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RESEARCH ARTICLE

CXCL9/10/11 as prognostic indicators for immunotherapy response in gastric cancer

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Abstract

Objective: Gastric cancer remains a major cause of cancer related mortality and only a group of patients benefits from immune checkpoint inhibitors. This study aimed to evaluate the expression profiles, immune associations, prognostic value, and predictive potential for immunotherapy responsiveness of CXCL9, CXCL10, CXCL11, and CXCR3.

Materials and Methods: Gene expression levels of CXCL9, CXCL10, CXCL11, and CXCR3 in gastric tumor tissues and normal tissues were analyzed using GEPIA. Co-expression among CXCL9/10/11 were performed using cBioPortal. Correlations and immunotherapy prediction analyses were performed using TIMER3. Overall survival analyses were assessed using Kaplan-Meier Plotter.

Results: A significant increase in CXCL9, CXCL10, CXCL11, and CXCR3 expression was observed in gastric tumor tissues versus normal tissues with no stage-dependent differences. High expression of CXCL9, CXCL10, and CXCL11 were correlated with higher infiltration of CD8+ T cells, M1 macrophages, and lower infiltration of M2 macrophages and myeloid-derived suppressor cells. Increased expressions of IFNG, GZMB, TBX21, and STAT1 were positively correlated with CXCL9/10/11 expression. Patients with high CXCL9/10/11 expression demonstrated favorable overall survival and improved immunotherapy response.

Conclusion: CXCR3-CXCL9/10/11 axis is associated with an immune-inflamed phenotype, improved survival, and enhanced immunotherapy responsiveness in gastric cancer. These results indicate that CXCL9/10/11 may function as potential biomarkers of immunotherapy responsiveness.

Keywords: gastric cancer, immunotherapy, tumor microenvironment

Introduction

Gastric cancer is one of the most lethal malignancies, and standard treatment strategies for most of the gastric cancer cases involve surgical operations, chemotherapy and/or targeted therapies. With the emergence of immunotherapies especially immune checkpoint inhibitors, treatment strategies for gastric cancer have been evolving [1]. However, not all patients benefit from the immunotherapies, and the reason is mostly because of the heterogeneity in the tumor microenvironment. To understand immunotherapy responsiveness in gastric cancer, the distinction between “hot” and “cold” tumors has become central. Hot tumors are characterized by high CD8+ T cell infiltration, high tumor mutational burden, and elevated PD-L1 expression whereas cold tumors are characterized by low CD8+ T cell infiltration, high tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC) infiltration [2].

Trafficking of immune cells from blood to tissue is maintained by the secreted chemokines. Since chemokines have role to determine the entrance

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Received: February 27, 2026 **Accepted:** March 11, 2026 **Published online:** August 14, 2026

and position of the immune cells in the solid tumors, they actively shape the tumor microenvironment as hot versus cold [3]. The heterogeneity of the tumor microenvironment alters the migration pattern of immune cells and even in the same tumor types, variations are observed among the patients. Accumulating evidence across multiple cancer types suggests that high intratumoral expression of CXCR3 ligands (CXCL9, CXCL10, and CXCL11) correlates with enhanced immune cell infiltration, improved survival, and increased sensitivity to immune checkpoint blockade [4-9]. CXCR3 is mostly expressed on activated $CD8^+$ cytotoxic T lymphocytes, Th1-polarized $CD4^+$ T cells, and NK cells [10,11]. CXCL9/10/11 are produced by multiple cellular compartments within the tumor microenvironment such as M1-macrophages, conventional dendritic cells, and cancer-associated fibroblasts [12-14]. CXCR3 serves as the primary receptor mediating cellular responsiveness to CXCL9, CXCL10, and CXCL11 gradients and high intratumoral CXCR3 expression may indicate the presence of effector immune cells [15].

CXCL9/10/11 production is mainly regulated by IFN-g/STAT1 pathway, forming a positive feedback loop in which infiltrating effector T cells amplify chemokine expression, thereby reinforcing immune cell recruitment and retention within the tumor microenvironment [16]. Conversely, tumors with defective interferon signaling, dominant immunosuppressive cytokines, or lacking CXCR3 ligands often remain immunologically cold [17-19].

The expression patterns, immune associations, and clinical relevance of the CXCR3 ligand–receptor axis in gastric cancer remain incompletely understood. Therefore, in this study, the expression profiles, immune correlates, prognostic significance, and immunotherapy predictive value of CXCL9, CXCL10, CXCL11, and CXCR3 in gastric cancer were comprehensively investigated. It was aimed to elucidate whether the CXCR3 chemokine axis functions as a molecular determinant of hot versus cold gastric tumors, and whether it may serve as a clinically relevant biomarker to guide immunotherapeutic strategies.

Materials and methods

Gene expression analysis

Gene expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) was used to analyze the expressions of CXCL9, CXCL10, CXCL11, and CXCR3 in tumor samples of gastric cancer patients and adjacent healthy tissue. In GEPIA, RNA sequencing data from the The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) Projects were integrated. Differential expression analysis between gastric tumor tissues and normal tissues were performed using log2-transformed transcripts per million (TPM) values.

Correlation analysis

The correlations between regulatory T cells (Tregs), cytotoxic CD8+ T cells, NK cells, M2 macrophages, and M1 macrophages and CXCL9/10/11 were analyzed via Tumour Immune Estimation Resource TIMER3 (https://compbio.cn/timer3/). CIBERSORT algorithm was used. Correlation analyses among CXCL9, CXCL10, and CXCL11 cBioportal database (https://www.cbioportal.org) was used. Evaluation was performed based on log2-transformed Transcript per Million (TPM) values of transcribed genes in stomach adenocarcinoma tumor tissues.

Receiver operating characteristic curve (ROC curve) and therapy response

To predict immunotherapy response depending on the expressions of CXCL9, CXCL10, and CXCL11 in gastric cancer cohort, TIMER 3.0 immunotherapy response prediction module was used. To analyze the immunotherapy responses, TIMER 3.0 immunotherapy module was used (Dataset STAD_PRJEB25780).

Survival analysis

Gastric cancer patients were classified into high and low expressions of CXCL9, CXCL10, CXCL11, and CXCR3 by best cutoff in Kaplan-Meier Plotter (https://kmplot.com/analysis/). Overall survival analyses were assessed, and statistical significance was obtained when a log rank p value <0.05. In Kaplan-Meier Plotter, publicly available gastric cancer cohorts were used, and patients were stratified into high- and low-expression groups using the best cutoff option provided by the platform.

Results

CXCL9, CXCL10, CXCL11 and their receptor CXCR3 were upregulated in gastric tumors

In silico transcriptomic analysis revealed that CXCL9, CXCL10, CXCL11, and CXCR3 were drastically upregulated in the tumor tissues of gastric cancer patients compared to the healthy tissues (Figure 1 A). No significant differences in CXCL9, CXCL10, CXCL11, and CXCR3 expressions were found depending on different

stages of gastric cancer (Figure 1 B). Additionally, in the tumor samples of gastric cancer patients, strong positive correlation was obtained among CXCL9, CXCL10, and CXCL11 (Figure 1 C).

High expressions of CXCL9, CXCL10, and CXCL11 indicated "hot" tumor phenotype

High expressions of CXCL9, CXCL10, and CXCL11 were associated with an increased infiltration of CD8+ T cells and M1-like macrophages but decreased infiltration of Tregs, and M2-like macrophages into the gastric tumors. However, for NK cell infiltration there was no association found among CXCL9, CXCL10, and CXCL11 expressions (Figure 2 A, B, C).

In addition to the infiltration of immune cells, elevated expressions of CXCL9, CXCL10, and CXCL11 were associated with the higher expressions of IFNG, GZMB, TBX21, and STAT1 genes, indicating that Th1-dominant immunity and inflammatory milieu of the tumor microenvironment (Figure 3 A, B, C).

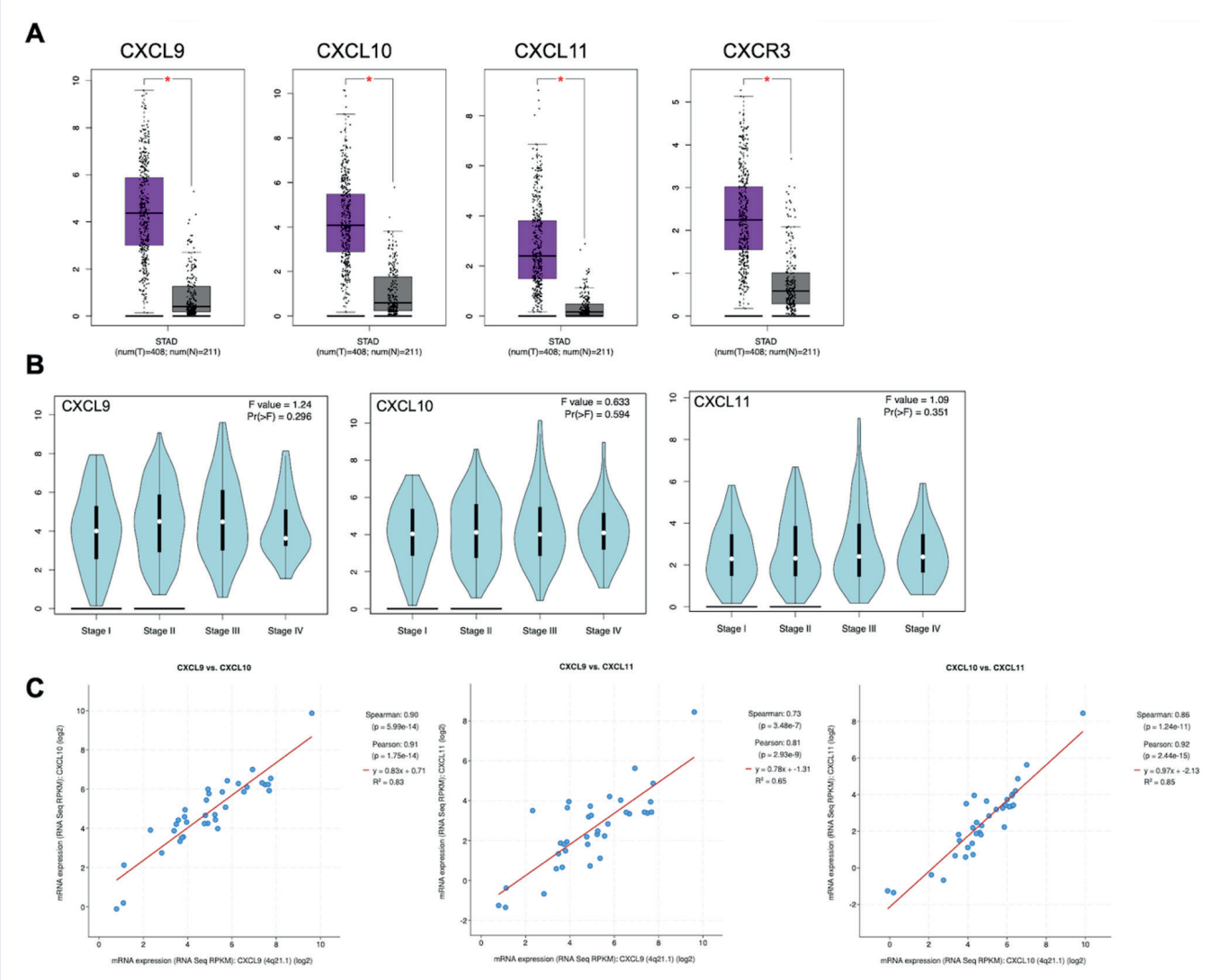

Figure 1. A) Differential expression of CXCL9, CXCL10, CXCL11, and CXCR3 in gastric cancer tumor tissue compared with adjacent normal tissues (T:tumor, N: normal tissue). B) Expression levels of CXCL9, CXCL10, and CXCL11 across different pathological stages of gastric cancer. C) Correlation analysis among CXCL9, CXCL10, and CXCL11 expression in gastric cancer tumor samples. Statistical significance is indicated where applicable.

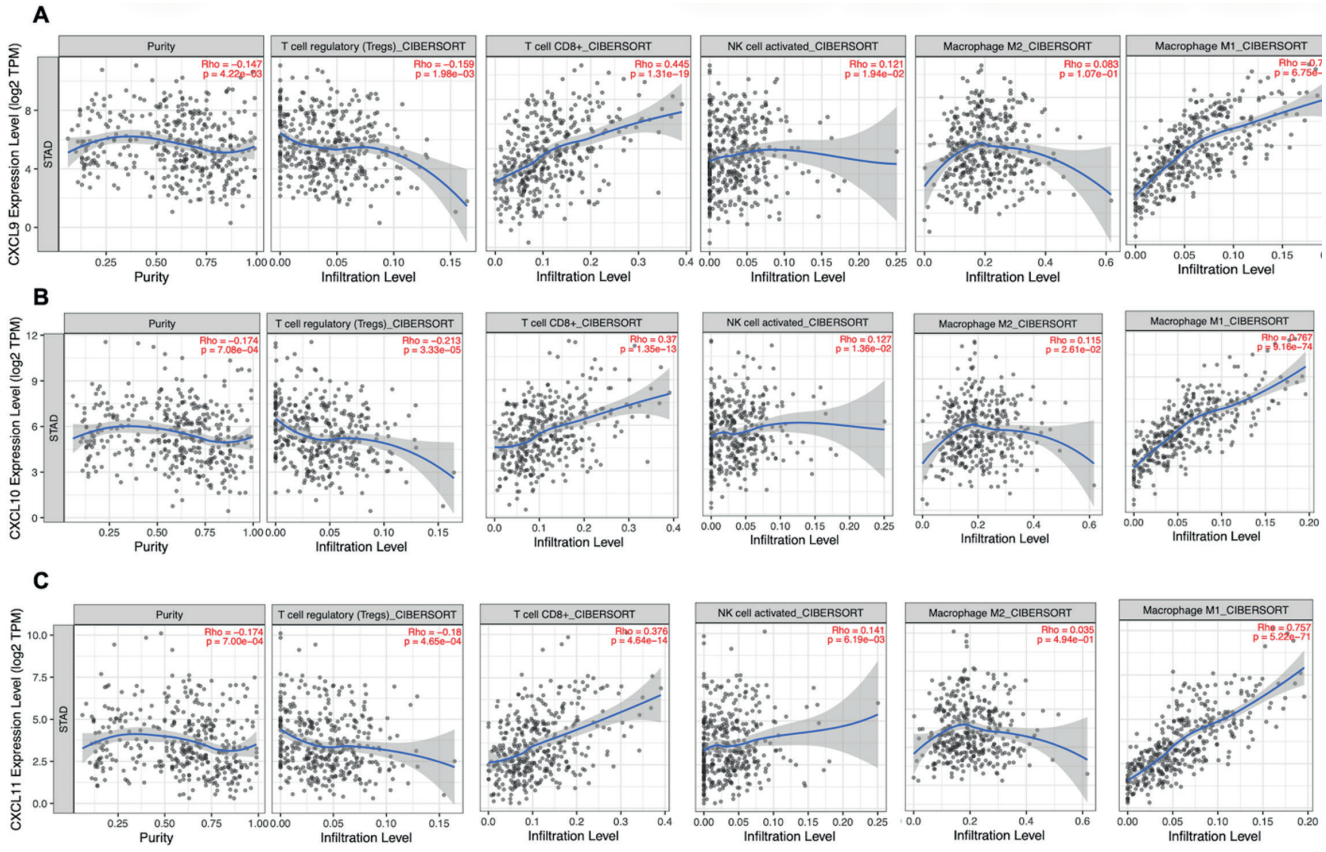

Figure 2. Correlation analyses between expression of CXCL9 (A), CXCL10 (B), and CXCL11 (C) and immune cell infiltration in gastric cancer tumors. Immune cell types include regulatory T cells, CD8+ T cells, NK cells, M2 macrophages, and M1 macrophages. Statistical significance is indicated where applicable

Expressions of CXCL9, CXCL10, and CXCL11 predicted improved clinical outcomes

Gastric cancer patients who received immune checkpoint inhibitors with high CXCL9, CXCL10, and CXCL11 expression in their tumor exhibited superior immunotherapy response (Figure 4 A). The ROC analysis demonstrated that CXCL9, CXCL10, and CXCL11 expressions may be used to predict immune response in gastric cancer (AUC values are 0.719, 0.692, 0.697; Figure 4 B). They demonstrated comparable predictive performance. Patients with high CXCL9, CXCL10, CXCL11, and CXCR3 expression exhibited favorable overall survival (OS) (Figure 4 C).

Discussion

Despite the expanding use of immune checkpoint inhibitors, not all gastric cancer patients benefit from immunotherapies. Although expression of PD-L1, microsatellite instability (MSI), tumor mutational burden (TMB), and Epstein–Barr virus (EBV) status can be commonly used as biomarkers, they are not fully sufficient to identify the patients who respond to immunotherapies better. Therefore, reliable biomarkers that precisely predict which patients will respond to immunotherapy are needed. Since chemokines and their receptors impact the immune cell trafficking and shape the characteristics of the tumor microenvironment as hot or cold, they may act as prognostic biomarkers in different cancers. In this work, expression, immune associations, prognostic value, and immunotherapy predictive capacity of CXCL9, CXCL10, CXCL11, and CXCR3 in gastric cancer were analyzed. Based on the existing literature, in gastric cancer, majority of the CXCR3+ tumor-infiltrating T cells are effector memory and accumulate in lymphocyte-rich area of the tumor. CXCR3+ tumor-infiltrating T cells have been in proximity with CXCL9 expressing cells [20]. Additionally, in

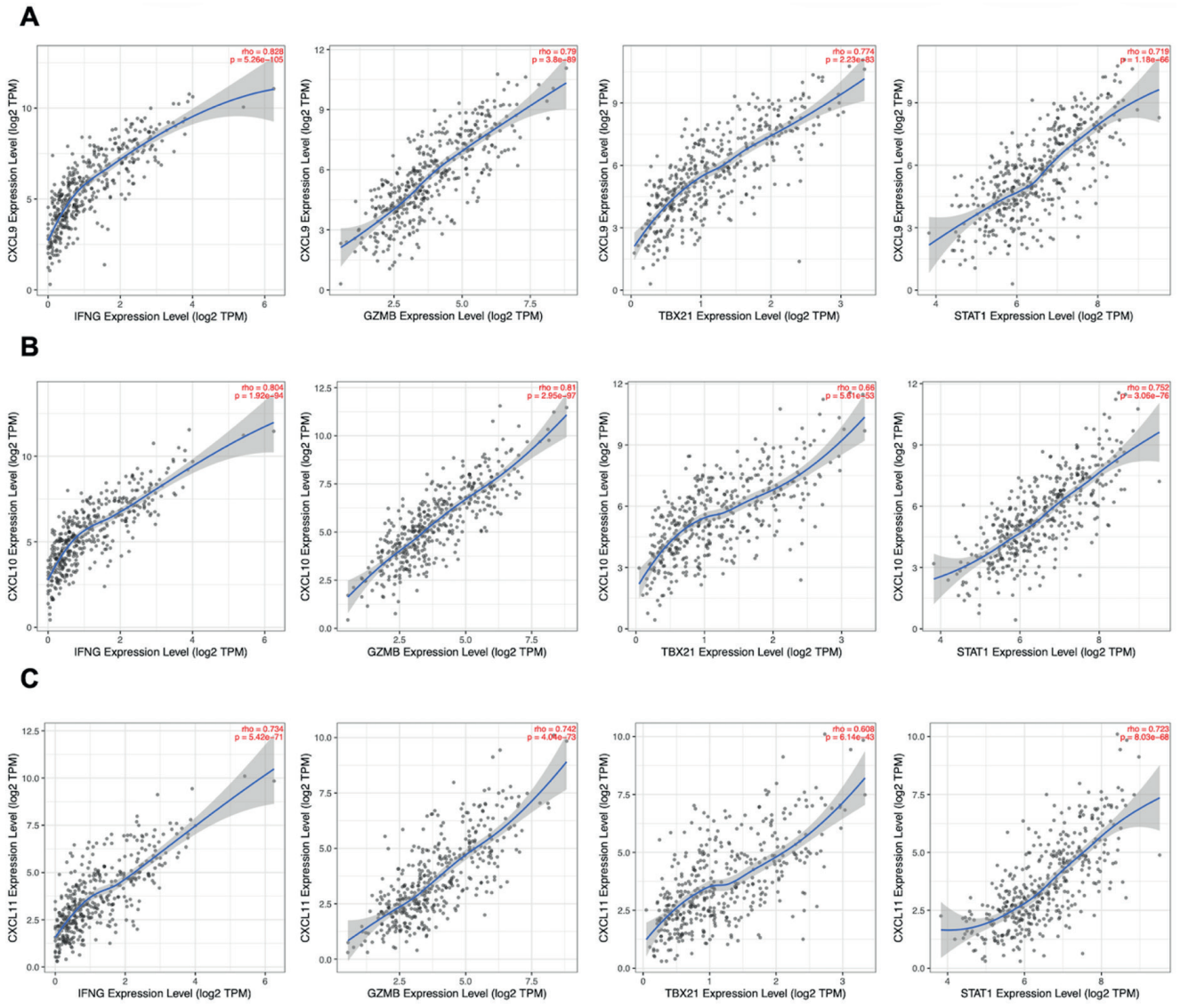

Figure 3. Correlation analyses of CXCL9 (A), CXCL10 (B), and CXCL11 (C) expression with immune related genes including IFNG, GZMB, TBX21, and STAT1 in gastric cancer tumor tissues. Statistical significance is indicated where applicable

tumor-bearing mice, cxcr3 deficiency promotes tumor progression and M2 macrophage polarization [21]. Intratumoral injection of CXCL9 and CXCL10 expressing dendritic cells into the mice tumors increased T cell infiltration and improved efficacy of anti-PD-1 therapy [22]. In various cancers, increased expression of CXCR3 ligands have been associated with good prognosis and immunotherapy response, whereas a lack of expression of CXCR3 ligands disrupts T cell trafficking into the tumor and resulting in ineffective anti-tumor immune responses [4,6,23,24]. Consistent with these results, our study demonstrated that elevated expression of CXCR3 ligands is associated with increased accumulation of CD8+ T cells, M1 macrophages, and decreased accumulation of M2 macrophages in gastric cancer. Effector immune cells such as cytotoxic CD8+ T lymphocytes have the ability to recognize and kill the tumor cells. These cells can also generate anti-tumor responses by producing cytokines such as IFN-g and Granzyme-B (GZMB). STAT1 transcription factor induces T-bet transcription factor and differentiation of Th1 cells, thereby supporting anti-tumor immune responses [25,26]. Moreover, T-bet regulates the differentiation of CD8+ T cells, which produce Granzyme B to kill the

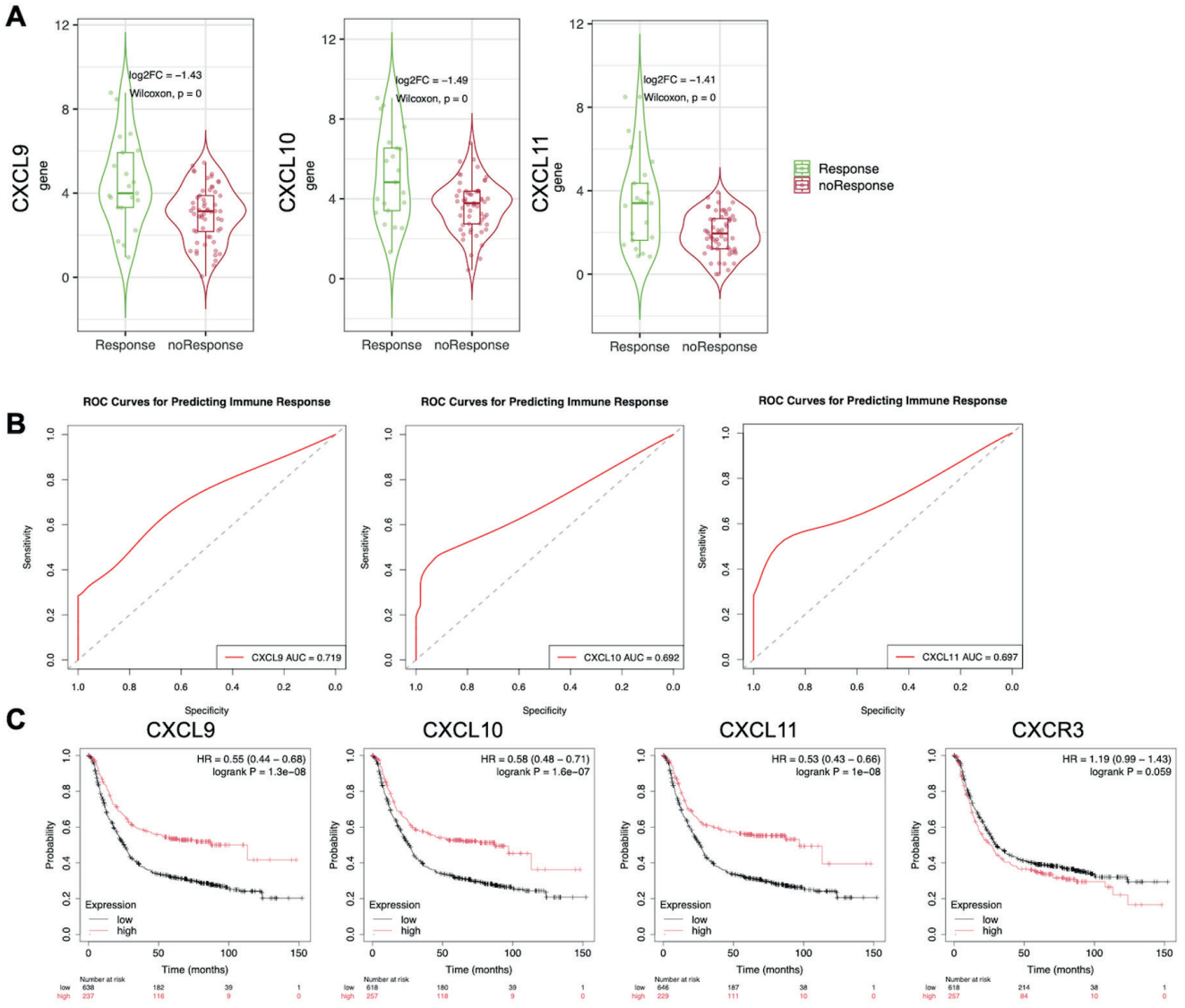

Figure 4. A) Immunotherapy response comparison between gastric cancer patients with high versus low expression of CXCL9, CXCL10, and CXCL11. B) ROC curve analysis evaluating the predictive performance of CXCL9, CXCL10, and CXCL11 expression for immune responses, with corresponding area under the curve (AUC) values. C) Kaplan-Meier overall survival analysis of gastric cancer patients based on high and low expression levels of CXCL9, CXCL10, CXCL11, and CXCR3

tumor cells [27]. Therefore, increased expression of STAT1, T-bet, IFN-g, and Granzyme B may indicate an immune-inflamed microenvironment in tumors. Our results show that gastric cancer patients with high CXCL9/10/11 expression had high T-bet, STAT1, Granzyme B, and IFN-g expressions and demonstrated superior immunotherapy response rates, consistent with their association with an inflamed and cytotoxic tumor microenvironment.

Ethical approval

This study did not include direct human participants. Publicly available datasets were used, any data from any hospital was not used. Gastric cancer patient data from GEPIA, TIMER3, cBioPortal, and Kaplan-Meier Plotter platforms were used, and all analyses were conducted through using these websites. Therefore, ethical approval is not applicable.

Author contributions

Conception and design: E.T.; Data acquisition: E.T.; Data analysis: E.T.; Data interpretation: E.T.; Drafting of the manuscript: E.T.; Critical revision of the manuscript: E.T. The author reviewed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of this study.

Ethical approval

Ethics committee approval and informed consent were not required for this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The author declares that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The author declares that this study received no funding.

Generative AI statement

The author declares that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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