Journal for ImmunoTherapy of Cancer

Intratumoral IL-1R1 expression delineates a distinctive molecular subset with therapeutic resistance in patients with gastric cancer

Puran Zhang,[1] Yun Gu,[1] Hanji Fang,[2] Yifan Cao,[2] Jieti Wang ,[3] Hao Liu,[2] Heng Zhang,[2] He Li,[2] Hongyong He,[2] Ruochen Li ,[2] Chao Lin,[2] Jiejie Xu [1]

To cite: Zhang P, Gu Y, Fang H, *et al*. Intratumoral IL-1R1 expression delineates a distinctive molecular subset with therapeutic resistance in patients with gastric cancer. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004047. doi:10.1136/jitc-2021-004047

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-004047).

PZ, YG and HF contributed equally.

Accepted 03 January 2022

[1]Department of Biochemistry and Molecular Biology, Fudan University School of Basic Medical Sciences, Shanghai, China
[2]Department of General Surgery, Zhongshan Hospital Fudan University, Shanghai, China
[3]Department of Gastric Surgery, Fudan University Shanghai Cancer Center, Shanghai, China

Correspondence to
Dr Ruochen Li;
rcli12@fudan.edu.cn

Dr Chao Lin;
lin.chao@zs-hospital.sh.cn

Professor Jiejie Xu;
jjxufdu@fudan.edu.cn

ABSTRACT

Background With the essential role of interleukin-1 signaling in cancer-related inflammation, IL-1R1, the main receptor for both IL-1α and IL-1β, demonstrated therapeutic potential in several types of cancer, which has been put into clinical trials. However, the expression profile and critical role of IL-1R1 in gastric cancer (GC) remain obscure. This study aimed to investigate the prognostic significance of IL-1R1 expression and its predictive value for chemotherapy and immunotherapy in GC.

Methods The study enrolled three cohorts, consisting of 409 tumor microarray specimens of GC patients from Zhongshan Hospital, 341 transcriptional data from *The Cancer Genome Atlas*, and 45 transcriptional data from patients treated with pembrolizumab. IL-1R1 mRNA expression was directly acquired from public datasets, and we also detected IL-1R1 protein expression on tumor microarray by immunohistochemistry. Finally, the associations of IL-1R1 expression with clinical outcomes, immune contexture, and genomic features were analyzed.

Results High IL-1R1 expression predicted poor prognosis and inferior responsiveness to both 5-fluorouracil-based adjuvant chemotherapy (ACT) and immune checkpoint blockade (ICB). IL-1R1 fostered an immunosuppressive microenvironment characterized by upregulated M2 macrophages and exhausted $CD8^+$ T cells infiltration. Moreover, the expression of IL-1R1 was intrinsically linked to genomic alterations associated with targeted therapies in GC.

Conclusions IL-1R1 served as an independent prognosticator and predictive biomarker for ACT and ICB in GC. Furthermore, IL-1R1 antagonists could be a novel agent alone or combined with current therapeutic strategies in GC.

INTRODUCTION

Gastric cancer (GC) ranks the fifth most frequently diagnosed cancer and the fourth leading cause of cancer-associated mortality worldwide.[1] Though radical gastrectomy is considered the most effective treatment,[2] patients with advanced GC tend to relapse even with surgical interventions. Accordingly, 5-fluorouracil-based adjuvant chemotherapy (ACT) has been widely applied as first-line therapy to reduce postoperative recurrence rate.[3 4] However, a significant fraction of patients failed to gain survival benefit due to acquired chemoresistance.[5 6] Further investigation of novel therapeutic opportunities is urgently needed to prolong survival and reduce drug resistance in GC.

Fortunately, recent advances in immunotherapy, especially immune checkpoint blockade (ICB), have shed light on new strategies for GC treatment.[7] Nevertheless, the current ICB only provides survival benefits for less than 20% of patients with GC. Due to the ineffectiveness of both therapeutic strategies aforementioned in a substantial proportion of GC patients, it is of great clinical significance to investigate emerging biomarkers for further patient stratification and improved treatment tactics.

Prior studies have demonstrated that the efficacy of ACT and ICB is inextricably correlated with the tumor microenvironment (TME), within which the interleukin (IL) family plays dynamic roles in various tumor biological activities.[8 9] Our previous studies have demonstrated that IL-9, IL-10 and IL-17 play critical roles in predicting therapeutic effectiveness.[10–12] As the earliest discovered member of the IL family, IL-1 has been long known for its pleiotropic effects on inflammation, which promotes progression and metastasis in multiple cancers, primarily through the IL-1R signaling pathway.[13–19] IL-1R1, as an essential participant in the IL-1R signaling pathway, is the only receptor that can bind to both agonistic ligands, IL-1α and IL-1β, and subsequently mediates positive signaling transduction via NF-κB and MAP kinase pathways.[20–22] Existing literature has elucidated the potential value of IL-1R1 antagonists and anti-IL-1 monoclonal antibodies for inhibiting

primary tumor growth and reversing the acquired resistance to chemotherapy and ICB in multiple models.[23–27] So far, several clinical trials have been recently carried out for evaluating the therapeutic value of targeting IL-1R1 and the synergetic effect of IL-1R1 antagonists, such as anakinra, with existing therapeutic strategies.[27–29] In GC, studies showed that both IL-1α and IL-1β were correlated with tumor initiation and progression.[30 31] Nevertheless, the prognostic and predictive value of IL-1R1 in GC remains obscure.

Here, we indicated that high IL-1R1 expression predicted poor prognosis and inferior responsiveness to ACT and ICB. Meanwhile, we confirmed that IL-1R1 fostered an immunosuppressive microenvironment featured by upregulated M2 macrophages and exhausted $CD8^+$ T cells infiltration. Moreover, the expression of IL-1R1 was intrinsically related to specific molecular subtypes and genomic alterations in GC. In a word, our study has shed light on the clinical and translational significance of IL-1R1 as a stratification biomarker and potential therapeutic target to facilitate personalized therapy in GC.

PATIENTS AND METHODS

Patients and gastric tissue samples

This study enrolled three independent patient cohorts, as illustrated in online supplemental figure S1. The ZSHS cohort consisted of 496 patients recruited from the Zhongshan Hospital, Fudan University (Shanghai, China). However, 87 of them were excluded due to dot loss, incomplete clinicopathological data, or suffering from metastatic diseases. The remaining 409 patients underwent radical gastrectomy and standard D2 lymphadenectomy between 2007 and 2008. All tumor samples were formalin fixed and paraffin embedded. Patient clinicopathological characteristics, including age, sex, tumor location, tumor size, tumor grade, Lauren classification, T classification, N classification, tumor–node–metastasis (TNM) stage, and application of fluorouracil-based ACT, were retrospectively collected. The TNM stage and T and N classifications were evaluated based on the 2010 International Union Against Cancer TNM staging system.[32] According to National Comprehensive Cancer Network guidelines and patients' will, postoperative ACT was applied to patients with TNM II/III stage GC. The overall survival (OS) and disease-free survival (DFS) were calculated from the day of surgery to the day of death, relapse, or last follow-up. *The Cancer Genome Atlas* (TCGA) cohort recruited 412 patients from TCGA. However, 71 of them were excluded due to incomplete clinicopathological data or suffering from metastatic diseases. The data of the remaining 341 patients were downloaded on August 20, 2020 (http://www.cbioportal.org), and 237 of whom had accessible DFS information. Furthermore, 61 patients treated with pembrolizumab were recruited from *the European Nucleotide Archive* as the ICB cohort. However, 16 of them were excluded due to loss of transcriptional data.[33] The remaining 45 patients with information of drug response were further analyzed, and 43 of whom had accessible clinical information, including OS and progression-free survival (PFS). The clinical data of the ICB cohort were generously provided by the research team of Professor Jeeyun Lee, Division of Hematology-Oncology, Samsung Medical Center.

Immunohistochemistry and evaluation of immunostaining

Prior to immunohistochemistry (IHC), the tissue microarrays (TMAs) were constructed by Shanghai Outdo Biotech Co, Ltd. The protocol of TMA construction and IHC staining has been described detailedly in our previous study.[34 35] The associated antibodies were listed (online supplemental table S1). In our study, all TMA samples were evaluated separately by two independent pathologists (Dr Lingli Chen and Dr Yunyi Kong) who were blinded to the clinicopathological data. Both of them scored independently according to the proportion of stained cells and the cellular staining intensity. Briefly, the proportion of stained cells was defined as the percentage of positive cells, whereas the cellular staining intensity was stratified as 0 (negative staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow-brown), and 3 (strong staining, brown). The mean score of their evaluation was adopted for further analysis. The median value of IL-1R1 IHC score was determined as the cut-off point. The representative images were displayed in online supplemental figure S2. Variations in IL-1R1 IHC score, exceeding 10, were re-evaluated separately by both pathologists to reach a final consensus. The Image-Pro-Plus software V.6.2 was used to further validate the scoring results from two independent pathologists. The processed IHC staining of IL-1R1 with Image-Pro-Plus was displayed in online supplemental figure S3. The IL-1R1 IHC score was closely related to the Image-Pro-Plus mean integrated optical density as demonstrated in online supplemental figure S4.

Statistical analysis

Pearson's χ^2 test and Fisher's exact test were applied to compare categorical variables. Mann-Whitney U test was applied to compare continuous variables. One-way analysis of variance followed by Tukey multiple comparisons test was applied for the correlation between IL-1R1 expression and TNM stages. OS, DFS, and PFS were analyzed by Kaplan-Meier curves, log-rank test, and multivariate analysis based on Cox regression analysis. The cut-off value for the classification of $IL\text{-}1R1^{high}$ and $IL\text{-}1R1^{low}$ subgroups was the median value. All analyses were conducted using IBM SPSS Statistics V.20.0, MedCalc 15.6.1, and R 4.0.2 software. The CIBERSORT algorithm was constructed to calculate the relative proportion of 22 immune cell types with the LM22 expression signature. The single sample gene sets enrichment analysis (ssGSEA) implanted in the 'GSVA' package was implemented to calculate signature scores. The stromal score, immune score, and ESTIMATE score were directly acquired from https://bioinformatics.

mdanderson.org/estimate/index.html to assess the overall stromal and immune content. The differential gene expression analysis was conducted via the 'limma' package. The mutational signatures were calculated with the 'deconstructSigs' package. The copy number variation (CNV) analysis was conducted using GISTIC2.0 software. The statistical analysis was two tailed, and p<0.05 was considered statistically significant.

RESULTS

IL-1R1 expression yields a poor prognosis and is involved in tumor progression in GC

To elucidate the clinical significance of IL-1R1 expression in GC, Kaplan-Meier curves and log-rank test were applied to assess OS and DFS between IL-1R1 high/low subgroups in the ZSHS cohort and TCGA cohort, respectively. In both cohorts, patients with high levels of IL-1R1 expression had significantly worse OS (p<0.001 and p=0.030; Figure 1A,B). However, the association between high levels of IL-1R1 expression and worse DFS was only observed in the Zhongshan Hospital (ZSHS) cohort (p<0.001 and p=0.110; Figure 1A,B). Furthermore, multivariate Cox regression analysis showed IL-1R1 was an independent prognosticator for worse OS and DFS in ZSHS cohort after adjustment for confounders (HR: 2.300, 95% CI 1.684 to 3.141, p<0.001 and HR: 2.463, 95% CI 1.812 to 3.346, p<0.001; figure 1A), and a merely independent prognosticator for worse OS in the TCGA cohort (HR: 1.398, 95% CI 0.964 to 2.029, p=0.078; figure 1B). Moreover, since previous studies have demonstrated that IL-1R1 expression was highly correlated with GC formation,[18 19] we wondered whether the expression of IL-1R1 might differ across TNM stages. Notably, we found that in both the ZSHS cohort and TCGA cohort, TNM stage III patients demonstrated more intensive IL-1R1 expression than TNM stage I patients (online supplemental figure S5A,B). Other clinicopathological characteristics of GC patients with high/low IL-1R1 expression in the ZSHS cohort and TCGA cohort were summarized (online supplemental table S2). Conclusively, these results showed that IL-1R1 serves as an independent adverse prognosticator and might be associated with tumor progression in GC.

IL-1R1 predicts inferior responsiveness to ACT and ICB in GC

Previous studies have demonstrated that the IL-1R signaling pathway was detrimental for 5-fluorouracil-based antitumor efficacy.[23 24] Considering the limited therapeutic response of GC patients to 5-fluorouracil-based ACT,[2 5 6] we wondered if IL-1R1 could be used to select suitable candidates for ACT. In the ZSHS cohort, ACT application predicted significantly better OS, rather than DFS in stage II/III patients (p<0.001 and p=0.230; online supplemental figure S6). However, such overall beneficial effect was only observed in IL-1R1^{low} subgroup after dividing patients based on IL-1R1 expression (p<0.001 and p=0.180; p=0.001 and p=0.110; figure 2A), which suggested that IL-1R1 expression might have a predictive effect on the responsiveness of GC patients to ACT. A test for interaction between IL-1R1 expression and ACT revealed that the therapeutic effectiveness observed in IL-1R1^{high} subgroup was significantly inferior to that in IL-1R1^{low} subgroup (p for interaction=0.001 and p for interaction=0.001; figure 2A).

Furthermore, we enrolled the ICB cohort consisting of patients treated with pembrolizumab to evaluate the predictive value of IL-1R1 for immunotherapy (table 1). We found that patients in IL-1R1^{high} subgroup demonstrated a significantly decreased response rate compared with those in IL-1R1^{low} subgroup (figure 2B). Meanwhile, patients in IL-1R1^{high} subgroup demonstrated a significantly worse OS and PFS compared with those in IL-1R1^{low} subgroup (p=0.027 and p=0.010; figure 2C). Since existing research has demonstrated that CD274 (PD-L1) mRNA expression was correlated with the efficacy of pembrolizumab,[33] we further stratified patients based on PD-L1 mRNA expression within IL-1R1 high/low subgroups. Interestingly, the IL-1R1^{low}/PD-L1^{high} group showed the highest objective response rate (ORR), while the ORR of IL-1R1^{high}/PD-L1^{high} group was the lowest (figure 2D). This result indicated that IL-1R1 could be a crucial factor causing attenuated responsiveness to pembrolizumab, even with high PD-L1 expression. The associations of IL-1R1/PD-L1 expression and molecular parameters were summarized (table 2). Cumulatively, our findings suggested that IL-1R1 could be a potential efficacy predictor for both ACT and ICB in GC.

IL-1R1 fosters an immunosuppressive microenvironment in GC

Prior studies have shown that the IL1-R signaling pathway could mobilize myeloid-derived suppressor cells and tumor-associated macrophages, subsequently fostering an immunosuppressive microenvironment,[16 31 36] which was predominantly relevant to prognosis and responsiveness to chemotherapy and immunotherapy.[8] Thus, we used the CIBERSORT algorithm to calculate the relative proportion of 22 human hematopoietic cell phenotypes (LM22) within the TCGA database. We found that memory B cells, monocytes, M0 macrophages, M2 macrophages, and Mast cells resting were significantly enriched in IL-1R1^{high} subgroup, whereas the enrichment of T cells follicular helper, regulatory T cells, NK cells resting, and Mast cells activated was observed in IL-1R1^{low} subgroup. We also noticed that the overall immune and stromal content were significantly increased in IL-1R1^{high} subgroup (figure 3A). To validate the result from the CIBERSORT algorithm, we evaluated the related immune cells infiltration based on IL-1R1 expression in the ZSHS cohort. Notably, only M2 macrophages demonstrated elevated infiltration in IL-1R1^{high} subgroup, which was consistent with the result from the CIBERSORT algorithm (figure 3B and online supplemental figure S7). Then, to further explore the relationship between IL-1R1 expression and M2 macrophages infiltration, we confirmed that

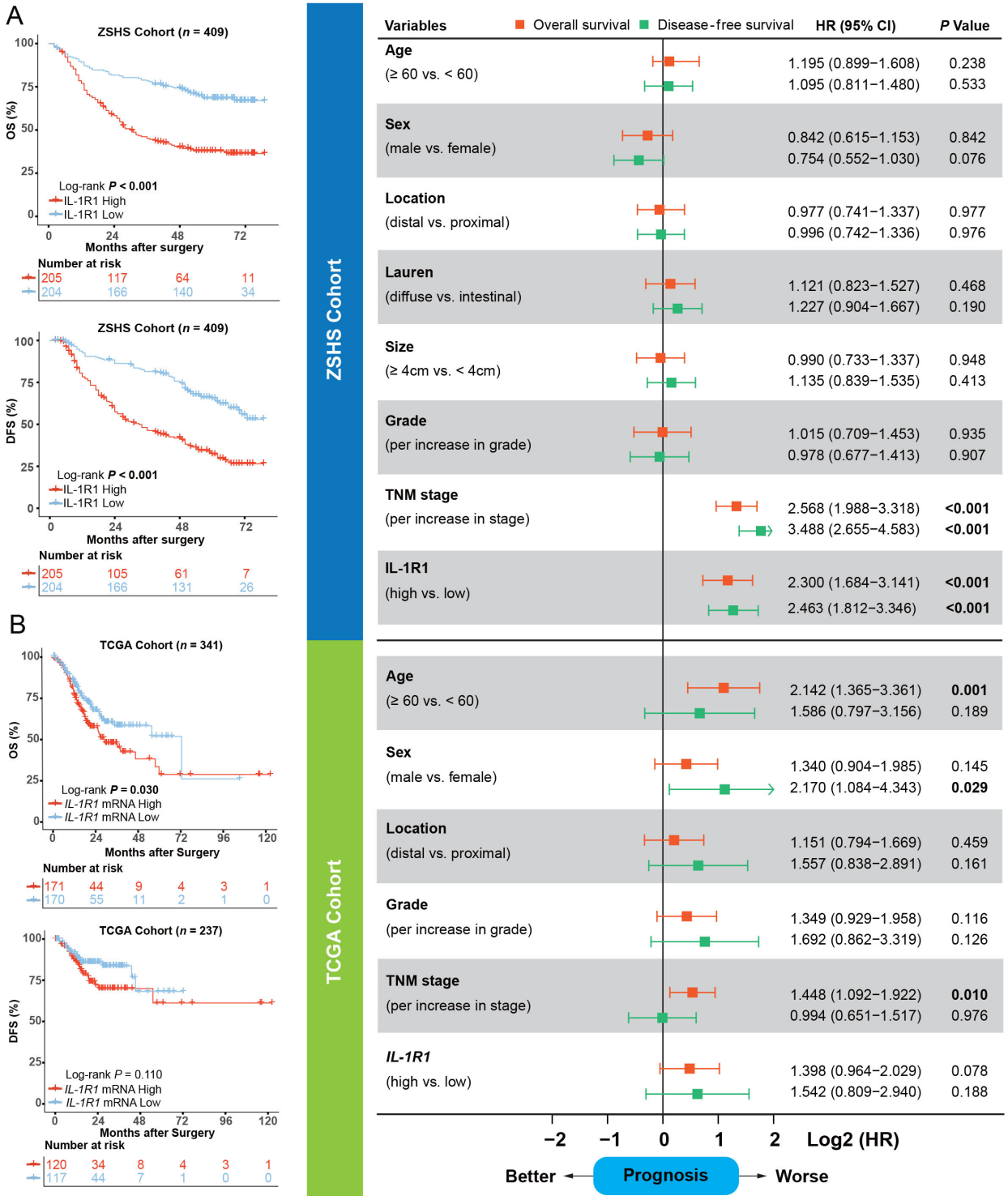

Figure 1 IL-1R1 yields a poor prognosis in patients with gastric cancer. (A–B) Kaplan-Meier curves and multivariate Cox regression analysis of overall survival (OS) and disease-free survival (DFS) based on IL-1R1 expression in the Zhongshan Hospital (ZSHS) cohort (n=409) (A) and the Cancer Genome Atlas (TCGA) cohort (n=341) (B) (log-rank test). P<0.05 marked in bold font shows statistical significance. IL, interleukin.

both M2 macrophages recruitment and activation associated genes, including CCL2, CSF1R, and IL6ST, and signatures including angiogenesis, hypoxia, EMT, IL6, TGF-β, and IL10 pathway were significantly upregulated in IL-1R1^{high} subgroup through differential gene expression analysis and ssGSEA (online supplemental figure S8A,C and table S3). However, Kaplan-Meier curves showed that there were no significant differences of OS and DFS based on CD163^{+} M2 macrophages infiltration within IL-1R1 high/low subgroups (p=0.990 and p=0.170; p=0.500 and p=0.280; online supplemental figure S8B). Furthermore, we explored the expression of immune

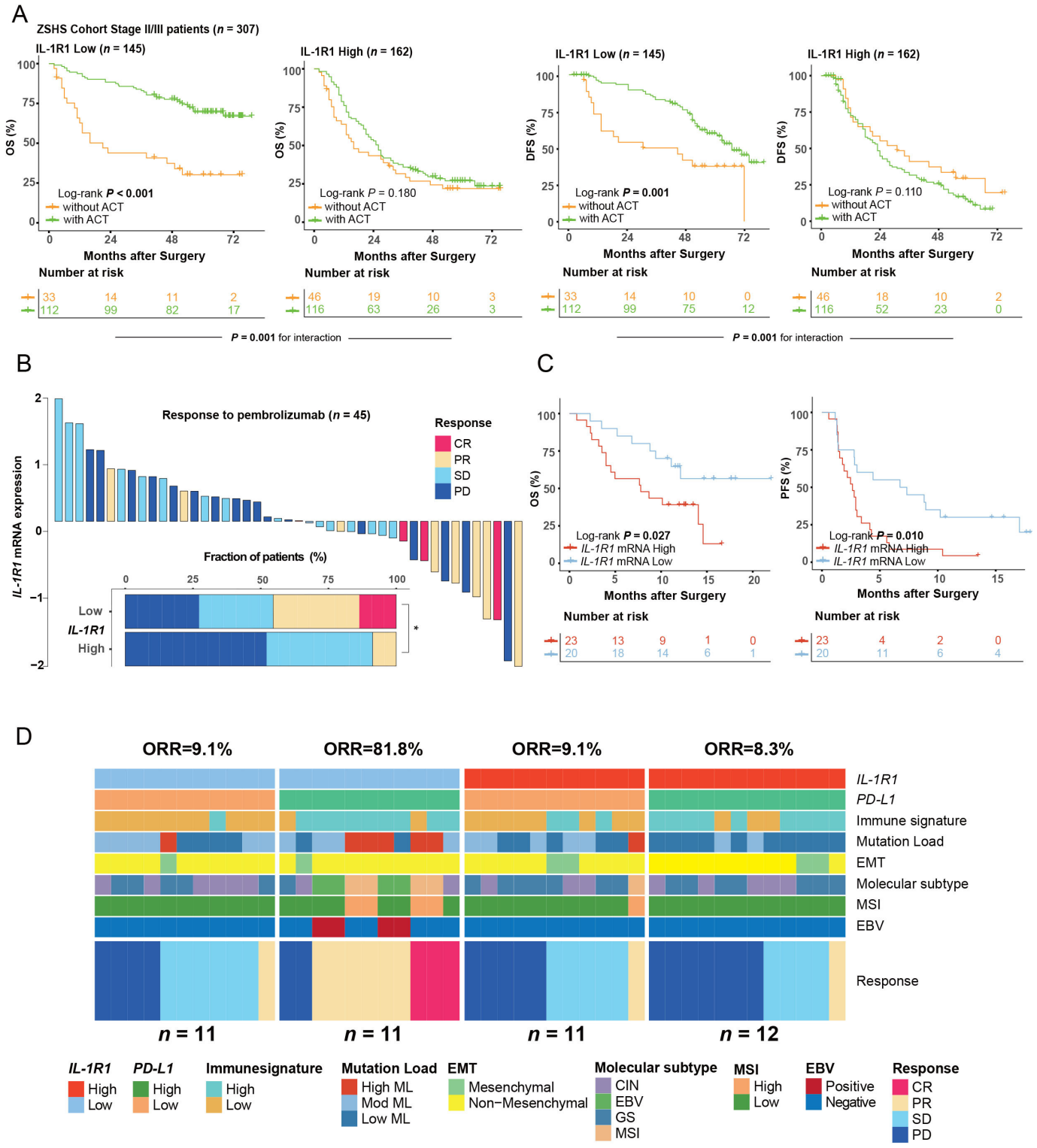

Figure 2 IL-1R1 expression predicts inferior responsiveness to 5-fluorouracil-based ACT and ICB in gastric cancer. (A) For stage II/III patients in the ZSHS cohort (n=307), Kaplan-Meier curves demonstrated responsiveness to 5-fluorouracil-based ACT in patients stratified by IL-1R1 expression. (B) The waterfall plot and stacked bar plot demonstrated responsiveness to pembrolizumab based on IL-1R1 expression in the ICB cohort (n=45). (Pearson's χ^2 test). (C) Kaplan-Meier curves of overall survival (OS) and progression-free survival (PFS) based on IL-1R1 expression in the ICB cohort (n=43). (D) Heatmap demonstrated responsiveness to pembrolizumab and molecular parameters based on PD-L1 mRNA expression within IL-1R1 high/low subgroups in the ICB cohort (n=45). ICB, immune checkpoint blockade. P<0.05 marked in bold font shows statistical significance. ACT, adjuvant chemotherapy; CIN, chromosomal instability; CR, complete response; EBV, EBV positive; EMT, epithelial-mesenchymal transition; GS, genomically stable; ICB, immune checkpoint blockade; ML, mutation load; MSI, microsatellite instability; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; ZSHS, Zhongshan Hospital.

Table 1 Objective patients' response to pembrolizumab

	All patients (n=45)		IL1R1^{high} (n=23)		IL1R1^{low} (n=22)	
Best overall response	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**
Objective response (CR+PR)	12	26.7 (15 to 45)	2	8.7 (1 to 28)	10	45.5 (24 to 68)
Disease control						
CR	3	6.7 (2 to 18)	0	0 (0 to 15)	3	13.6 (3 to 35)
PR	9	20.0 (9 to 34)	2	8.7 (1 to 28)	7	31.8 (14 to 55)
SD	15	33.3 (20 to 49)	9	39.1 (20 to 62)	6	27.3 (11 to 50)
PD	18	40.0 (26 to 56)	12	52.2 (31 to 73)	6	27.3 (11 to 50)

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 2 Association between IL1R1/CD274 (PD-L1) expression and molecular parameters

Factors	IL1R1^{hi} CD274^{hi}	IL1R1^{lo} CD274^{hi}	IL1R1^{hi} CD274^{lo}	IL1R1^{lo} CD274^{lo}	P value
All patients	12	11	11	11	
Immune signature					**0.001**
High	9	9	3	1	
Low	3	2	8	10	
Mutation load					**0.016**
High	0	5	1	1	
Moderate	2	4	5	6	
Low	10	2	5	4	
EMT status					1.000
Mesenchymal	2	1	2	1	
Non-mesenchymal	10	10	9	10	
Molecular subtype					**0.002**
CIN	5	2	3	6	
EBV	0	4	0	0	
GS	7	1	7	5	
MSI	0	4	1	0	
MSI status					**0.020**
High	0	4	1	0	
Low	12	7	10	11	
EBV status					**0.007**
Positive	0	4	0	0	
Negative	12	7	11	11	
Response					**0.003**
PD	7	2	5	4	
SD	4	0	5	6	
PR	1	6	1	1	
CR	0	3	0	0	

P<0.05 marked in bold font shows statistical significance.
CIN, chromosomal instability; CR, complete response; EBV, EBV positive; EMT, epithelial-mesenchymal transition; GS, genomically stable; hi, high; lo, low; MSI, microsatellite instability; PD, progressive disease; PR, partial response; SD, stable disease.

checkpoints and inhibitory molecules in IL-1R1 high/low subgroups within the TCGA database. We found significantly higher expression of PDCD1, CTLA4, HAVCR2, LAG3, TIGIT, TGFB1, IL10, and PDCD1LG2 in IL-1R1^{high} subgroup (figure 3A). To validate this result, we used IHC staining and found that the expression of IL10, TGF-β, and LAG3 were elevated in IL-1R1^{high} subgroup, which was consistent with the result from the TCGA database (figure 3B). Conclusively, we inferred that IL-1R1 might foster an immunosuppressive microenvironment via mobilizing M2 macrophages infiltration and inducing the elevated expression of multiple immune checkpoints and inhibitory molecules.

Characterization of CD8$^+$ T cells functions based on IL-1R1 expression in GC

As is known to all, CD8$^+$ T cells are regarded as the primary effector cells in antitumor immunity.[37] However, no significant difference in the quantity of CD8$^+$ T cells was observed between IL-1R1 high/low subgroups. We wondered whether IL-1R1 expression was correlated with the functional status of CD8$^+$ T cells. Through GSEA, we found that exhausted CD8$^+$ T cells signatures were upregulated in IL-1R1^{high} subgroups (figure 3C). Additionally, Kaplan-Meier curves showed that CD8$^+$ T cells infiltration predicted improved OS and DFS only in IL-1R1^{low} subgroup, rather than IL-1R1^{high} subgroup (p=0.005 and p=0.280; p<0.001 and p=0.290; figure 3D). Since CD8$^+$ T cells infiltration failed to serve as a prognosticator in IL-1R1^{high} subgroup, we further trichotomized patients into various risk subgroups, defined as the low-risk group (IL-1R1^{low} CD8$^+$ T cells^{high}), intermediate-risk group (IL-1R1^{low} CD8$^+$ T cells^{low}), and high-risk group (IL-1R1^{high}). Consistent with our hypothesis, the low-risk group demonstrated the most optimal prognosis, whereas the high-risk group demonstrated the worst prognosis regarding OS and DFS (online supplemental figure S9A,C). Furthermore, the results of Cox regression analysis showed that our novel risk stratification model could be used as an independent prognosticator regarding OS and DFS (online supplemental figure S9B,D). Moreover, we sought to evaluate whether various risk subgroups indicated distinct chemotherapeutic responsiveness in stage II/III GC patients. Cox regression analysis was applied,

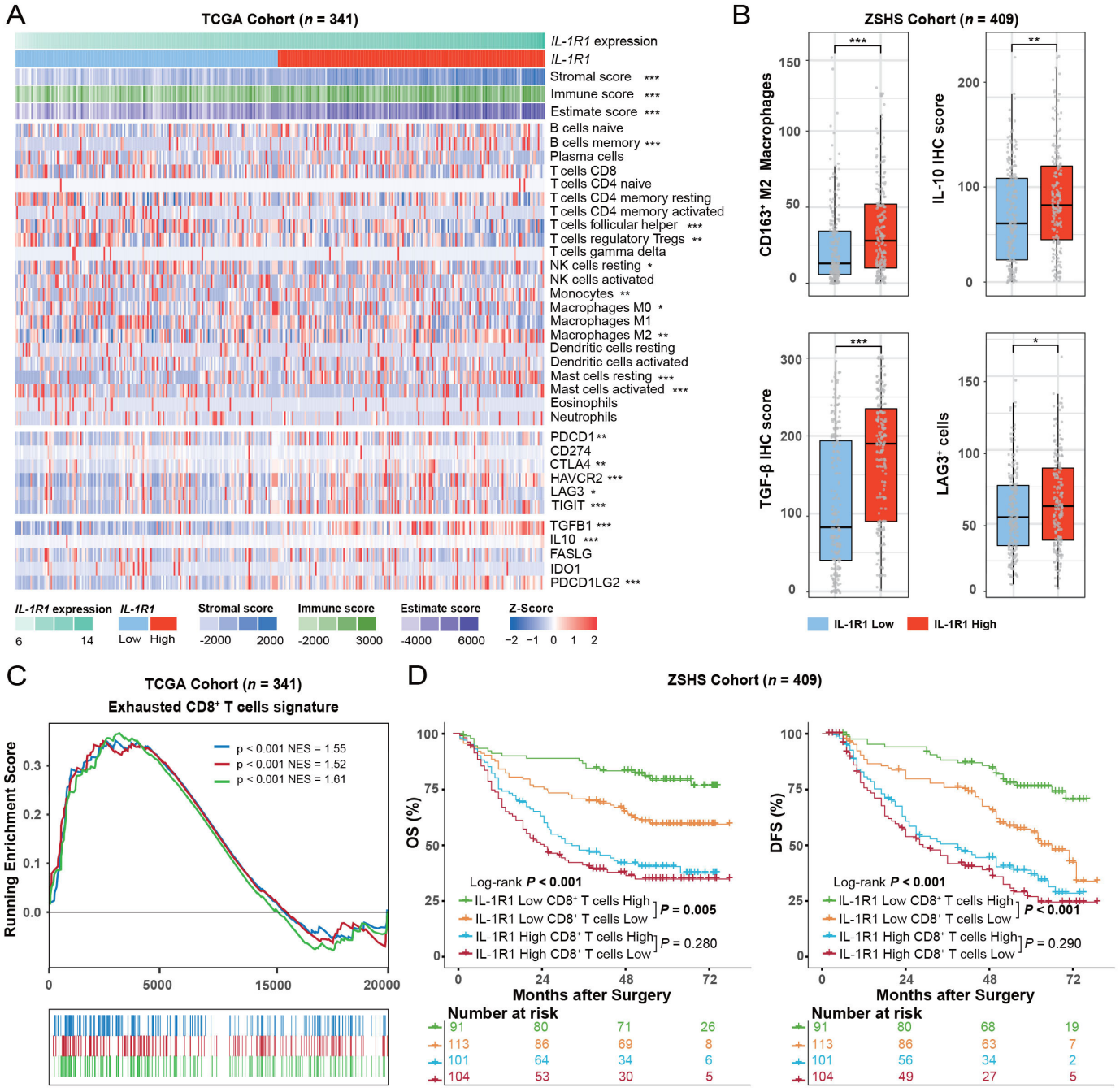

Figure 3 IL-1R1 indicates an immunosuppressive microenvironment and dysfunctional CD8+ T cells phenotype in gastric cancer. (A) Heat map demonstrated the comprehensive immune landscape containing estimate score, 22 types of immune cells generated by the CIBERSORT algorithm, immune checkpoints, and inhibitory molecules in the TCGA cohort (n=341) (Mann-Whitney U test). (B) IHC staining of significant immune cells, immune checkpoints, and inhibitory molecules in the Zhongshan Hospital (ZSHS) cohort (n=409) (Mann-Whitney U test). (C) GSEA analysis indicated an enrichment of exhausted CD8+ T cells genes in IL-1R1^{high} subgroup in the Cancer Genome Atlas (TCGA) cohort (n=341). (D) Kaplan-Meier curves of overall survival (OS) and disease-free survival (DFS) based on CD8+ T cells infiltration in IL-1R1 high/low subgroups in the ZSHS cohort (n=409). *P<0.05, **p<0.01, ***p<0.001. P<0.05 marked in bold font shows statistical significance. IHC, immunohistochemistry; IL, interleukin; NES, Normalized Enrichment Score.

and the results implied that higher risk subgroups might have attenuated responsiveness to 5-fluorouracil-based ACT (online supplemental figure S9E). Conclusively, our findings implied that IL-1R1 might impede the antitumor immunity of CD8+ T cells via shaping a dysfunctional phenotype.

Characteristics of IL-1R1 mRNA expression across molecular subtypes and targetable genomic alterations in GC

Since the progressive gene alterations accumulated throughout life are considered a driving force of cancer,[38] we next investigated the differential distributions of somatic gene mutations and GC molecular subtypes

between IL-1R1 high/low subgroups. To profile a comprehensive landscape of genomic features associated with IL-1R1 expression, we delineated the top 10 gene mutations within GC (figure 4A). Among the top 10 mutated genes, the mutational frequencies of TTN, MUC16, ARID1A, LRP1B, CSMD3, FAT4, FLG, and PCLO were significantly decreased in IL-1R1^{high} subgroup, along with tumor mutational burden (figure 4A). To further elucidate whether the inferior prognostic merit of IL-1R1 was correlated with certain genomic features among the top 10 mutated genes, we used Cox regression analysis and found that only in TTN, TP53, and CSMD3 mutation subgroup and SYNE1, FLG, and PCLO wildtype subgroup can IL-1R1 expression act as a prognosticator for worse OS (online supplemental figure S10). Since growing evidence has revealed the molecular subtypes of GC as a novel avenue for precision therapy and patient stratification,[39] we subsequently explored the distribution of different molecular subtypes between IL-1R1 high/low subgroups. Notably, within the IL-1R1^{high} subgroup, the proportion of the genomically stable subtype was significantly higher, while the proportion of the microsatellite instability (MSI) subtype was significantly lower than that of the IL-1R1^{low} subgroup (figure 4B). Recently, advances in cancer biology have enabled patient selection for targeted precision therapy.[40] Here, based on our findings that certain genomic features demonstrated different patterns between IL-1R1 high/low subgroups, we wondered if IL-1R1 expression was associated with potential therapeutic targets in GC. Thus, we further delineated a comprehensive landscape of genomic features associated with multiple targeted therapies evaluated in clinical trials.[40] First and foremost, we used the COSMIC mutational signatures related to the APOBEC family and DNA damage repair[41] and found that only signature 6, which represented mutational patterns of mismatch repair, demonstrated decreased occurrence in IL-1R1^{high} subgroup (figure 4C). The gene mutation analysis showed a decreased mutational frequency of PIK3CA and KRAS in IL-1R1^{high} subgroup (figure 4C). However, no significant differences were observed in CNV between IL-1R1 high/low subgroups (figure 4C). At the transcriptional level, we found that ERBB, EGFR, and VEGF signaling pathways were significantly upregulated in IL-1R1^{high} subgroup, whereas the homologous recombination repair pathway was significantly downregulated (figure 4C). Conclusively, our findings implied that IL-1R1 might be applied to optimize patient selection and as a potential target to improve the efficacy of current targeted therapies in GC.

DISCUSSION

TME represents a pivotal component of cancer,[8] and inflammation is a crucial component of TME.[42] Tumor-promoting inflammation is mainly orchestrated by multiple inflammatory cytokines and chemokines.[43] As a prototypic inflammatory cytokine, IL-1 is involved in a complex cascade that serves an essential role in the initiation and regulation of innate and adaptive immunity.[42 43] Existing literature has elucidated that the IL-1R signaling pathway shapes an immunosuppressive TME primarily through the mobilization and activation of myeloid-derived suppressor cells and tumor-associated macrophages.[16 31 36] In this study, we verified that GC patients with more intensive IL-1R1 expression exhibited inferior OS and DFS. We also found that IL-1R1 expression was positively associated with M2 macrophages and exhausted CD8$^+$ T cells infiltration, highlighting the significance of IL-1R1 as a potent TME modifier in GC. Since IL-1R1 was also expressed in tumor cells (online supplemental figure S2), we believed that the IL-1R signaling pathway might be related to specific biological properties of GC. Herein, we discovered that TNM stage III tumors demonstrated more intensive IL-1R1 expression than stage I tumors. Moreover, tumors with high levels of IL-1R1 expression tended to undergo epithelial-mesenchymal transition. Previous studies have revealed that the IL-1R signaling pathway was involved in the induction of EMT phenotype in an NF-κB/AKT/Wnt-dependent manner.[44] These results indicated that the IL-1R signaling pathway might be associated with the intrinsic aggressiveness of GC. Furthermore, we found that IL-1R1 expression was associated with particular genotypes, especially loss of MSI status and increased genomic stability. This implied that the IL-1R signaling pathway might affect or be affected by specific molecular properties of GC.

Since chemotherapeutic agents also harness the host's immune system in addition to their direct cytotoxic effects,[45] altered TME via IL-1R1 might blunt its antitumor activity. Prior studies have revealed that 5-fluorouracil triggered activation of inflammasomes in myeloid-derived suppressor cells leading to the production of IL-1β was a crucial mechanism of chemoresistance.[23 24] Consistent with these theories, our study revealed that patients with more intensive IL-1R1 expression exhibited attenuated responsiveness to 5-fluorouracil-based chemotherapy in GC, highlighting the potential value of IL-1R1 for patient stratification. Moreover, since multiple clinical trials have been carried out to evaluate the therapeutic value of IL-1R1 antagonists alone or in combination with existing chemotherapeutic agents in a large variety of cancers,[27 28] IL-1R1 blockade might be available as a novel tactic for GC treatment in the near future.

ICB, which reactivates tumoricidal T cells via the PD-1/PD-L1 axis, has emerged as a novel and promising therapeutic strategy to eradicate cancer cells.[46] Nevertheless, the ORR remains unsatisfying, especially in GC.[47] Recently, multiple preclinical models have been carried out to evaluate the synergetic effect of anti-IL-1 mAbs with ICB.[25 26] For instance, in triple-negative breast cancer, treatment with anti-IL-1β mAbs significantly potentiated anti-PD-1 therapy.[25] Remarkably, our study revealed that compared with responders to pembrolizumab in GC, non-responders demonstrated more intensive IL-1R1 expression, indicating IL-1R1 expression could be used

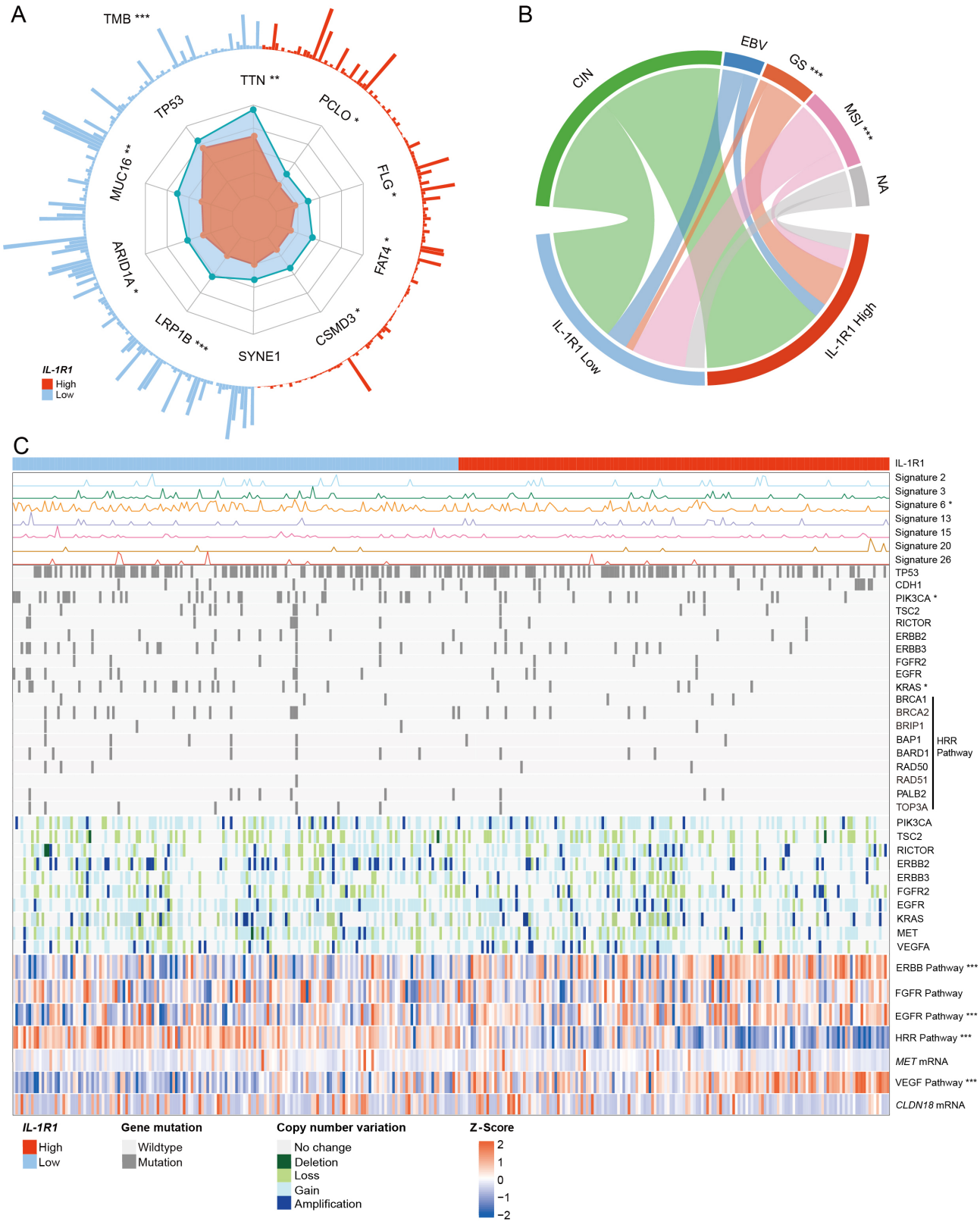

Figure 4 Characteristics of IL-1R1 mRNA expression across molecular subtypes and targetable genomic alterations in gastric cancer. (A) The radar chart demonstrated the distribution of the mutational frequencies of the top 10 mutated genes and tumor mutational burden based on IL-1R1 expression (Pearson's χ^2 test and Mann-Whitney U test). (B) Chord diagram demonstrated the distribution of different GC molecular subtypes based on IL-1R1 expression (Pearson's χ two test). (C) Heatmap demonstrated the genomic alterations of potential therapeutic targets in gastric cancer based on IL-1R1 expression (Pearson's χ^2 test and Mann-Whitney U test). *P<0.05, **p<0.01, ***p<0.001. CIN, chromosomal instability; EBV, EBV-positive; GS, genomically stable; HRR, homologous recombination repair; MSI, microsatellite instability; NA, not available; TMB, tumor mutational burden.

as a stratification biomarker for anti-PD-1 therapy. Moreover, since the success of anti-IL-1 mAbs with ICB in multiple models aforementioned, IL-1R1 might also be a potential target for evaluation to complement existing ICB strategies in GC.

Advances in sequencing technology have broadened the horizon of understanding the tumor biological properties and therapeutic guidance.[48] The development of emerging therapeutic strategies such as targeted therapy has enabled personalized precision treatment of multiple solid tumors.[49] Nevertheless, only three molecular biomarkers have been identified to predict response to novel therapies in GC.[40] Recently, in a biomarker-guided trial, VIKTORY, patients with GC who were assigned to different groups based on eight biomarkers (RAS aberrations, TP53 mutations, PIK3CA mutations and/or amplification, MET amplification, MET overexpression, all negative, TSC2 deficiency, or RICTOR amplification) demonstrated significantly prolonged OS and PFS, highlighting the essential role of biomarker-guided targeted therapy for GC patients.[50] In this study, we found that compared with IL-1R1^{low} subgroup, IL-1R1^{high} subgroup demonstrated significantly decreased mutational frequency of several targetable genes, whereas several targetable pathways were significantly upregulated, suggesting the potential possibility of using IL-1R1 as a novel companion stratification biomarker for targeted precision therapy in GC.

Considering the retrospective design of our study, further validation is required to confirm our results within the framework of more extensive, multicentered clinical trials.

In conclusion, our study demonstrated that IL-1R1 was an adverse independent prognosticator and yielded inferior responsiveness to both ACT and ICB in GC. Furthermore, IL-1R1 fostered an immunosuppressive TME and was associated with certain genomic features. Moreover, IL-1R antagonists, such as anakinra, might be applied alone or as complementary therapy to reinvigorate ACT and ICB in GC.

Acknowledgements We would like to thank Dr Lingli Chen (Department of Pathology, Zhongshan Hospital, Fudan University, Shanghai, China) and Dr Yunyi Kong (Department of Pathology, Shanghai Cancer Center, Fudan University, Shanghai, China) for their excellent pathological technology help.

Contributors PZ, YG and HF for acquisition of data, analysis and interpretation of data, statistical analysis and drafting of the manuscript; YC, JW, HL, HZ, HL and HH for technical and material support; RL, CL and JX for study concept and design, analysis and interpretation of data, drafting of the manuscript, obtained funding and study supervision. JX acts as the guarantor for the overall content. All authors read and approved the final manuscript.

Funding This study was funded by grants from National Natural Science Foundation of China (31770851, 81871930, 81902402, 81902901, 81972219, 82003019, 82103313) and Shanghai Sailing Program (18YF1404600, 19YF1407500, 21YF1407600).

Disclaimer All the sponsors have no roles in the study design, in the collection, analysis and interpretation of data.

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval Our study followed the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University, Shanghai. Signed informed consent was obtained from each patient. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iDs
Jieti Wang http://orcid.org/0000-0002-7251-4225
Ruochen Li http://orcid.org/0000-0003-4013-3281
Jiejie Xu http://orcid.org/0000-0001-7431-9063

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