department, Hubei Provincial Hospital of TCM, Hubei Institute of Traditional Medicine, Wuhan, China.

**\*Correspondence:**

Sheng Li, Department of Biological Repositories, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuhan 430071, China. Email address: [lisheng-znyy@whu.edu.cn](mailto:lisheng-znyy@whu.edu.cn);

Shuang Zhou, Radiology Department, Hubei Provincial Hospital of TCM, Hubei Institute of Traditional Medicine, Wuhan, China, 430071. Email: [zshbhtcm@sina.com](mailto:zshbhtcm@sina.com)

Yingying Hu, Department of Obstetrics and Gynecology, The fourth affiliated hospital, Zhejiang University School of Medicine, Yiwu, China; 8615202@zju.edu.cn

**Abstract**

**Background** Ovarian cancer (OC) is one of the most common types of female cancers and its prognosis remains dismal. **Methods** OC gene expression studies were used to construct immune related gene pairs (IRGPs). Log-rank based survival analysis and least absolute shrinkage and selection operator (LASSO) Cox proportional hazards regression model (CoxPH) were conducted to identify prognostic IRGPs to construct an IRGP index (IRGPI) based prognostic signature. An immune-clinical prognostic index (ICPI) was generated by applying CoxPH. An ICPI-containing nomogram was developed and validated. Decision curve analysis (DCA) of the nomogram was performed. **Results** 21 OC gene expression studies were obtained and 23 prognostic IRGPs containing 34 immune related genes (IRGs) were identified. Survival analyses suggested that IRGPI was an independent prognostic factor in the training and test set. Subgroup analysis suggested that patients with advanced stage OC, low grade OC and high grade OC in the IRGPI low risk group had better OS compared with those in the IRGPI high risk group in the training and test set. The prognostic performance of IRGPI was significantly better compared with that of three other prognostic signatures. The C-index of the ICPI-containing nomogram for predicting the OS was 0.671. Internal and external validation of the nomogram confirmed the robustness of the nomogram. External calibration analysis showed good agreement between nomograms’ prediction and observed outcomes. DCA suggested that the nomogram could be translated into clinical practice. **Conclusions** A prognostic immune related, prognostic signature and nomogram for OC was developed, which could be translated into clinical practice.

**Novelty & Impact Statements** 21 OC gene expression studies including 2,777 OC patients were obtained and 23 prognostic immune related gene pairs containing 34 immune related genes were identified. An immune related, prognostic signature and nomogram for OC were developed, which could be translated into clinical practice.

**Keywords:** ovarian cancer; immune related gene; prognostic signature; nomogram

**Introduction**

Ovarian cancer (OC), which originates from the patient’s fallopian tube, represents the 5th most common type of cancer in females in the United States[1]. OC accounts for more deaths than any other types of cancers of the female reproductive system[2]. It was reported that there were about 22,240 newly diagnosed cases of OC and nearly 14,070 patients died from OC each year[1; 3]. Owning to the fact that nearly 75% OC patients were diagnosed with advanced stage disease (stage Ⅲ and stage Ⅳ) and more than 75% OC patients would relapse with chemoresistance even though they were initially sensitive to current management (taxane/platinum-based chemotherapies and cytoreduction), the 5-year survival rate of patients with OC remained extremely poor (<25%)[1; 4; 5]. Thus, novel biomarkers or prognostic signatures are required to change the status quo.

Immune system, which is made up of special cells, proteins, tissues, and organs, plays a role of defensive mechanism of the body[6]. Increasing evidences show that the host immune system plays an important role in the control of tumor growth, and tumor infiltrating lymphocytes have been repeatedly associated with improved survival in multiple cancers including OC[7; 8; 9]. Meanwhile, the introduction of immunotherapy including immune checkpoint blockade, cancer vaccines, and adoptive cell therapy has significantly improved the therapeutic efficacy of OC patients and laid the foundation of future studies for OC[4; 9]. ImmPort, created and maintained by NIAID and other NIH programs and privately funded investigators, is a curation and distribution portal for facilitating reutilization of immunological research data[10]. Thanks to high throughput technologies, lots of gene expression studies have been conducted to study the pathogeny and management of OC. Nevertheless, few studies have successfully translated their findings into clinical practice due to overfitting on small sample studies.

In the present study, we retrospectively and integratedly analyzed a total number of 21 OC[11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29] gene expression studies to investigate the role of the immune related genes (IRGs)[8] on the clinical outcomes of OC patients, and develop an immune prognostic signature.

**Methods**

**Collection and characteristics of OC gene expression profiles**

A total number of 21 OC gene expression studies including 2,777 samples were obtained from gene expression omnibus (GEO) database (n=19), ArrayExpress (n=1), and the Cancer Genome Atlas (TCGA) (n=1) (supplementary table 1). Only those samples with survival information were included in our study. We removed normal ovarian tissues in GSE18520 (n=10) and GSE26712 (n=10) and overlapped samples in GSE8841 and GSE8842, respectively. Giving that relative ranking of gene expression levels could eliminate the requirement for data preprocessing (explained blow), we downloaded and analyzed the previously normalized gene expression profiles of these 21 OC gene expression studies regardless of what methods they used for scaling and normalization. Entrez IDs were used to represent genes across different platforms. We selected the probe ID with the largest interquartile range (IQR) of expression values among all samples to represent the gene when multiple probe IDs corresponded to the same Entrez ID. Details of OC gene expression studies were summarized in supplementary table 1.

**Construction of immune-related prognostic signature**

As mentioned above, the immune related genes (IRGs) were downloaded from ImmPort, and these IRGs were classified into several subgroups including antigen processing and presentation, antimicrobials, BCR signaling pathway, chemokine receptors, chemokines, cytokine receptors, cytokines, interferon receptor, interferons, interleukins, interleukins receptor, natural killer cell cytotoxicity, TCR signaling pathway, TGF β family member, TNF family members, TNF family members receptors. Only those IRGs measured by all of the 21 OC microarrays were included for subsequent analysis.  Relative expressions of IRGs in each OC samples were used to construct immune related gene pairs (IRGPs) using relative ranking of gene expression method: pairwise comparison of the relative expression of IRGs were performed, An IRGP score of 1 was assigned if IRG 1 was less than IRG 2; otherwise the IRGP score was 0[30]. IRGPs with constant values (0 or 1) were removed to minimize bias caused by platform-dependent preferential measurement.

**Statistical analysis**

The whole samples in the 21 OC microarray studies were randomly (in a 1:1 ratio) categorized into training set and test set. As described previously[8], log-rank test was used to analyze the correlations between each IRGPs and the overall survival (OS) of OC patients to select prognostic IRGPs. IRGPs with a familywise error rate less than 0.05 were selected to conduct 10-fold cross-validation in a least absolute shrinkage and selection operator (LASSO) penalized Cox regression model in the training set[31]. IRGPs with non-zero coefficients were selected to construct the IRGP index (IRGPI), and then the risk scores for each patient in the training set and test set were calculated. The LASSO penalized Cox regression model was performed using the R package “glmnet”[31]. Time dependent receiver operating characteristic (ROC) analysis was performed to identify the optimal cutoff value of IRGPI to classify patients into high risk group and low risk group using the R package “survivalROC”( https://cran.r-project.org/package=survivalROC).

Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of IRGs were conducted using the web-based tool the Database for Annotation, Visualization and Integrated Discovery (DAVID) (version 6.8)[32], and the protein-protein interaction (PPI) network of IRGs were conducted using String database[33].

The relationship between IRGPI and the OS of OC patients was analyzed through univariate and multivariable Cox proportional hazards regression model (CoxPH) in the training set and test set using the R package “survival”( https://cran.r-project.org/package=survival). Stage 1A was coded as 1, 1B as 2, 1C as 3, and the range between 1A, 1B, and 1C was coded as 2; 2A as 4, 2B as 5, 2C as 6, and the range between 2A, 2B, and 2C was coded as 5; 3A as 7, 3B as 8, 3C as 9, and the range 3A, 3B and 3C was coded as 8; stage 4 was coded as 10. Well differentiated OC was coded as grade 1, moderately differentiated OC was coded as grade 2, and poorly differentiated OC was code as grade 3. Meanwhile, according to national comprehensive cancer network (NCCN) guidance, stage 1 and 2 were equal to early stage, stage 3 and 4 were equal to late (advanced) stage, grade 1 and 2 were equal to low grade and grade 3 was equal to high grade. In the CoxPH, age, stage and grade were treated as continuous variable.

To enhance the prognostic performance of IRGPI, we integrated independent prognostic variables (including age and IRGPI risk score) to construct an immune-clinical prognostic index (ICPI) by applying CoxPH in the training set. Similarly, OC samples were divided into high risk group and low risk group by ICPI using time-dependent ROC analysis. Concordance index (C-index) calculated using the R/bioconductor package “survcomp”[34] was used to evaluate the prognostic performance of IRGPI, ICPI and other OC prognostic signatures[35; 36; 37].

On the basis of multivariable survival analysis, variables significantly correlated with the OS of OC patients were selected along with ICPI to construct a nomogram in the training set using the R package “rms”( https://cran.r-project.org/web/packages/rms/). The performance of the nomogram was evaluated using C-index, and higher C-index was associated with better model performance. Internal and external validation of the nomogram was performed using bootstrapping method (1,000 bootstrap resamples). External calibration analysis of the nomogram for 2- and 4- year OS was conducted in the test set with the use of 1000 bootstrap resamples. Decision curve analysis (DCA), a method to estimate the clinical benefit of the nomogram in routine clinical practice, was conducted in the test set as introduced by Vickers AJ et al[38]. Differences at P<0.05 were considered to be statistically significant. All the statistical analyses were conducted using R software (version 3.4.4).

**Results**

**Determination of immune related gene pair index (IRGPI)**

A total number of 2,777 OC patients were included in our study, including 1,389 subjects in the training set and 1,388 subjects in the test set. In the training set and test set, 980 patients were less than or equal to 60 years old, and 801 patients were older than 60 years old. A total of number of 304 early stage OCs and 2,414 advanced stage OCs. For the disease grade, 685 patients were low grade OC and 1,727 patients were high grade OC. The baseline characteristics of the OC patients in training set and test set were not significantly different (supplementary table 2). A total of 1,811 IRGs were obtained from the ImmPort, and 458 IRGs could be detected across all the platforms of the 21 OC microarrays, and then 104,653 IRGPs were generated. After log-rank based survival analysis, 38 IRGPs were found to be significantly correlated with OS of OC patients. Finally, 23 IRGPs including 34 IRGs were selected to construct the IRGPI based on the results of LASSO penalized CoxPH (supplementary table 3). According to the results of time-dependent ROC analysis, the OC patients were categorized into high risk group and low risk group with the optimal cutoff of IRGPI equaled to 1.106 (supplementary figure 1).

**Prognostic role IRGPI in OC**

As shown in figure 1, the OS of patients in the IRGPI low risk group was significantly better than that in the IRGPI high risk group in the training set (HR=0.482, 95% CI: 0.4112-0.565, P<0.0001) and test set (HR=0.6699, 95% CI: 0.5798-0.7739). Moreover, univariate and multivariable analysis further confirmed that OS of the IRGPI low risk group was significantly better than that of the IRGPI high risk group (supplementary table 4). Owing to the fact that some microarray studies classified OC into early stage, advanced stage, low grade and high grade, and some studies classified OC into stage Ⅰ-Ⅳ, and grade 1-3, we evaluated the prognostic value of IRGPI in early stage, advanced stage, low grade, and high grade in the training set and test set. As shown in figure 2 and supplementary figure 2, patients with early OC, advanced OC, low grade OC and high grade OC in the IRGPI low risk group had better OS compared with those in the IRGPI high risk group in the training set. Meanwhile, as shown in figure 2 and supplementary figure 3, patients with advanced OC, low grade OC, high grade OC in the IRGPI low risk group were demonstrated to have better OS compared with those in IRGPI high risk group in the test set.

**Functional analysis of IRGs**

To get a preliminary understanding of the IRGs, GO analysis and KEGG pathway analysis were conducted. As shown in supplementary table 5, IRGs were enriched in GO terms related with proteometabolism, cell proliferation and signal transduction. KEGG analysis suggested that IRGs were mostly enriched in some well-known cancer related pathways including TGF-β, NF-κB and MARK signaling pathways. Protein-protein interaction network analysis suggested that PSMC6, BDNF, JAK2, LYN, PSMD4, CX3CR1, IGF1, PSMC5, TGFB2, and VCAM1 (those genes interacted with no less three other genes) were at the hub of the network (supplementary figure 4).

**Construction of ICPI and its prognostic role**

As mentioned above, we constructed an ICPI by integrating the IRGPI and clinical features using the following formula: ICPI= age × 0.02421 + stage ×0.16979 + IRGPI score × 1.55408. Patients in the training set and test set were also classified into ICPI high risk group and ICPI low risk group based on the cutoff of 4.555 (supplementary figure 5A). Supplementary figure 5B suggested that the ICPI had higher separation ability than IRGPI alone in the test set. Next, we compared the prognostic performance of the IRGPI and ICPI with C-index. **As shown figure 3A,** the C-indexes for ICPI were significantly higher than those for IRGPI in the training set (C-indexIRGPI=0.62 vs C-indexICPI=0.67, P<0.0001) and test set (C-indexIRGPI=0.60 vs C-indexICPI=0.66, P<0.0001). Finally, the performance of IRGP was also compared with other prognostic signatures in OC. As shown in figure 3B, the prognostic performance of IRGPI were significantly better than that of 10-gene signature (C-index: 0.63 vs 0.56, P=0.003), 11-gene signature (C-index: 0.63 vs 0.59, P=0.04), and 4-gene signature (C-index: 0.63 vs 0.53, P<0.0001) in the TCGA ovarian cancer cohort.

**Development and validation of ICPI related nomogram for OS**

In order to translate our findings into clinical use, we constructed a nomogram by integrating these independent prognostic variables including ICPI, age, and staging in the training cohort (figure 4), The usage of the nomogram can be referred to the suggestions of Vassiliki L et al.[39] . Briefly, the nomogram could be interpreted by summing up the points assigned to each variable, and a vertical line should be drawn from the total points row to obtain the proportion of 2- year and 4- year OS. The C-index of the nomogram for predicting the OS was 0.671 (standard deviation=0.029, p<0.0001) in the training set. Internal validation (1,000 bootstrap resamples) of the nomogram suggested that corrected C-index was 0.668 for the OS. In the test set (external validation cohort), both original C-index and corrected C-index (1,000 bootstrap resamples) were 0.660. External calibration plots for the probability of 2- and 4-year OS also showed good agreement between nomograms’ prediction and observed outcomes in the test set (figure 5).

**Clinical relevance of the ICPI related nomogram**

The results of DCA of the nomogram, as shown in figure 6, suggested that if the threshold probability of a patient was 3%-68%, prediction of 3-year OS based on the nomogram showed more benefit than either the treat-all-patients scheme or the treat-none scheme.

**Discussions**

Owning to the lack of methods for screening and early diagnosis of OC and the absence of early warning symptoms, approximately 70% OC are diagnosis at advanced stage, making the disease incurable for the majority of cases[40]. Immune system has been demonstrated to be closely related to the proliferation and progression of various tumors including OC[7; 41; 42]. Immunotherapy has begun to emerge in the treatment of OC and has achieved good clinical benefits[4]. In the present study, we developed and validated an IRG-based prognostic signature by retrospectively analyzing OC gene expression studies (supplementary figure 6). One of the strengths of our study is that it incorporates as many OC patients whose survival times have been well documented as possible, which makes our conclusion more robust. The other strengths of our study, as discussed previously[8; 43]**,** is that the signature is constructed on the basis of the relative ranking of gene expression values and only involves pairwise comparison within the gene expression profile of a sample, which is unnecessary to normalize the data. Therefore, our prognostic signature could be used as a personalized, single-sample estimate of OS of OC patients. Meanwhile, the OS of OC patients (especially patients with advanced stage and high grade disease) favored patients in IRGPI low risk group over those in IRGPI high risk group, and when the prognostic performance of the IRGPI was compared the previously established prognostic signatures, it still showed better performance. Thus, our prognostic signature is very suitable to be translated to clinical application.

Functional enrichment analysis of IRGs suggested that the IRGs were mostly enriched in protein metabolism, cell proliferation, and several signaling pathways that were correlated with tumor growth. Meanwhile, the literature review on the hub genes (including PSMC6[44], BDNF[45; 46], JAK2[47], LYN[48], PSMD4[49], CX3CR1[50], IGF1[51], PSMC5[52], TGFB2[53], and VCAM1[54]) in the PPI network suggested that they were involved in the proliferation of tumor growth, which was consistent with the results of survival analysis on the IRGPI-based signature.

At present, the etiology of OC remains unclear, many factors have been demonstrated to be involved in the occurrence and development of OC. Nomograms, which always takes multiple clinicopathological factors into consideration, have been shown to be more accurate than the established staging systems for predicting prognosis and guiding treatment in some cancers[39; 55; 56]. Thus, to translate our findings into clinical practice, we constructed a nomogram by integrating in independent prognostic variables (age, stage, and ICPI) in the multivariable survival analysis model. The original and corrected C-indexes for the nomogram in the training set and test set were 0.66-0.67, suggesting that the prognostic performance of the nomogram was relatively good. Moreover, calibration analysis in the test set further confirmed the robustness of the prognostic performance of the nomogram. The result of DCA provided a graphic tool to help the physicians and patients use the nomogram with more convenience.

At the same time, some limitations exist in our study. Firstly, this is a retrospective analysis of previously published OC studies, we could not control the exposure and outcome measurements and selection bias might not be excluded. Secondly, a total of 1,811 IRGs were obtained from the ImmPort, and only 458 IRGs were measured by all the 21 OC studies, thus some important information might be inevitably missed. Thirdly, the exact mechanisms regarding the impact of IRGs on the growth of OC cells have not been validated by experiments in cellular and molecular biology.

In conclusions, we developed of immune related prognostic signature (including 23 IRGPs) for patients with OC patients. The prognostic performance of the signature is better compared with 3 established prognostic signatures. An ICPI based nomogram was constructed and validated to translate the immune related signature into clinical practice.

**Acknowledgements**

The authors thank Bailiang Li and Ruijiang Li for help with manuscript preparation.

**Funding**

None

**Availability of data and materials**

All data generated or analyzed during this study are included in this article and its Additional files.

**Authors’ contributions**

Liu X designed the study, collected and analyzed the data and wrote the manuscript. Wu S, Shi H, Yang Li and Wang G participated in statistical analysis. Chen C, Gang L participated in data collection and analysis. Gong Q, Gan L participated in the manuscript writing and review. Zhou S and Hu Y, Li S participated in designed the study.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

This is not applicable for this study

**Competing interests**

The authors declare no conflicts of interest

**References**

[1] B. Davidson, and C.G. Trope, Ovarian cancer: diagnostic, biological and prognostic aspects. Womens Health (Lond) 10 (2014) 519-33.

[2] H.T. Lynch, C.L. Snyder, J.F. Lynch, B.D. Riley, and W.S. Rubinstein, Hereditary breast-ovarian cancer at the bedside: role of the medical oncologist. J Clin Oncol 21 (2003) 740-53.

[3] D.W. Cramer, The epidemiology of endometrial and ovarian cancer. Hematol Oncol Clin North Am 26 (2012) 1-12.

[4] E. Zsiros, J. Tanyi, K. Balint, and L.E. Kandalaft, Immunotherapy for ovarian cancer: recent advances and perspectives. Curr Opin Oncol 26 (2014) 492-500.

[5] J. Liu, and U.A. Matulonis, New strategies in ovarian cancer: translating the molecular complexity of ovarian cancer into treatment advances. Clin Cancer Res 20 (2014) 5150-6.

[6] K.M. Yatim, and F.G. Lakkis, A brief journey through the immune system. Clin J Am Soc Nephrol 10 (2015) 1274-81.

[7] Y. Zakharia, O. Rahma, and S.N. Khleif, Ovarian cancer from an immune perspective. Radiat Res 182 (2014) 239-51.

[8] B. Li, Y. Cui, M. Diehn, and R. Li, Development and Validation of an Individualized Immune Prognostic Signature in Early-Stage Nonsquamous Non-Small Cell Lung Cancer. JAMA Oncol 3 (2017) 1529-1537.

[9] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12 (2012) 252-64.

[10] S. Bhattacharya, P. Dunn, C.G. Thomas, B. Smith, H. Schaefer, J. Chen, Z. Hu, K.A. Zalocusky, R.D. Shankar, S.S. Shen-Orr, E. Thomson, J. Wiser, and A.J. Butte, ImmPort, toward repurposing of open access immunological assay data for translational and clinical research. Sci Data 5 (2018) 180015.

[11] S. Marchini, P. Mariani, G. Chiorino, E. Marrazzo, R. Bonomi, R. Fruscio, L. Clivio, A. Garbi, V. Torri, M. Cinquini, T. Dell'Anna, G. Apolone, M. Broggini, and M. D'Incalci, Analysis of gene expression in early-stage ovarian cancer. Clin Cancer Res 14 (2008) 7850-60.

[12] A.P. Crijns, R.S. Fehrmann, S. de Jong, F. Gerbens, G.J. Meersma, H.G. Klip, H. Hollema, R.M. Hofstra, G.J. te Meerman, E.G. de Vries, and A.G. van der Zee, Survival-related profile, pathways, and transcription factors in ovarian cancer. PLoS Med 6 (2009) e24.

[13] C. Denkert, J. Budczies, S. Darb-Esfahani, B. Gyorffy, J. Sehouli, D. Konsgen, R. Zeillinger, W. Weichert, A. Noske, A.C. Buckendahl, B.M. Muller, M. Dietel, and H. Lage, A prognostic gene expression index in ovarian cancer - validation across different independent data sets. J Pathol 218 (2009) 273-80.

[14] K. Yoshihara, A. Tajima, T. Yahata, S. Kodama, H. Fujiwara, M. Suzuki, Y. Onishi, M. Hatae, K. Sueyoshi, H. Fujiwara, Y. Kudo, K. Kotera, H. Masuzaki, H. Tashiro, H. Katabuchi, I. Inoue, and K. Tanaka, Gene expression profile for predicting survival in advanced-stage serous ovarian cancer across two independent datasets. PLoS One 5 (2010) e9615.

[15] S.C. Mok, T. Bonome, V. Vathipadiekal, A. Bell, M.E. Johnson, K.K. Wong, D.C. Park, K. Hao, D.K. Yip, H. Donninger, L. Ozbun, G. Samimi, J. Brady, M. Randonovich, C.A. Pise-Masison, J.C. Barrett, W.H. Wong, W.R. Welch, R.S. Berkowitz, and M.J. Birrer, A gene signature predictive for outcome in advanced ovarian cancer identifies a survival factor: microfibril-associated glycoprotein 2. Cancer Cell 16 (2009) 521-32.

[16] V. Vathipadiekal, V. Wang, W. Wei, L. Waldron, R. Drapkin, M. Gillette, S. Skates, and M. Birrer, Creation of a Human Secretome: A Novel Composite Library of Human Secreted Proteins: Validation Using Ovarian Cancer Gene Expression Data and a Virtual Secretome Array. Clin Cancer Res 21 (2015) 4960-9.

[17] J.S. Ferriss, Y. Kim, L. Duska, M. Birrer, D.A. Levine, C. Moskaluk, D. Theodorescu, and J.K. Lee, Multi-gene expression predictors of single drug responses to adjuvant chemotherapy in ovarian carcinoma: predicting platinum resistance. PLoS One 7 (2012) e30550.

[18] D. Spentzos, D.A. Levine, S. Kolia, H. Otu, J. Boyd, T.A. Libermann, and S.A. Cannistra, Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol 23 (2005) 7911-8.

[19] K. Yoshihara, T. Tsunoda, D. Shigemizu, H. Fujiwara, M. Hatae, H. Fujiwara, H. Masuzaki, H. Katabuchi, Y. Kawakami, A. Okamoto, T. Nogawa, N. Matsumura, Y. Udagawa, T. Saito, H. Itamochi, M. Takano, E. Miyagi, T. Sudo, K. Ushijima, H. Iwase, H. Seki, Y. Terao, T. Enomoto, M. Mikami, K. Akazawa, H. Tsuda, T. Moriya, A. Tajima, I. Inoue, K. Tanaka, and G. Japanese Serous Ovarian Cancer Study, High-risk ovarian cancer based on 126-gene expression signature is uniquely characterized by downregulation of antigen presentation pathway. Clin Cancer Res 18 (2012) 1374-85.

[20] D. Pils, G. Hager, D. Tong, S. Aust, G. Heinze, M. Kohl, E. Schuster, A. Wolf, J. Sehouli, I. Braicu, I. Vergote, I. Cadron, S. Mahner, G. Hofstetter, P. Speiser, and R. Zeillinger, Validating the impact of a molecular subtype in ovarian cancer on outcomes: a study of the OVCAD Consortium. Cancer Sci 103 (2012) 1334-41.

[21] K.M. Lisowska, M. Olbryt, V. Dudaladava, J. Pamula-Pilat, K. Kujawa, E. Grzybowska, M. Jarzab, S. Student, I.K. Rzepecka, B. Jarzab, and J. Kupryjanczyk, Gene expression analysis in ovarian cancer - faults and hints from DNA microarray study. Front Oncol 4 (2014) 6.

[22] S. Bentink, B. Haibe-Kains, T. Risch, J.B. Fan, M.S. Hirsch, K. Holton, R. Rubio, C. April, J. Chen, E. Wickham-Garcia, J. Liu, A. Culhane, R. Drapkin, J. Quackenbush, and U.A. Matulonis, Angiogenic mRNA and microRNA gene expression signature predicts a novel subtype of serous ovarian cancer. PLoS One 7 (2012) e30269.

[23] D.C. Marchion, H.M. Cottrill, Y. Xiong, N. Chen, E. Bicaku, W.J. Fulp, N. Bansal, H.S. Chon, X.B. Stickles, S.G. Kamath, A. Hakam, L. Li, D. Su, C. Moreno, P.L. Judson, A. Berchuck, R.M. Wenham, S.M. Apte, J. Gonzalez-Bosquet, G.C. Bloom, S.A. Eschrich, S. Sebti, D.T. Chen, and J.M. Lancaster, BAD phosphorylation determines ovarian cancer chemosensitivity and patient survival. Clin Cancer Res 17 (2011) 6356-66.

[24] B. Mateescu, L. Batista, M. Cardon, T. Gruosso, Y. de Feraudy, O. Mariani, A. Nicolas, J.P. Meyniel, P. Cottu, X. Sastre-Garau, and F. Mechta-Grigoriou, miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. Nat Med 17 (2011) 1627-35.

[25] P.A. Konstantinopoulos, D. Spentzos, B.Y. Karlan, T. Taniguchi, E. Fountzilas, N. Francoeur, D.A. Levine, and S.A. Cannistra, Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. J Clin Oncol 28 (2010) 3555-61.

[26] R.W. Tothill, A.V. Tinker, J. George, R. Brown, S.B. Fox, S. Lade, D.S. Johnson, M.K. Trivett, D. Etemadmoghadam, B. Locandro, N. Traficante, S. Fereday, J.A. Hung, Y.E. Chiew, I. Haviv, G. Australian Ovarian Cancer Study, D. Gertig, A. DeFazio, and D.D. Bowtell, Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res 14 (2008) 5198-208.

[27] B.Y. Karlan, J. Dering, C. Walsh, S. Orsulic, J. Lester, L.A. Anderson, C.L. Ginther, M. Fejzo, and D. Slamon, POSTN/TGFBI-associated stromal signature predicts poor prognosis in serous epithelial ovarian cancer. Gynecol Oncol 132 (2014) 334-42.

[28] N. Cancer Genome Atlas Research, Integrated genomic analyses of ovarian carcinoma. Nature 474 (2011) 609-15.

[29] B. Winterhoff, H. Hamidi, C. Wang, K.R. Kalli, B.L. Fridley, J. Dering, H.W. Chen, W.A. Cliby, H.J. Wang, S. Dowdy, B.S. Gostout, G.L. Keeney, E.L. Goode, and G.E. Konecny, Molecular classification of high grade endometrioid and clear cell ovarian cancer using TCGA gene expression signatures. Gynecol Oncol 141 (2016) 95-100.

[30] F. Peng, R. Wang, Y. Zhang, Z. Zhao, W. Zhou, Z. Chang, H. Liang, W. Zhao, L. Qi, Z. Guo, and Y. Gu, Differential expression analysis at the individual level reveals a lncRNA prognostic signature for lung adenocarcinoma. Mol Cancer 16 (2017) 98.

[31] N. Simon, J. Friedman, T. Hastie, and R. Tibshirani, Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent. J Stat Softw 39 (2011) 1-13.

[32] W. Huang da, B.T. Sherman, and R.A. Lempicki, Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37 (2009) 1-13.

[33] D. Szklarczyk, J.H. Morris, H. Cook, M. Kuhn, S. Wyder, M. Simonovic, A. Santos, N.T. Doncheva, A. Roth, P. Bork, L.J. Jensen, and C. von Mering, The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45 (2017) D362-D368.

[34] M.S. Schroder, A.C. Culhane, J. Quackenbush, and B. Haibe-Kains, survcomp: an R/Bioconductor package for performance assessment and comparison of survival models. Bioinformatics 27 (2011) 3206-8.

[35] H.S. Isaksson, B. Sorbe, and T.K. Nilsson, Whole genome expression profiling of blood cells in ovarian cancer patients -prognostic impact of the CYP1B1, MTSS1, NCALD, and NOP14. Oncotarget 5 (2014) 4040-9.

[36] J.P. Gillet, A.M. Calcagno, S. Varma, B. Davidson, M. Bunkholt Elstrand, R. Ganapathi, A.A. Kamat, A.K. Sood, S.V. Ambudkar, M.V. Seiden, B.R. Rueda, and M.M. Gottesman, Multidrug resistance-linked gene signature predicts overall survival of patients with primary ovarian serous carcinoma. Clin Cancer Res 18 (2012) 3197-206.

[37] D.J. Cheon, Y. Tong, M.S. Sim, J. Dering, D. Berel, X. Cui, J. Lester, J.A. Beach, M. Tighiouart, A.E. Walts, B.Y. Karlan, and S. Orsulic, A collagen-remodeling gene signature regulated by TGF-beta signaling is associated with metastasis and poor survival in serous ovarian cancer. Clin Cancer Res 20 (2014) 711-23.

[38] A.J. Vickers, and E.B. Elkin, Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making 26 (2006) 565-74.

[39] V.L. Tsikitis, K.C. Lu, J.S. Kim, K.G. Billingsley, C.R. Thomas, Jr., and D.O. Herzig, Nomogram for Predicting Overall Survival and Salvage Abdominoperineal Resection for Patients with Anal Cancer. Dis Colon Rectum 59 (2016) 1-7.

[40] A.J. Cortez, P. Tudrej, K.A. Kujawa, and K.M. Lisowska, Advances in ovarian cancer therapy. Cancer Chemother Pharmacol 81 (2018) 17-38.

[41] A. Ribas, Adaptive Immune Resistance: How Cancer Protects from Immune Attack. Cancer Discov 5 (2015) 915-9.

[42] S.R. Woo, L. Corrales, and T.F. Gajewski, Innate immune recognition of cancer. Annu Rev Immunol 33 (2015) 445-74.

[43] M. Heinaniemi, M. Nykter, R. Kramer, A. Wienecke-Baldacchino, L. Sinkkonen, J.X. Zhou, R. Kreisberg, S.A. Kauffman, S. Huang, and I. Shmulevich, Gene-pair expression signatures reveal lineage control. Nat Methods 10 (2013) 577-83.

[44] C.X. Shi, K.M. Kortum, Y.X. Zhu, L.A. Bruins, P. Jedlowski, P.G. Votruba, M. Luo, R.A. Stewart, J. Ahmann, E. Braggio, and A.K. Stewart, CRISPR Genome-Wide Screening Identifies Dependence on the Proteasome Subunit PSMC6 for Bortezomib Sensitivity in Multiple Myeloma. Mol Cancer Ther 16 (2017) 2862-2870.

[45] L. Cao, X. Liu, E.J. Lin, C. Wang, E.Y. Choi, V. Riban, B. Lin, and M.J. During, Environmental and genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition. Cell 142 (2010) 52-64.

[46] B. Chen, Y. Liang, Z. He, Y. An, W. Zhao, and J. Wu, Autocrine activity of BDNF induced by the STAT3 signaling pathway causes prolonged TrkB activation and promotes human non-small-cell lung cancer proliferation. Sci Rep 6 (2016) 30404.

[47] P.G. Talati, L. Gu, E.M. Ellsworth, M.A. Girondo, M. Trerotola, D.T. Hoang, B. Leiby, A. Dagvadorj, P.A. McCue, C.D. Lallas, E.J. Trabulsi, L. Gomella, A.E. Aplin, L. Languino, A. Fatatis, H. Rui, and M.T. Nevalainen, Jak2-Stat5a/b Signaling Induces Epithelial-to-Mesenchymal Transition and Stem-Like Cell Properties in Prostate Cancer. Am J Pathol 185 (2015) 2505-22.

[48] L.J. Schwarz, E.M. Fox, J.M. Balko, J.T. Garrett, M.G. Kuba, M.V. Estrada, A.M. Gonzalez-Angulo, G.B. Mills, M. Red-Brewer, I.A. Mayer, V. Abramson, M. Rizzo, M.C. Kelley, I.M. Meszoely, and C.L. Arteaga, LYN-activating mutations mediate antiestrogen resistance in estrogen receptor-positive breast cancer. J Clin Invest 124 (2014) 5490-502.

[49] M.S. Fejzo, L. Anderson, H.W. Chen, E. Guandique, O. Kalous, D. Conklin, and D.J. Slamon, Proteasome ubiquitin receptor PSMD4 is an amplification target in breast cancer and may predict sensitivity to PARPi. Genes Chromosomes Cancer 56 (2017) 589-597.

[50] K.M. Hart, E.J. Usherwood, and B.L. Berwin, CX3CR1 delineates temporally and functionally distinct subsets of myeloid-derived suppressor cells in a mouse model of ovarian cancer. Immunol Cell Biol 92 (2014) 499-508.

[51] M. Koti, R.J. Gooding, P. Nuin, A. Haslehurst, C. Crane, J. Weberpals, T. Childs, P. Bryson, M. Dharsee, K. Evans, H.E. Feilotter, P.C. Park, and J.A. Squire, Identification of the IGF1/PI3K/NF kappaB/ERK gene signalling networks associated with chemotherapy resistance and treatment response in high-grade serous epithelial ovarian cancer. BMC Cancer 13 (2013) 549.

[52] J.H. Yim, H.S. Yun, S.J. Lee, J.H. Baek, C.W. Lee, J.Y. Song, H.D. Um, J.K. Park, J.S. Kim, I.C. Park, and S.G. Hwang, Radiosensitizing effect of PSMC5, a 19S proteasome ATPase, in H460 lung cancer cells. Biochem Biophys Res Commun 469 (2016) 94-100.

[53] P. Bragado, Y. Estrada, F. Parikh, S. Krause, C. Capobianco, H.G. Farina, D.M. Schewe, and J.A. Aguirre-Ghiso, TGF-beta2 dictates disseminated tumour cell fate in target organs through TGF-beta-RIII and p38alpha/beta signalling. Nat Cell Biol 15 (2013) 1351-61.

[54] H.C. Tai, A.C. Chang, H.J. Yu, C.Y. Huang, Y.C. Tsai, Y.W. Lai, H.L. Sun, C.H. Tang, and S.W. Wang, Osteoblast-derived WNT-induced secreted protein 1 increases VCAM-1 expression and enhances prostate cancer metastasis by down-regulating miR-126. Oncotarget 5 (2014) 7589-98.

[55] Y.Q. Huang, C.H. Liang, L. He, J. Tian, C.S. Liang, X. Chen, Z.L. Ma, and Z.Y. Liu, Development and Validation of a Radiomics Nomogram for Preoperative Prediction of Lymph Node Metastasis in Colorectal Cancer. J Clin Oncol 34 (2016) 2157-64.

[56] Y. Wang, J. Li, Y. Xia, R. Gong, K. Wang, Z. Yan, X. Wan, G. Liu, D. Wu, L. Shi, W. Lau, M. Wu, and F. Shen, Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. J Clin Oncol 31 (2013) 1188-95.

**Figure 1** Overall survival of OC patients in the training set (A) and test set (B)

**Figure 2** Univariate Cox proportional hazards regression analysis of IRGPI signature for OS in different OC subgroups.

**Figure 3** C-index comparisons between IRGPI and other prognostic model. (A) C-index comparison between IRGPI and ICPI. (B) C-index comparison between IRGPI and 10-gene signature, 11-gene signature, and 4-gene signature.

**Figure 4** To obtain the predicted probability 2- and 4- year OS of OC patients, locate the patient values on each axis. Draw a straight line upward to the ‘Points’ axis to determine the points of the variable. Sum the points for all variables and locate the sum on the ‘Total points’ axis. Draw a vertical line down to the ‘Probability of 2- and 4- year survival probability’ axis to find the probability of survival at 2 and 4 years. The equation of each variable as follows: Points for age = 0 \* Age ^3 + 0 \* Age ^2 + -0.095701946 \* Age + 9.570194592; Points for stage = 0 \* Stage ^2 + 0.080584323 \* Stage + 0; Points for ICPI= -22.222222222 \* ICPI + 133.333333333; 2-year Survival Probability = -1.76e-06 \* points ^3 + 7.9012e-05 \* points ^2 + 0.011958347 \* points + 0.299978225; 4-year Survival Probability = -1.891e-06 \* points ^3 + 0.000195695 \* points ^2 + 0.006382286 \* points + 0.127737235;

**Figure 5** Calibration plots depicting the correlation between predicted and actual probability of 2year OS (A) and 4 year OS in the test set.

**Figure 6** Decision curve analyses for overall survival predictions. Decision curve analyses depicting the net benefit associated with the use of the nomograms.

**Supplementary figure1** Optimal cutoff of IRGPI to classify OC patients into IRGPI low risk group and IRGPI high risk group based on the result of time dependent survival analysis.

**Supplementary figure 2** Kaplan- Meier survival analysis of early stage (A), advanced stage (B), low grade (C) and high grade (D) OC patients according to the IRGPI status in the training set.

**Supplementary figure 3** Kaplan- Meier survival analysis of early stage (A), advanced stage (B), low grade (C) and high grade (D) OC patients according to the IRGPI status in the test set.

**Supplementary figure 4** Protein –to protein interaction network of IRGs.

**Supplementary figure 5 (A)** Optimal cutoff of IRGPI to classify OC patients into ICPI low risk group and ICPI high risk group based on the result of time dependent survival analysis. (B) Kaplan-Meier survival analysis of OC patients in the whole population in the training set and test set according to IRGPI and ICPI status.