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Implications of PD-L1 expression on the immune microenvironment in HER2-positive gastric cancer

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Abstract

In the KEYNOTE-811 study, anti-HER2 and immunotherapy treatments resulted in longer survival in HER2-positive gastric cancer patients with CPS≥1, whereas CPS<1 patients lacked notable benefits. We studied this in a real-world cohort of 106 HER2-positive, CPS<1 patients and found no survival differences between those treated with anti-HER2 therapy alone or with added immunotherapy. Thus, we investigate the tumor microenvironment variations in 160 HER2-positive patients, CPS≥1 cases exhibited elevated spatial effective scores of immune cells, including CD4, CD8 subtypes, and NK cells, compared to CPS<1. Furthermore, through single-cell sequencing in eight HER2-positive individuals, gene expressions revealed regulation of T-cell co-stimulation in CPS≥1 and IL-1 binding in CPS<1 cases. Notably, we discovered a CPS<1 subtype marked by CXCR4^{+}M2 macrophages, associated with poor prognosis, whose proportion and expression were reduced when benefiting from anti-HER2 therapy. These findings suggest CPS≥1 patients, due to their immune microenvironment composition, may respond better to anti-PD-1/PD-L1 therapy.

Introduction

Phase 3 KEYNOTE-811 study convincingly demonstrated the promising efficacy of the therapeutic combination of Pembrolizumab, Trastuzumab, and chemotherapy in improving progression-free survival rates and inducing a higher tumor response for the first-line treatment of cancer [1]. This therapeutic combination was found to be particularly effective in a subset of patients with HER2-positive and PD-L1-positive tumors. Interestingly, this combined therapy regimen showed increased effectiveness in a particular cohort of patients who presented with HER2-positive and PD-L1-positive tumors. Remarkably, the benefits were amplified for patients whose tumors displayed a PD-L1 combined positive score (CPS) of 1 or higher. However, patients possessing a PD-L1 CPS less than 1 experienced negligible improvement.

Preclinical research continues to make breakthroughs in understanding of cancer treatments, revealing the potential of deploying tumor-specific antibodies in tandem with PD-1 inhibitors, such as Pembrolizumab and Trastuzumab, in association with traditional chemotherapy. The synergy between these treatments resulted in an influx of immune cells infiltrating tumor sites, heightened

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T-cell reactivity, and counteracting the potentially harmful effects of tolerogenic dendritic cells [2]. Nevertheless, a patient subset with a CPS below 1 seemed to derive minimal benefit from these combined immunotherapies. To understand this stark discrepancy by CPS scores, our research aims to delve into an in-depth exploration of the tumor microenvironment within these specific patient groups.

Results and discussion

We delineate three distinct cohorts as demonstrated in Fig. 1a. The assembly of the first cohort was carried out retrospectively, comprising 106 patients diagnosed with HER2-positive cancer and a CPS less than 1. Within this cohort, 73 patients were prescribed a treatment plan involving both anti-HER2 agents and chemotherapy, while 33 patients were assigned a trio of therapies - anti-HER2, chemotherapy, and immunotherapy. The baseline characteristics of the first cohort are tabulated comprehensively in Supplementary Table 1. Interestingly, even with the inclusion of an additional immunotherapeutic approach, there was no identifiable enhancement in progression-free survival (PFS) and overall survival (OS) measures for this group of patients, as depicted in Fig. 1b. Our study outcomes concur with the KEYNOTE-811 study findings, suggesting that among HER2-positive groups possessing a CPS below 1, the incorporation of immunotherapy fails to lengthen survival [1].

In the second cohort for this investigation, our primary concentration gravitated towards scrutinizing the tumor microenvironment within HER2-positive tumors, with a specific focus on the spatial associations apparent within the groups with CPS≥1 and CPS<1, as detailed in Supplementary Table 2. We amassed samples from 160 gastric cancer patients who were HER2-positive and performed an immune microenvironment analysis via multiplex immunohistochemistry. Out of these, 121 patients had a CPS surpassing 1, whereas 39 patients had a CPS beneath 1. The foundational clinical pathological traits, which include the tumor location, tumor differentiation, LAUREN categorization, Mismatch Repair (MMR) status, and Epstein-Barr Virus (EBV) status, exhibited no marked discrepancies between the CPS<1 and CPS≥1 groups.

Beyond the immune cell density, we contrived two innovative analytical methodologies, termed "effective score" and "effective percent". These methods precisely describe the spatial positioning of various immune cell subtypes, as detailed in our previous studies [3] and delineated in Extended Data Figs. 1, 2 and 3. A higher effective score or percentage signifies a greater density of immune cells surrounding tumor cells within a specific distance, facilitating interactions that can either enhance antitumor responses or contribute to immunosuppressive effects. A comparison between CPS≥1 and CPS<1 cohorts revealed no substantial discrepancies in the occurrence of $CD3^+$ T cells, $CD20^+$ B cells, or total macrophages among HER2-positive patients. Conversely, an intriguing pattern emerged during the exploration of the effective scores for Natural Killer (NK) cells, $CD8^+$ T cells, $CD4^+$T cells, $CD4^+FoxP3^-$ T cells, $CD4^+PD\text{-}L1^+$ T cells, $PD\text{-}L1^+CTLA\text{-}4^-$ cells, and M2 macrophages ($CD68^+CD163^+$). Patients within the CPS<1 group consistently demonstrated diminished effective scores for these specific cell subtypes in comparison to those in the CPS≥1 group, a trend that consistently reverberated in both effective percentage and density measurements. Hence, it is evident that the tumor microenvironment in HER2-positive patients with a CPS greater than 1 not only exhibits a higher density of NK cells and CD4 and CD8 subgroups, but these cells also demonstrate a tighter spatial distribution with tumor cells.

In the third cohort of this study, we executed single-cell sequencing analysis on samples from eight patients, yielding a total of 10 samples for study (shown in Extended Data Fig. 4, Supplementary Table 3). The gene expression signatures of both immune and tumor cells were examined (Extended Data Figs. 5 and 6). In the patient group presenting with a CPS of ≥1, a regulation of T-cell co-stimulation and CXCR3 chemokine receptor binding was observed in CD4 T cells. CXCR3, a chemokine receptor, facilitates the migration and functioning of T cells by assisting in their interaction with ligand-producing dendritic cells, leading to their differentiation into effector cells, and thereby potentially boosting anti-tumor responses [4]. Concurrently, there was an active positive regulation of immature T-cell proliferation in CD8 T cells and NK cells, accompanied by an upregulation of dendritic cell antigen processing and presentation in NK cells. This gene expression pattern suggests a state of specific immune response activation. Conversely, in patients with a CPS of <1, the gene expression profile was largely defined by characteristics such as IL-1 binding and receptor activity, negative regulation of IL-5 production in $CD4^+$ T cells, and a positive regulation of the VEGF signaling pathway in both $CD4^+$ and $CD8^+$ T cells. Here, the IL-1 promotes inflammation, and aids in tumor growth, whereas, IL-5, primarily influencing eosinophils and B cells, indirectly enhances the anti-tumor immune response [5, 6]. Additionally, the profile included augmented macrophage proliferation, and positive regulation of Epithelial-to-Mesenchymal Transition (EMT) in NK cells.

In our study population of gastric cancer patients testing positive for HER2 with a CPS<1, we identified of certain ligand-receptor pairs that illuminate a unique pattern of intercellular communication, as depicted in Extended Data Fig. 7. We observed that the interaction

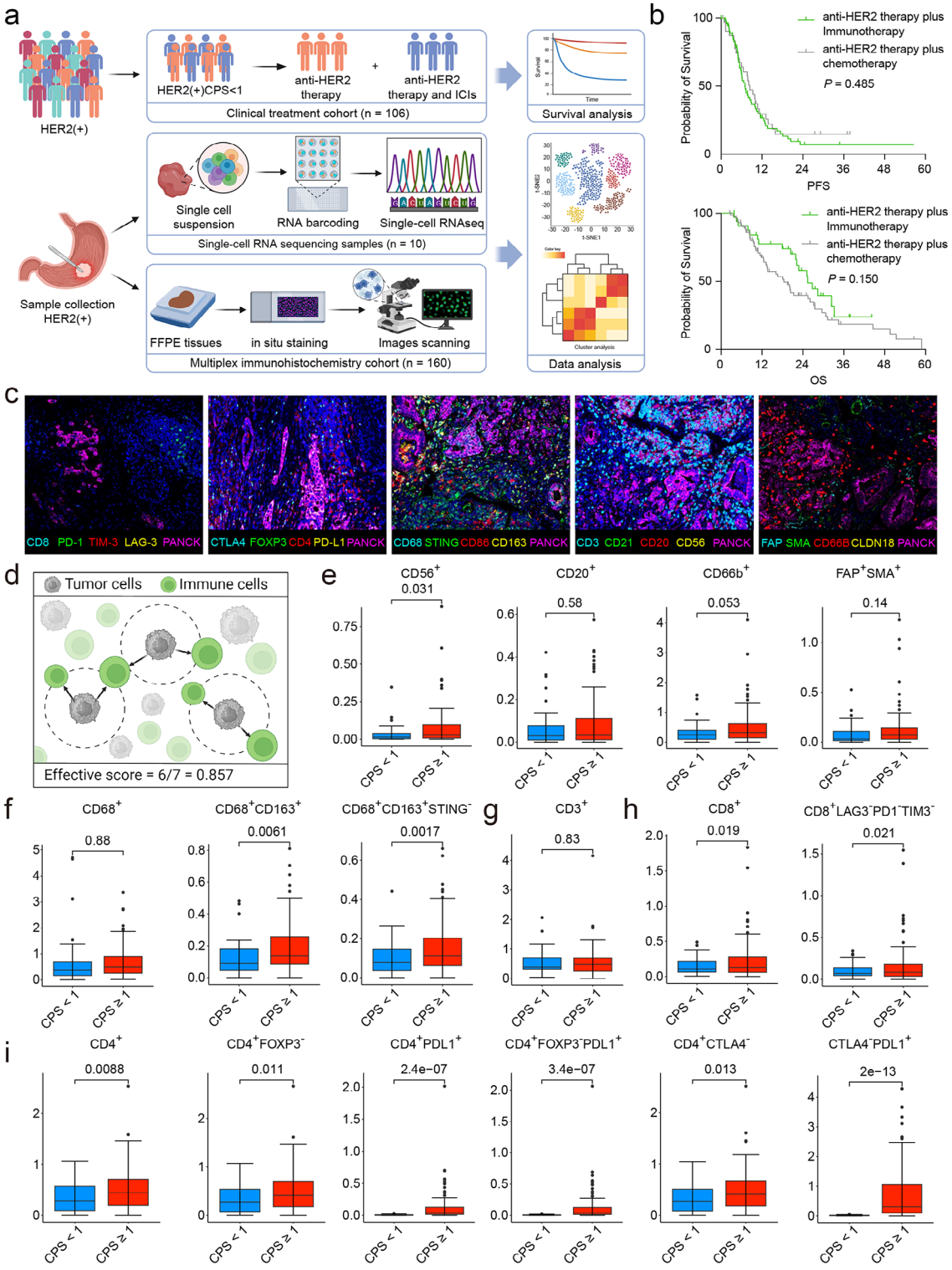

Fig. 1 Study design and the spatial immune features of HER2-positive gastric cancer. **a** The illustration of cohorts, including the clinical cohort, the single-cell RNA sequencing cohort, and the multiplex immunohistochemistry cohort. **b** Survival analysis of irPFS and irOS between the treatment regimen of anti-HER2 plus chemotherapy and anti-HE2 therapy plus immunotherapy in HER2-positive gastric cancer with CPS < 1 (Log-rank test). **c** Multiplex immunohistochemical staining labels 21 markers through 5 panels. **d** The illustration of the method for calculating the effective score, which quantifies the extent to which central tumor cells are surrounded by peripheral immune cells. (**e**-**i**) Comparisons of spatially efficient scores among different immune cells and their respective functional subtypes. Abbreviations: HER2, human epidermal growth factor receptor 2; CPS, combined positive score; FFPE, formalin-fixed paraffin-embedded

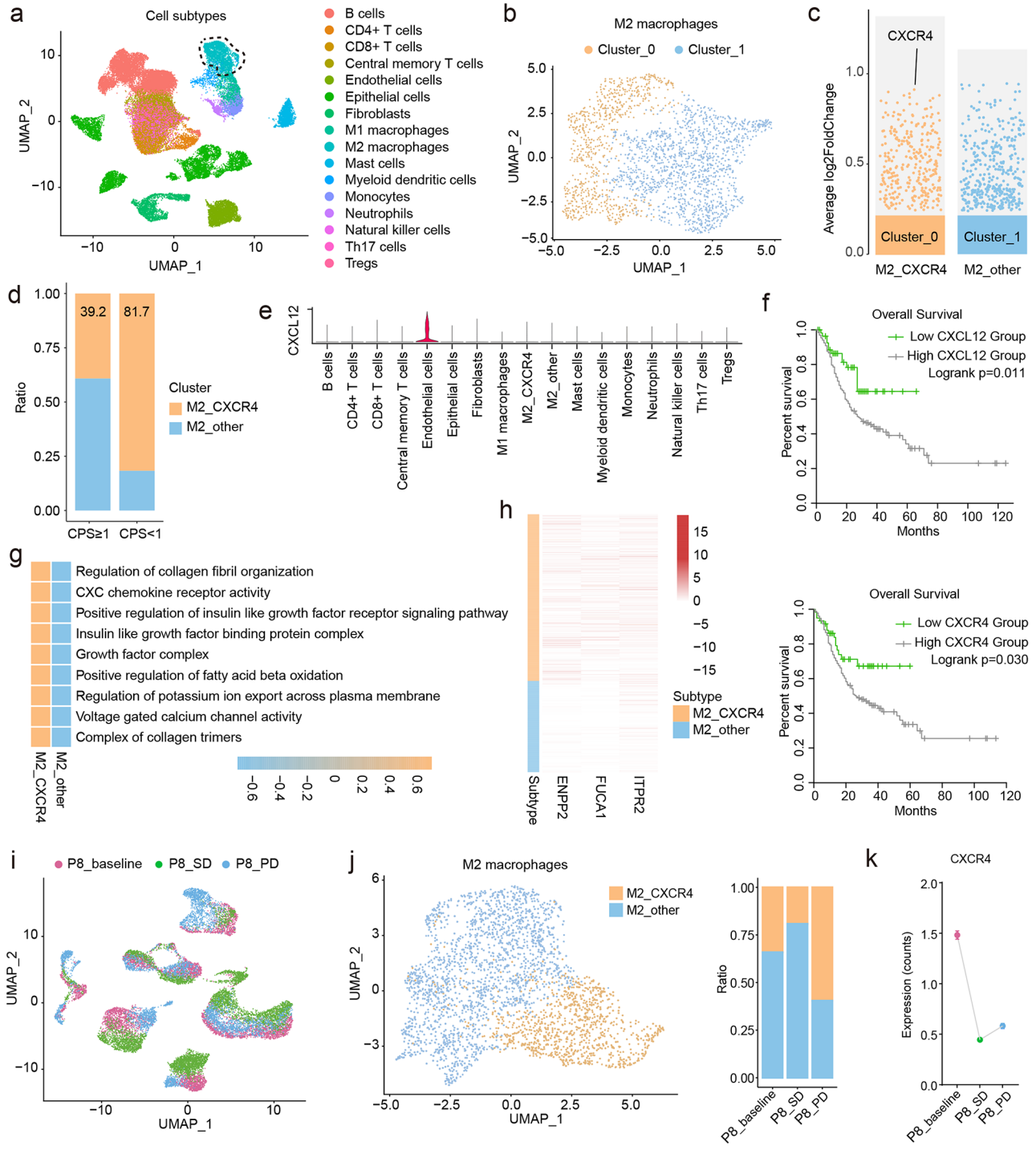

Fig. 2 CXCR4$^+$ M2 macrophages were a unique immunosuppressive subtype and were enriched in HER2-positive gastric cancer with CPS < 1. **a** The landscape of annotated cell subpopulations in baseline samples from eight HER2-positive gastric cancer patients. **b** The UMAP plot presents two distinct M2 macrophages with different transcriptomic features. **c** Differential gene expression is presented between the two subpopulations of M2 macrophages. **d** Proportion of CXCR4$^+$ M2 macrophages in HER2-positive gastric cancer with different PD-L1 CPS. (**e**) Expression levels of CXCL12, the ligand of CXCR4, in various cell subpopulations. (**f**) Survival analysis of CXCL12 and CXCR4 in gastric cancer cohort from TCGA database (Log-rank test). (**g**) Gene expression-based pathway scoring reveals functional characteristics of CXCR4$^+$M2 macrophages. (**h**) Genes ITPR2, FUCA1, and ENPP2 were upregulated in CXCR4$^+$ M2 macrophages. (**i**-**j**) Dynamic changes in the proportion of CXCR4$^+$ M2 macrophages are depicted throughout the course of treatment. (**k**) The comparison of CXCR4 expression in M2 macrophages between baseline, SD, and PD samples. Abbreviations: SD, stable disease; PD, progressive disease

between COL1A1-ITGAV-ITGB8 has the potential to stimulate tumor matrix proliferation and fibrosis [7]. This interaction, in turn, could adversely affect the immunities directed against the tumor. Additionally, the bond between CD34 and SELP hints towards vascular genesis and cell adhesion, which could inadvertently contribute to tumor progression. The relationship exhibited by CXCL3 and CXCR2 was seen to correspond with tumor invasion and subsequent advancement. Contrarily, upon examining the group of HER2-positive gastric cancer patients with a CPS of 1 and above, the revealed ligand-receptor pairings portrayed contrasting behavior. Here, potential collaborations were noticed between LGALS9 and CD45, ostensibly bolstering the activities of NK cells as well as T cells [8]. Similarly, the cooperative function of LTA-LTB-LTBR appears to play a significant role in both the recruitment and chemoattraction of T and B lymphocytes [9]. Lastly, the ITGAV-ITGB5 pair seemed to augment the migration of M2 macrophages, which in consequence, might significantly affect the tumor microenvironment [10]. Hence, our study elucidates that distinct intercellular communications could be observed within different patient groups, each personifying different CPS statuses. This diversity serves to underscore the intricate and multifarious nature of HER2-positive gastric cancer.

Furthermore, M2 macrophages associated with pro-tumor but higher in CPS ≥ 1 and HER2 positive patients. Building on our understanding of the M2 macrophage subset, we discovered a compelling association between M2 macrophages, known for their pro-tumor properties, and patients with a CPS over 1 who also tested positive for HER2. Following our exploration into M2 macrophage clustering, our analysis identified a novel cell subtype labelled as $CXCR4^{+}$M2 (Fig. 2a-d). This particular subtype showed a significant enrichment in Gastric Cancer patients exhibiting a CPS score of less than 1, and it displayed unique transcriptomic features. We deduced that $CXCR4^{+}$M2 cells could be influenced by endothelial cells via the CXCR4-CXCL12 signaling pathway, which has been associated with poor patient prognosis (Fig. 2e-f). CXCL12-mediated stimulation of cancer cells and macrophages may initiate and amplify a GM-CSF/HBEGF paracrine feedback loop, thereby facilitating macrophage-driven cancer survival and proliferation [11]. The CXCL12-CXCR4 axis is pivotal in monocyte recruitment at tumor sites, regulation of cancer metastasis, cancer cell migration, epithelial-mesenchymal transition (EMT), and intra-tumor regulatory T cell (Treg) recruitment [12]. Furthermore, several signaling pathways within these $CXCR4^{+}$M2 cells appeared to be noticeably upregulated (Fig. 2g). These include pathways regulating collagen fibril organization, positive regulation of the insulin-like growth factor receptor signaling pathway and voltage gated calcium channel activity [13, 14]. Within the context of CXCR4 + M2 macrophages, we observed an intriguing genetic response. This particular behavior was typified by heightened activity levels of ITPR2, FUCA1, and ENPP2 genes (Fig. 2h). However, it is imperative to note that such upregulation in gene activity potentially fuels the process of tumorigenesis while concurrently debilitating the efficacy of anti-tumor immunity.

In a more dynamic evaluation, we analyzed the changes in the proportion of $CXCR4^{+}$ M2 macrophages within HER2-positive patients who received anti-HER2 therapy, comparing baseline measures with those observed during the stable disease (SD) phase and the progression disease (PD) phase (Fig. 2i-j). Encouragingly, we noted a decline in both the proportion of $CXCR4^{+}$ M2 macrophages and the expression of the CXCR4 in M2 macrophages when patients appeared to benefit from anti-HER2 therapy. We conducted a detailed gene expression pattern analysis and functional assessment of CXCR4 + M2 macrophages in the context of anti-HER2 treatment. Functional enrichment analysis revealed that, in patients who responded favorably to anti-HER2 therapy, the predominant pathways were those associated with lymphocyte-mediated immunity. Conversely, in cases of tumor progression, the enriched pathways were predominantly related to macrophage migration and collagen-containing extracellular matrix formation (Extended Data Fig. 8).

Therefore, our initial results suggest the complicated yet significant role of the novel cell subtype $CXCR4^{+}$ M2 in gastric cancer progression and the potential influence of anti-HER2 therapy on it. The findings from the COMBAT phase II trial indicate that, for patients suffering from metastatic pancreatic cancer, a combination therapy incorporating the CXCR4 antagonist BL-8040 with pembrolizumab and chemotherapy exhibits not only notable safety and efficacy, but also augments immunobiological responses and overall survival rate, thereby potentially amplifying the advantages conferred by chemotherapy [15].

The careful delineation into three cohorts allowed for a fine-grained analysis. In these patients, the addition of immunotherapy provided no discernible improvement in PFS and OS. These findings raise important questions about the value of CPS and the role of specific immune cell populations. We analyzed the complex and multifaceted nature of the tumor microenvironment in HER2-positive tumors. With our primary focus centered on analyzing the tumor microenvironment, some intriguing spatial patterns emerged. Upon comparing the CPS ≥ 1 and CPS < 1 groups, there was a consistently lower effective score for specific cell types including NK cells, $CD8^{+}$ T cells, and M2 macrophages in the CPS < 1 category. These differences in the microenvironment, related to

CPS scores, may be influential in shaping the response to therapy and underscore the importance of immune cell infiltration patterns in potential treatment efficacy.

In our attempt to bridge the gap between genetic and microscopic manifestations of tumors, we executed single-cell sequencing analysis. This established a correlation between CPS and gene expression profiles, illuminating unique patterns of intercellular communication within different patient groups. Particularly, our analysis uncovered a unique cell subtype labeled as $CXCR4^{+}M2$ in gastric cancer patients exhibiting a CPS score of less than 1, which we found to be enriched significantly. This subtype indicates a potential influence on disease progression and response to treatment, as demonstrated by the declining proportion of $CXCR4^{+}$ M2 macrophages and reduced expression in response to anti-HER2 therapy. Therefore, our results suggest the intricate, significant contribution of immune cell-related factors within the context of HER2-positive gastric cancer progression.

Conclusion

Through immune microenvironment composition, the response to anti-PD-1/PD-L1 therapy is likely to be considerably more favorable among patients with a CPS greater than 1. Furthermore, our results suggest that anti-HER2 therapy may have potential influence over the CXCR4+M2 macrophages, therefore possibly shaping the clinical outcomes in HER2-positive gastric cancer. This study provides a wealth of information for future research in enhancing personalized medicine and therapeutic strategies in treating HER2-positive gastric cancer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12943-024-02085-w.

Supplementary Material 1

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Author contributions

L.S., X.Z., and Y.C. conceived and supervised the study. Y.C., K.J., and C.X. contributed to sample collection and collection of patient clinical information. Y.S. contributed to pathology review. K.J. and Y.C. contributed to data processing, integrative analyses, and generating figures and tables. Y.X., H.P., L.J., D.L., J.Y., Y.L., X.F., and J.L. assisted with data processing and analysis. Y.C. and K.J. wrote the manuscript. L.S. revised the manuscript.

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Data availability

All data relevant to the study are included in the article or uploaded as supplementary information.

Declarations

Ethical approval

This study was approved by the Ethics Committee of Peking University Cancer Hospital (2020KT08). All the participants and their legal guardians provided informed consent.

Competing interests

The authors declare no competing interests.

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