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Reviewer A

Comment 1: First of all, the predictive model based on the gene prognostic signature only has poor accuracy for predicting the prognosis, so I suggest the authors to clearly indicate the combination of gene and clinical factors in the title. Please also indicate the development and validation of the prognosis prediction model in the title.

Reply 1: Thank you for your suggestion. The title of this study has been revised with added information regarding inflammatory response–related genes, clinical factors, and model development and validation.

Changes in the text: We have modified our text as advised to "Development and validation of an inflammatory response–related gene and clinical factor-based signature for predicting prognosis in gastric cancer" (See page 1, line 2-3).

Comment 2: Second, the abstract needs some revisions. The background did not describe the need to combine clinical factors and the genes related to inflammation. They also did not explain why the proposed model can accurately predict the prognosis. In the method, please describe the clinical factors in the datasets, how they were identified as potential predictors, and the prognosis outcomes to be predicted in the datasets, as well as the measures of treatment response. Main statistical methods for assessing the predictive accuracy of the model were not described. In the results, please describe the clinical factors used in the predictive model, its predictive accuracy in both the training and validation datasets, as indicated by AUC values. I do not agree with the reporting of HR values, since the focus is predicting. In the conclusion, the authors need more detailed comments for the clinical implications of the findings.

Reply 2: Thank you for your suggestion. The abstract has been revised with the following context added: 1). The unsatisfactory performance of clinical factors in predicting GC prognosis and the need to combine clinical factors and the genes related to inflammation; 2). The common clinical factors identified in the univariable analysis across the training and validation sets were involved in the nomogram for better verification; 3). Cox analysis of overall survival (OS) was performed to assess prognostic value, while the risk scores were compared between responders to immunotherapy and non-responders for measurement of treatment response; 4) Clinical factors including sex, age, and tumor stage were included in the predictive model, together with the AUCs indicating the predictive accuracy in both the training and the validation datasets; 5) The association of the risk score with response to immunotherapy, together with the potential utilization of the novel signature in the management of GC.

Changes in the text: We have rewritten the abstract with the AUC value of the predictive model reported. Besides, description of the predictive efficacy of single clinical factors and a summarized table of the AUC values were also added to the Result section of the main text accordingly. (See page 12, lines 359-368, 375-377, and Page 34, Table 2). The revised Abstract is as follows:

Background: Gastric cancer (GC) is an aggressive disease that requires prognostic tools to aid in clinical management. The prognostic power of clinical features is unsatisfactory, which might be improved by combining mRNA-based signatures. Inflammatory response is widely associated with cancer development and treatment response. It is worth exploring the prognostic performance of inflammatory-related genes plus clinical factors in GC.

Methods: An 11-gene signature was trained using the least absolute shrinkage and selection operator (LASSO) based on the messenger RNA (mRNA) and overall survival (OS) data of The Cancer Genome Atlas-stomach adenocarcinoma (TCGA-STAD) cohort. A nomogram was established using the signature and clinical factors with a significant linkage with OS and was validated in 3 independent cohorts (GSE15419, GSE13861, and GSE66229) via calculating the area under the receiver operator characteristic curve (AUC). The association between the signature and immunotherapy efficacy was explored in the ERP107734 cohort.

Results: A high risk score was associated with shorter OS in both the training and the validation sets (the AUC for 1-, 3-,5-year in TCGA-STAD cohort: 0.691, 0.644, and 0.707; GSE15459: 0.602, 0.602, and 0.650; GSE13861: 0.648, 0.611, and 0.647; GSE66229: 0.661, 0.630, and 0.610). Its prognostic power was improved by combining clinical factors including age, sex, and tumor stage (the AUC for 1-, 3-,5-year in TCGA-STAD cohort: 0.759, 0.706, and 0.742; GSE15459: 0.773, 0.786, and 0.803; GSE13861: 0.749, 0.881, and 0.795; GSE66229: 0.773, 0.735, and 0.722). Moreover, a low risk score was associated with a favorable response to pembrolizumab monotherapy in the advanced setting (AUC=0.755, P=0.010).

Conclusions: In GCs, the inflammatory response-related gene-based signature was related to immunotherapy efficacy, and its risk score plus clinical features yielded robust prognostic power. With prospective validation, this model may improve the management of GC by enabling risk stratification and the prediction of response to immunotherapy. (See page 3, lines 71-98)

Comment 3: Third, in the introduction of the main text, the authors need to briefly review known prognostic biomarkers and predictive models for GC and its treatment response, have comments on their predictive accuracy and predictors used, analyze the limitations of existing models, and explain why the inflammation genes based models can accurately predict the prognosis and treatment response. It is also necessary to emphasize the need to combine biomarkers and clinical factors, since the genes only model is failed in this study.

Reply 3: Thank you for your suggestion. The mRNA-based models for GC prognosis have been described in the introduction (See page 5, lines 137-142), and we further added limitations of these models to the manuscript, including their unsatisfactory efficiency, insufficient evidence for clinical application by validation in limited populations, and the high cost due to a large number of features involved in the model. researches exploring the potential of other clinicopathologic features beyond tumor stage as prognostic factors.

The importance of inflammatory response in tumors was described, together with previous studies exploring the role of hematological inflammatory parameters in GC prognosis (See page 6, lines 157-161). Besides, we have further added the context regarding the prognostic value of a novel systemic immune-inflammatory index in GC. Taken together, the inflammatory-related genes are of great potential to predict the prognosis and treatment response accurately.

To emphasize the need to combine biomarkers and clinical factors, we added context regarding the advantages of nomograms, together with previously published nomogram models in GC prognosis.

Changes in the text: We have modified our text as advised to as follows:

In addition to tumor stage, previous studies have attempted to explore the potential of other clinicopathologic features as prognostic factors, although the predictive efficacies were unsatisfactory in GC patients, including the histologic grade (PMID: 11013353), abnormal tumor markers (PMID: 37007099), and lymph node invasion (PMID: 36999785, 37007147). (See page 5, lines 124-128).

The unsatisfactory predictive accuracies, the prognostic value validated in limited populations, and the high cost of tests owing to a large number of features involved in the model have limited their clinical applications. (See page 5, lines 142-145).

Prognostic nomograms are pictorial and quantitative models with high precision and predicting capacity, which have been established in clinical practice to assess cancer survival, including GC (PMID: 37007147, 36994190). By considering crucial prognostic indicators, nomograms can more correctly estimate survival for individual patients than the AJCC staging method. (PMID: 29759555). (See page 5, lines 147-151).

Besides, the systemic immune-inflammatory index, which consists of lymphocytes, neutrophils, and platelets (platelets × neutrophils/lymphocytes), was identified as an effective prognostic signature in GC by various studies and meta-analysis (PMID: 34012630), suggesting the potential inflammatory-related mechanisms are of great potential to predict the GC prognosis and treatment response accurately. (See page 6, lines 157-161).

Comment 4: Fourth, in the methodology of the main text, the authors need to describe the identification of clinical predictors, describe the clinical factors and prognosis outcomes in the datasets, ensure P<0.05 is two-sided, and provide the threshold AUC values for a good predictive model.

Reply 4: Thank you for your suggestion. The methodology of the main text has been revised with the following context added: 1) the identification of clinical predictors; 2) the clinical factors and prognosis outcomes provided in the training and validation datasets; 3) two-sided P<0.05 for statistically significance; 4) AUC >0.70 for acceptable predictive efficacy.

Changes in the text: Several context has been added to the Methods section as follows:

Overall survival (OS) was reported as the prognostic outcomes in the training and validation datasets. Clinical features including age, sex, tumor stage, EBV infection status, microsatellite instability (MSI) status, TP53 mutation, H. pylori infection status, radiation therapy, and race were described in the TCGA-STAD cohort, while the other 3 validation cohorts provided only the age, sex, and tumor stage information. (See page 7, lines 206-211).

Univariable and multivariable analyses were implemented to evaluate the independence of the risk score and identify clinical features for nomogram development. The common clinical factors identified in the univariable analysis across the training and validation sets were involved in the construction and validation of the nomogram. (See page 8, lines 238-241).

A two-sided P value less than 0.05 was considered statistically significant unless otherwise specified. A predictive model with an AUC value >0.70 was considered as a good predictive efficacy. (See page 9, lines 277-279)

Reviewer B

The paper titled "Implications of inflammatory response-related genes in predicting prognosis and treatment response in gastric cancer" is interesting. The study provides a novel method for estimating the prognosis of patients with gastric cancer and may have the potential to predict the clinical benefit of targeted agents and immunotherapy. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: What is the relationship between the polymorphism of inflammatory response-related genes and the risk of gastric cancer? It is suggested to add relevant contents.

Reply 1: Thank you for your suggestion. We agree that the relationship between the polymorphisms of inflammatory response-related genes and the risk of gastric cancer

is of great importance for better interpretation of our results. Among the 11 key inflammatory-related genes involved in our model, previously published studies have reported an increased risk of GC in individuals with *SERPINE1* rs2227692 C>T, *TNFAIP2* rs8126 T>C, and *DNMT1* rs2228612 A>G polymorphisms, while there are hardly any researches reporting the association of GC risk with polymorphisms in other inflammatory-related genes. These contents have been summarized in the discussion section, together with recommendations for further research.

Changes in the text: We added contents accordingly in the discussion section regarding the inflammatory-related gene polymorphisms and gastric cancer risk as follows:

In addition to the association between GC prognosis and mRNA levels of inflammatory-related genes, previous studies have also explored the role of gene polymorphisms in GC. For instance, the rs2227692 C>T polymorphism in *SERPINE1* intron affecting gene expression is associated with diffuse-type gastric cancer susceptibility (PMID: 20549826). The *TNFAIP2* 3' UTR rs8126 T>C polymorphism, which might affect TNFAIP2 protein expression, is associated with GC risk in the Chinese population, especially in cases with males aged 60 years or older, *H. pylori*-negative, non-smoking and non-drinking individuals (PMID: 32793480). Besides, several researches and meta-analyses have also demonstrated the association between the *DNMT1* rs2228612 A>G polymorphism and GC risk (PMID: 27789275, 28473984, 31516756). These results emphasized the potential roles of inflammatory-related gene polymorphisms in GC, and the associations of polymorphisms of *CREB3L3*, *ADAMTS12*, *APOD*, *GFRA1*, *KIT*, *ZFP36*, *APOA1*, and *PVT1* with GC risk are of great potential for further researches. (see Page 15, lines 476-488).

Comment 3: The letter G appears in Figure 4E of this study, and the figures in Supplementary Figure 1 are skewed. The author is requested to carefully review the figures and make corrections.

Reply 4: Thank you for your suggestion. Figure 4 and Supplementary Figure 1 have been revised. The other figures have been carefully reviewed to ensure quality.

Changes in the text: We have deleted the letter G that appears in Figure 4, and the subfigures in Supplementary Figure 1 are aligned. (See revised Figure 4 and revised Supplementary Figure 1).

Comment 4: What are the biggest advantages and disadvantages of this model? What is the next research plan? It is recommended to add relevant content to the discussion.

Reply 4: Thank you for your question. One of the advantages is the wide utility of this model in different populations since it has been constructed and validated in 4 independent cohorts. Besides, the measurement of mRNA levels of 11 genes was relatively economic, and together with the easily accessible clinical information, this

model is of great potential for clinical practice. The disadvantages include the potential bias introduced by the retrospective design of the study, the lack of experimental validation of the results, and insufficient evidence regardless of the complex tumor microenvironment and multiple driving factors of the GC prognosis. Further researches are recommended with prospective cohorts, explanatory experiments, and multi-omic data in GC. The disadvantages and research plans for the future were described in the discussion section. The advantages of our model have been added in the revised manuscript.

Changes in the text:

We added contents accordingly in the discussion section regarding the advantages of our model as follows:

Constructed and validated in 4 independent cohorts, our model exhibited wide utility in different populations. Besides, the measurement of mRNA levels of 11 genes was relatively economic, and together with the easily accessible clinical information, this model is of great potential for clinical practice. However, some limitations to this study should also be mentioned. (See page 16, lines 519-522)

Comment 5: All figures are not clear enough. It is recommended to provide clearer figures again.

Reply 4: Thank you for your suggestion. The figures presented in the Word file of the manuscript are only demos. JPG files with high resolution were provided as separate files.

Changes in the text: The clearer figures have been provided as separate files with high resolution. Please kindly refer to the separate JPG files (See Figure 1-5 and Supplementary Figure 1-5).

Comment 6: How to determine the molecular subtypes related to inflammation based on the results of this study? If we can clarify this aspect, it may make the entire study more complete.

Reply 4: Thank you for your suggestion. We totally agree that the inflammation-related molecular subtypes of GC are of great importance for better interpretation of our results. There were four molecular subtypes of gastric cancer uncovered by The Cancer Genome Atlas (TCGA) project: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN, PMID: 25079317). Our results have compared the distribution of inflammation-related risk scores among the four subtypes in Supplementary Figure S4F. Compared to the CIN and GS subtypes, the EBV and MSI subtypes obtained lower risk scores, which were associated with improved prognosis. These results coincide with previously published studies reporting the worst prognosis in the GS subtype and the best prognosis in the EBV subtype (PMID: 28747339). Besides, the lower risk scores in the EBV and MSI subtypes were

associated with lower densities of immunosuppressive tumor-infiltrating immune cells (Supplementary Figure S5), lower levels of TGF-beta response (Supplementary Figure S4I), and response to pembrolizumab (Figure 5C), suggesting the potential of immunotherapies in EBV and MSI subtypes of GC with lower risk scores. Taken together, hopefully, these results can explain preferably the association between the risk score and the GC molecular subtypes, which may provide evidence for further research into the inflammatory features in GC molecular subtypes.

Changes in the text: We have added these contexts to the discussion section as follows:

The Cancer Genome Atlas (TCGA) project has uncovered four molecular subtypes of gastric cancer: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN). Based on our results, the EBV and MSI subtypes obtained lower risk scores compared to the CIN and GS subtypes (Figure S4F), which was associated with improved prognosis. These results coincide with previously published studies reporting the worst prognosis in the GS subtype and the best prognosis in the EBV subtype. Besides, the lower risk scores in the EBV and MSI subtypes were associated with lower densities of immunosuppressive tumor-infiltrating immune cells, lower levels of TGF-beta response, and response to pembrolizumab, suggesting the potential of immunotherapies in EBV and MSI subtypes of GC with lower risk scores. Taken together, hopefully, these results can explain preferably the association between the risk score and the GC molecular subtypes, which may provide evidence for further research into the inflammatory features in GC molecular subtypes. (See page 16, lines 506-518).

Comment 7: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Prognostic value of systemic immune-inflammatory index in survival outcome in gastric cancer: a meta-analysis, J Gastrointest Oncol, PMID:34012630". It is recommended to quote this article.

Reply 4: Thank you for your suggestion. This article has been added to the reference.

Changes in the text: We have added the following context:

Besides, the systemic immune-inflammatory index, which consists of lymphocytes, neutrophils, and platelets (platelets × neutrophils/lymphocytes), was identified as an effective prognostic signature in GC by various studies and meta-analysis (PMID: 34012630). (See page 6, lines 157-160).

Comment 8: How to regulate inflammatory response to improve the efficacy of gastric cancer treatment? What are the intervention strategies for targeted regulation of inflammatory response in the treatment of gastric cancer? It is suggested to add relevant contents.

Reply 4: Thank you for your suggestion. We totally agree that the inflammation regulation can strengthen the significance of our study. We have added the relevant contents to the discussion section, which include the “double-edged sword” feature of inflammation regulation in cancer treatment, anti-inflammation agents in other cancers and attempts in GC treatment, and additional considerations of inflammation regulation in cancer management. Last but not the least, inflammation regulation is a promising issue for precision medicine, which introduces the topic of our study.

Changes in the text: We have added the following context:

Inflammation, which enables epigenetic alterations and results in the production of growth factors, is a crucial cause of newly emergent tumors and malignant progression. Inflammation-reducing strategies that inhibit either the initiation or propagation of persistent inflammation (eg., anti-infective agents, nonsteroidal anti-inflammatory drugs (NSAIDs), and other inflammation-reducing drugs including statins and metformin) might therefore prevent or delay cancer initiation (47). In gastric cancer, antiviral therapies for Epstein-Barr virus (EBV) and antibacterial therapies for H. pylori are the main anti-inflammatory strategies in GC treatment. Besides, targeting interleukin-6 may overcome stroma-induced resistance to chemotherapy in GC (48). However, some pro-inflammatory cytokines or stimulators (TNF-α, cGAS-STING pathway activators) can promote the infiltration of immune cells into infected tissues and thus significantly improve the efficacy of tumor therapy (49), suggesting that inflammation is a "double-edged sword", making inflammation regulation an important issue to improve the efficacy of cancer therapy (50). Identification of the most critical drivers affecting inflammatory TME tumor, avoidance of the conversion of acute-to-chronic inflammation induced by anticancer therapies, and reduction of the severe inflammatory side effects of anticancer therapies (CAR-T therapy induced inflammatory storm) are important challenges involved in inflammation regulation (50,51). In addition, the different inflammatory responses of cancer patients should be considered in cancer management, and personalized treatment strategies regarding tumor-associated inflammation will help improve anti-cancer efficacy. (See page 14, lines 428-448).