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Blood TCTP as a potential biomarker associated with immunosuppressive features and poor clinical outcomes in metastatic gastric cancer

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ABSTRACT

Background No established biomarker exists for specific myeloid cell populations or in gastric cancer. This study aimed to explore the prognostic and immunological relevance of plasma translationally controlled tumor protein (TCTP) in patients with advanced gastric cancer treated with an immune checkpoint inhibitor and/or cytotoxic chemotherapy.

Methods Plasma samples were prospectively collected from the cohorts of patients with gastric cancer treated with first-line fluoropyrimidine plus platinum chemotherapy (n=143, cohort 1) and third-line nivolumab (n=165, cohort 2). Plasma TCTP levels were quantified using ELISA, and multiplex proteomic analysis (Olink) was conducted to assess expression levels of immune-related proteins. External single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics datasets were employed to validate the findings.

Results Patients with high plasma TCTP levels (TCTP-high group) exhibited poor progression-free survival (PFS) and overall survival (OS) with first-line chemotherapy compared with those with low levels (TCTP-low group) in cohort 1 (HR: 1.73 for PFS; 1.77 for OS). In the TCTP-high group, proteins associated with immunosuppressive myeloid cells, angiogenesis, and immune exclusion of T/natural killer (NK) cell function were upregulated, whereas proteins involved in T-cell activation/exhaustion were significantly upregulated in the TCTP-low group. scRNA-seq analyses identified a myeloid subset with high *TPT1* (encoding TCTP) expression and TCTP-related molecules, enriched with inhibitory myeloid inflammation gene signatures and providing inhibitory signals to T/NK cells (Macrophage-chemokine). Spatial transcriptomics analyses revealed a tumor-cell-enriched cluster co-localized with the Macrophage-chemokine subset, which exhibited the highest *TPT1* expression and a positive correlation between its abundance and average *TPT1* levels. In nivolumab-treated patients (cohort 2), the high TCTP group was associated with poor survival outcomes (HR: 1.39 for PFS; 1.47 for OS).

Conclusions Plasma TCTP is a prognostic biomarker, reflecting clinically relevant immunosuppressive myeloid signals in patients with gastric cancer.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immunosuppressive myeloid cells confer resistance against systemic treatments, including immune checkpoint inhibitors (ICIs) in cancers, including gastric cancer. However, no established biomarker represents specific myeloid cell populations and/or representative markers in gastric cancer. Translationally controlled tumor protein (TCTP) is associated with immune evasion driven by immunosuppressive myeloid cells and poor survival outcomes in certain types of cancer. Therefore, the plasma TCTP expression levels in patients with gastric cancer may provide a valuable tool for assessing the treatment efficacy and prognosis in gastric cancer.

BACKGROUND

Gastric cancer is the fourth leading cause of cancer-related mortality and the fifth most prevalent malignancy worldwide.[1] Over the past decade, immune checkpoint inhibitor (ICI)-based treatments emerged as a critical systemic therapy for patients with advanced gastric cancer. The phase 3 ATTRACTION-2 study first showed that nivolumab improved overall survival (OS) compared with that of a placebo in third or later lines of systemic treatment.[2] Subsequently, pivotal phase 3 studies confirm that adding ICIs to chemotherapy improves survival, particularly for those with tumors exhibiting high programmed death-ligand 1 (PD-L1) expression levels.[3–7] Despite identifying predictive biomarkers for ICI-based treatments, including the PD-L1 combined positive score (CPS), mismatch repair (MMR)/microsatellite instability (MSI) status, and Epstein-Barr virus (EBV) positivity, many patients with gastric cancer show no antitumor response,

WHAT THIS STUDY ADDS

⇒ This study is the first to examine the prognostic and immunological relevance of plasma TCTP in patients with gastric cancer treated with ICI and cytotoxic chemotherapy. This study showed that high plasma TCTP levels were associated with poor survival outcomes in patients treated with ICI and cytotoxic chemotherapy. Patients with high plasma TCTP levels showed enrichment of plasma proteins associated with immunosuppressive myeloid cells, angiogenesis, and immune exclusion of T/natural killer (NK) cell function. Single-cell RNA sequencing and spatial transcriptomics analyses identified a myeloid subset with high TPT1 expression levels and inhibitory functional features.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study indicates the unfavorable prognostic value of plasma TCTP in independent cohorts of patients with gastric cancer treated with ICI and cytotoxic chemotherapy. The association between TCTP levels and immunosuppressive myeloid cell inflammation, angiogenesis, and immune exclusion and/or suppression of T/NK cell function suggests that plasma TCTP may serve as a clinically relevant, non-invasive biomarker for reflecting immunosuppressive signals. Our findings also underscore the potential value of targeting TCTP to overcome poor survival outcomes with ICIs and chemotherapy, which may be applicable to other cancer types.

leading to further disease progression. Hence, identifying biomarkers that reflect resistance to these treatments is crucial for evaluating treatment efficacy and the prognosis of patients.

Immunosuppressive myeloid cells, such as myeloid-derived suppressor cells (MDSCs), are implicated in resistance to ICI-based treatments by inhibiting effective antitumor immune responses.[8 9] These cells are also associated with poor survival outcomes of patients with gastric cancer treated with cytotoxic chemotherapy and targeted therapy,[10] suggesting that they may indicate aggressive tumor features linked to poor prognosis. Although myeloid cells are present in the tumor microenvironment (TME) of gastric cancer and harbor immunosuppressive characteristics,[11–13] the absence of an established biomarker representing specific myeloid cell populations or representative markers hinders their identification in clinical practice.

Translationally controlled tumor protein (TCTP), encoded by *TPT1*, is involved in various cellular processes, including cell cycle, growth, and anti-apoptosis. In tumors, TCTP is overexpressed in various cancers, including colorectal, urothelial, and breast cancer, and is associated with poor clinical outcomes.[14–16] Specifically, elevated *TPT1* expression levels correlate with immune-refractory phenotypes and poor survival outcomes with ICIs in urothelial cancer.[15] Mechanistically, TCTP decreases T-cell trafficking to the tumors and impairs T-cell-mediated tumor cell killing.[15] Furthermore, TCTP released from tumor cells promotes the accumulation of MDSCs in the TME through its interaction with the TLR2 signaling pathway, which activates the CXCL1/2-CXCR2 pathway and enhances the suppressive function of polymorphonuclear (PMN) MDSCs.[17] These findings suggest that TCTP plays a role in resistance to anticancer treatments via immune evasion and may be a viable target to overcome such resistance. However, the expression levels of TCTP and its clinical and immunological implications in patients with gastric cancer remain poorly understood.

Therefore, this study aimed to investigate the prognostic and immunological significance of blood TCTP expression levels in patients with advanced gastric cancer treated with cytotoxic chemotherapy and ICIs via ELISA, multiplexed proteomic assay (Olink), and publicly available single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics data sets.

METHODS

Study design

This study was conducted employing prospective biomarker cohorts from patients with metastatic, recurrent, or locally advanced unresectable gastric cancer, whose plasma samples were archived at Asan Medical Center (Seoul, Korea). To assess the clinicopathologic characteristics and prognostic value of plasma TCTP levels, clinicopathologic data were extracted from electronic medical records and analyzed in relation to plasma TCTP levels. Baseline plasma samples were used to evaluate the prognostic value of TCTP levels in each therapeutic context. PD-L1 CPS, MMR status, and EBV positivity were assessed as previously described.[18]

Study patients

Figure 1A illustrates the study populations. Cohort 1 included patients with HER2-negative tumors treated with first-line fluoropyrimidine plus platinum (FP) chemotherapy between October 2014 and September 2019 (n=143). Cohort 2 comprised patients treated with third-line or later nivolumab monotherapy between August 2015 and May 2023 (n=165) to assess the clinical relevance of plasma TCTP levels in relation to ICIs.

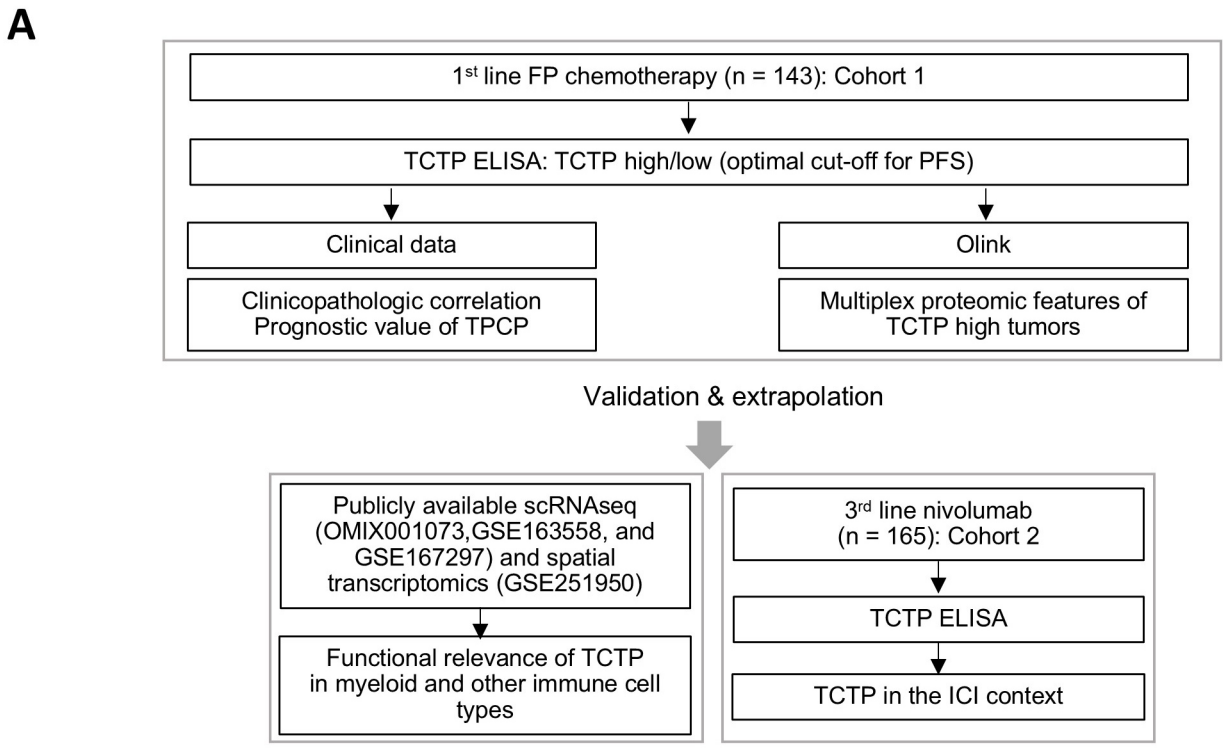
TCTP ELISA

Blood samples were collected in EDTA-coated tubes and centrifuged at 1,800 rpm for 10 min at room temperature. After centrifugation, 1 mL of plasma was aliquoted into cryotubes and stored at −80°C.

TCTP levels were quantified using a human TCTP ELISA kit (LS-F12706, LS Bio, Seattle, Washington, USA) following the instructions of the manufacturer, and the optical density was measured at 450 nm using a microplate reader (SpectraMax190, Molecular Devices).

Multiplex proteomic analysis based on the proximity extension immunoassay

Plasma protein levels were assessed using proximity extension immunoassay technology from Olink Proteomics (Uppsala, Sweden) using the Olink Target Immuno-Oncology 96-plex panel (V.3113). The proteomics data



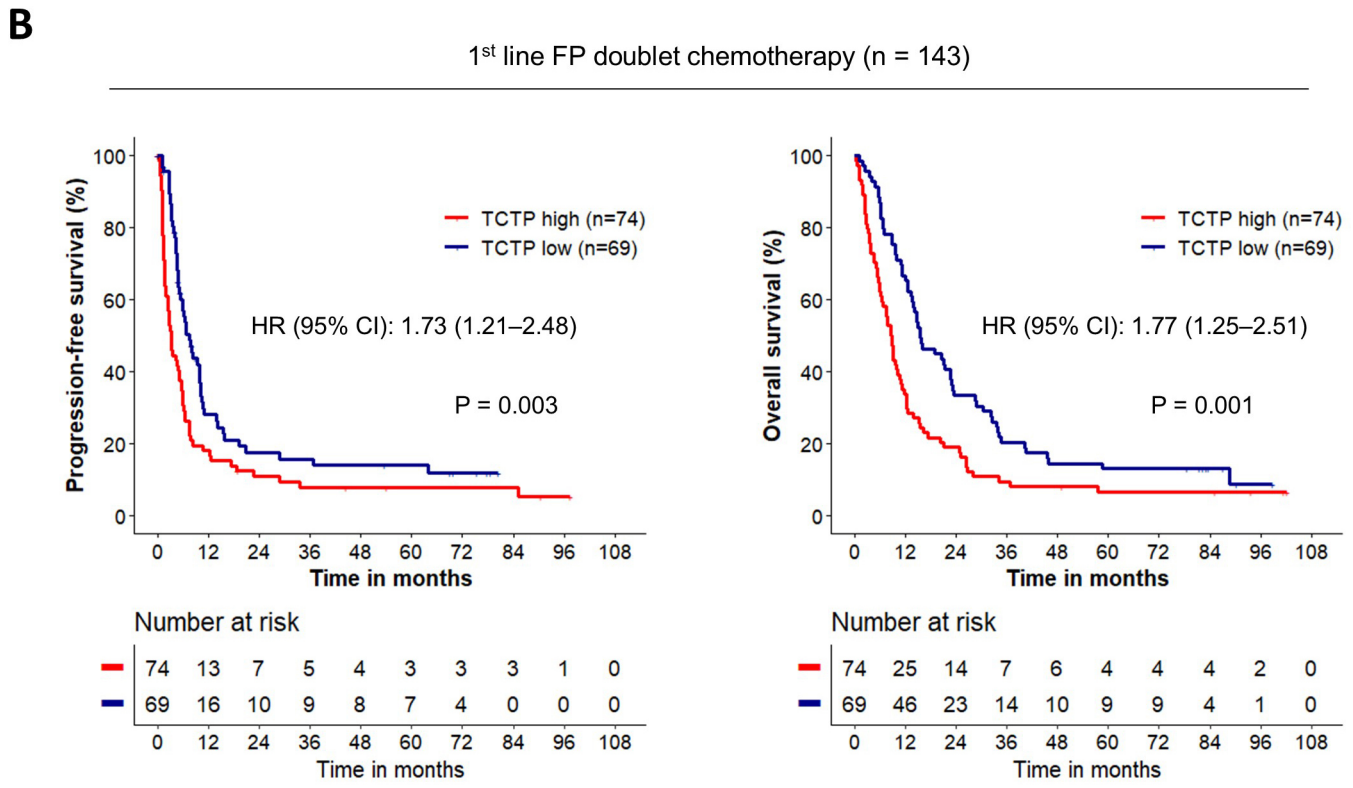


Figure 1 Study scheme. (A) Overall study scheme. (B) Survival outcomes of patients with gastric cancer treated with fluoropyrimidine plus platinum (FP) doublet chemotherapy (n=143), categorized according to plasma TCTP levels. ICI, immune checkpoint inhibitor; PFS, progression-free survival; scRNA-seq, single-cell RNA sequencing; TCTP, translationally controlled tumor protein.

generated were background-corrected, log2-transformed, and normalized on a normalized protein expression (NPX) scale. Samples deviating> ±0.3 NPX from the plate median were flagged with a quality control (QC) warning. Of the 96 proteins, 6 proteins (FGF2, IL-33, CD28, IL-4, IL-5, ARG1) were excluded from the analysis owing to

the QC warnings in >80% of samples. We performed a Spearman's rank correlation analysis to explore the relationship between expression levels of TCTP and immune-related proteins evaluated using Olink. Additionally, we identified proteins with significantly different NPX levels between the TCTP high and low groups (p<0.05, two-sided t-test) to link our findings to protein cascades. We performed an over-representation analysis of relevant pathways using the Gene Ontology Biological Process database (enrichR V.3.2).

Single-cell RNA sequencing

We analyzed 36 publicly available scRNA-seq data sets of gastric cancer tissues (OMIX001073,[11] GSE163558,[19] and GSE167297[20]). Additional filtering was performed in Seurat (V.4.4.0). We filtered cells based on expressed genes and mitochondrial percentage (nFeature_RNA>300 and per cent.mt<10). The filtered data matrix was normalized by the total number of unique molecular identifiers per cell and log2-transformed. Subsequently, we constructed a batch effects-corrected transformed "integrated" data matrix using Canonical Correlation Analysis integration (FindIntegrationAnchors function, number of feature genes=2,000) (online supplemental figure 1). After scaling, principal component analysis (PCA) (RunPCA function) was performed for dimensional reduction of the transformed "integrated" data matrix. Uniform Manifold Approximation and Projection (UMAP) and neighbors were determined using the top 30 principal components (PCs) with the RunUMAP and FindNeighbors functions. Cells were then clustered unsupervised based on the shared nearest neighbor (SNN) graph (FindClusters function, resolution=0.3) and visualized with UMAP. For subclustering analysis of myeloid and T_NK cells, each count matrix of the subcluster was preprocessed and clustered unsupervised using PCs as described above (myeloid subcluster: PCs=30, resolution=0.8; T_NK subcluster: PCs=30, resolution=0.4). Marker genes were identified by selecting Differentially expressed genes in each cluster relative to others using the Wilcoxon rank-sum test and the RunPrestoAll function (default parameter). Intercellular communication among cell types was analyzed using the CellChat package (V.2.1.2). A CellChat object was created based on the normalized count using the createCellChat function. Subsequently, overexpressed genes and the interactions between cell types were calculated using the identifyOverExpressedGenes, identifyOverExpressedInteractions, computeCommunProb, computeCommunProbPathway, and aggregateNet functions based on the CellChatDB database of ligand-receptor interactions in humans. The following gene signatures representing immunosuppressive features of myeloid cells were used: tumor-associated macrophage (TAM),[21 22] CSF1 response,[23] M2 macrophages,[24] myeloid inflammation,[25] CD73 TAM,[26] and protumorigenic signatures [27] (online supplemental file 1).

Spatial transcriptomics

For spatial transcriptomics analysis of gastric cancer, we analyzed the publicly available 10x Visium data set of tumor tissues from 10 patients (GSE251950).[28] Filtering was performed in Seurat (V.4.4.0) using expressed gene counts (nCount_Spatial >1,000). To deconvolute the Visium data sets, spacexr (V.2.2.1) was used to infer the cell type compositions of spots based on previously analyzed public scRNA-seq data sets. Cell-type decomposition was performed using the run. RCTD function (doublet_mode="full"). The coexistence of gene expression and deconvoluted cell types in each Visium data set was calculated using Spearman's rank correlation coefficient (cor.test function, stats V.4.1.3). For unsupervised clustering of Visium spots, samples were integrated using the top 2,000 variable genes from the SelectIntegrationFeatures function. After merging the data sets, PCA (RunPCA function) was performed for dimensional reduction of the transformed "SCT" data matrix. Batch effects were corrected using the RunHarmony function in harmony (V.1.0.3). UMAP and neighbors were determined using the top 30 principal PCs (RunUMAP and FindNeighbors functions). Visium spots then underwent unsupervised clustering based on the SNN graph (FindClusters function, resolution=0.4) and were visualized with UMAP.

Statistical analysis

Progression-free survival (PFS) was defined as the time from the initiation of chemotherapy (index date) to the date of disease progression, according to Response Evaluation Criteria in Solid Tumors V.1.1, or death, whichever occurred first. OS was defined as the time from the index to the date of death from any cause. The Kaplan-Meier method was used to estimate survival outcomes, and the log-rank test was used to compare survival outcomes between subgroups. Categorical variables among subgroups were compared using the χ^2 or Fisher's exact tests. To compare patients with different TCTP levels, the maximally selected rank statistics according to Lausen were used to determine the optimal cut-off value of TPCP levels that best segregated the PFS outcomes (249.2 ng/mL). Survival sensitivity analysis of the TCTP cut-off value was performed using the maximally selected rank statistics for OS, according to Lausen. Sensitivity analyses for the cut-off value were also performed using area under the receiver operating characteristic curve (AUROC) analysis for PFS events, maximizing Youden's Index and minimizing the distance to the upper left corner (244.4 ng/mL) and the median TCTP value (260.0 ng/mL).

A Cox proportional hazards model was employed to estimate HRs and corresponding 95% CIs. In the multivariate analysis, variables with a potential relationship (p<0.1) in the univariate analyses, together with demographic factors (age and sex), were included. Propensity score matching was performed using Eastern Cooperative Oncology Group performance status (ECOG PS) (0/1 vs 2) and a history of gastrectomy. Logistic regression was

used for propensity score estimation, and 1:1 nearest neighbor matching with a 0.3 caliper (R package "MatchIt") was applied. After matching, the data set contained 52 pairs of low and high TCTP patients, with an absolute standardized mean difference of 0.1 used to assess balance. All statistical analyses were performed using R V.4.2.3. A two-sided p value of <0.05 was considered statistically significant.

RESULTS

Clinical characteristics and prognostic value according to plasma TCTP levels

We analyzed plasma TCTP levels in patients with gastric cancer treated with first-line FP doublet chemotherapy (cohort 1, table 1). To compare patients with different TCTP levels, they were divided into high-TCTP and low-TCTP subgroups using a cut-off value (249.2 ng/mL), which optimally distinguished the PFS in this cohort. The cohort had a median age of 57 years, with 63.6% being men. Most patients exhibited ECOG PS of 0/1 (81.8%). While no difference was observed in the distribution of

Table 1 Baseline characteristics of cohort 1 stratified based on translationally controlled tumor protein levels

Characteristics	Overall (n=143)	TCTP-low (n=69)	TCTP-high (n=74)	P value*
Median age (range)	57 (24–86)	56 (33–86)	57 (24–77)	0.787
Sex				0.125
Female	52 (36.4)	30 (43.5)	22 (29.7)	
Male	91 (63.6)	39 (56.5)	52 (70.3)	
Disease status				0.053
Initially metastatic	101 (70.6)	42 (60.9)	59 (79.7)	
Recurrence	36 (25.2)	23 (33.3)	13 (17.6)	
Locally advanced unresectable	6 (4.2)	4 (5.8)	2 (2.7)	
Location				0.497
Gastric	141 (98.6)	69 (100.0)	72 (97.3)	
GEJ	2 (1.4)	0 (0.0)	2 (2.7)	
Histology	(n=141)	(n=69)	(n=72)	0.216
WD/MD	34 (24.1)	13 (18.8)	21 (29.2)	
PD/SRC	107 (75.9)	56 (81.2)	51 (70.8)	
ECOG PS				0.029
0/1	117 (81.8)	62 (89.9)	55 (74.3)	
≥2	26 (18.2)	7 (10.1)	19 (25.7)	
Gastrectomy				0.002
Done	53 (37.1)	35 (50.7)	18 (24.3)	
Not done	90 (62.9)	34 (49.3)	56 (75.7)	
Site of metastasis				
Lymph node	64 (44.8)	28 (40.6)	36 (48.6)	0.423
Peritoneum	92 (64.3)	43 (62.3)	49 (66.2)	0.755
Liver	18 (12.6)	5 (7.2)	13 (17.6)	0.108
Lung	7 (4.9)	0 (0.0)	7 (9.5)	0.014
Bone	12 (8.4)	3 (4.3)	9 (12.2)	0.167
Number of metastatic organs				0.091
≥2	59 (41.3)	23 (33.3)	36 (48.6)	
Chemotherapy regimen				0.334
FOLFOX	46 (32.2)	19 (27.5)	27 (36.5)	
XELOX	97 (67.8)	50 (72.5)	47 (63.5)	

Data are presented as median (range) or number (percentage).
*P value of TCTP low versus high.
CPS, combined positive score; dMMR, deficient mismatch repair protein; ECOG, Eastern Cooperative Oncology Group; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; GEJ, gastroesophageal junction; MD, moderately differentiated; PD, poorly differentiated; PS, performance status; SRC, signet ring cell carcinoma; WD, well differentiated; XELOX, Capecitabine and oxaliplatin.

age and sex between high and low TCTP groups, patients with high TCTP levels had a greater proportion with ECOG PS≥2 (25.7% vs 10.1%, p=0.029) and a lower proportion with a history of gastrectomy (24.3% vs 50.7%, p=0.002) (table 1).

Compared with patients with low TCTP levels, those with high plasma TCTP levels had significantly poor PFS (median PFS 3.2 vs 7.3 months, log-rank p=0.003) and OS (median OS 8.6 vs 15.6 months, log-rank p=0.001) (figure 1B). Among patients with measurable lesions (n=60), the high TCTP group demonstrated a lower objective response rate (ORR) compared with the low TCTP group (20.7% vs 48.4%, p=0.048). Multivariate analysis revealed elevated TCTP levels as an independent predictor of PFS (HR: 1.51, 95% CI: 1.04 to 2.20, p=0.029) and as being related to poor OS with marginal statistical significance (HR: 1.43, 95% CI: 0.99 to 2.06, p=0.054) (online supplemental table 1). Analysis of the propensity score-matched cohort, adjusted for ECOG PS and gastrectomy (online supplemental table 2), revealed that higher TCTP level was related to a trend of poor PFS (HR: 1.43, 95% CI: 0.94 to 2.17, p=0.097) and significantly linked to poor OS (HR: 1.52, 95% CI: 1.01 to 2.30, p=0.045) (online supplemental figure 2). Multivariate analysis revealed that a high TCTP level was independently related to poor PFS and OS (online supplemental table 3).

Sensitivity analyses were performed to determine the plasma TCTP cut-off value. The cut-off value based on maximally selected rank statistics according to Lausen for OS was identical to that for PFS (online supplemental figure 3A). AUROC analysis for PFS events identified 244.4 ng/mL as the cut-off value with an area under the curve of 0.582 and a positive predictive value of 77.6%, with comparable performance across parameters including the sum of sensitivity and specificity, Youden's Index, distance to the upper-left corner, and accuracy (online supplemental figure 3B). This cut-off value effectively distinguished survival outcomes between high and low TCTP groups (online supplemental figure 3C). Similar results were observed when using the median TCTP level (260 ng/mL) as a cut-off point (online supplemental figure 3D).

Proteomic features according to plasma TCTP levels

Given the prognostic relevance of plasma TCTP levels, we employed a multiplex proteomic approach (Olink) to explore the biology underlying elevated TCTP expression levels. In the high plasma TCTP group, proteins associated with immunosuppressive myeloid cell inflammation (ie, IL-6, IL-8, CXCL1, CSF-1, CCL3, CCL4 and CCL23), angiogenesis (ie, VEGF, ANGPT1 and PDGFB) and immune exclusion or suppression of T/natural killer (NK) cell function (ie, transforming growth factor-β 1 and interleukin-10) were upregulated (figure 2A). CXCL1, which is produced from myeloid MDSCs when TCTP binds to TLR2 and activates PMN-MDSCs to induce immunosuppression,[14] was significantly higher in the high TCTP group. TNFSF14 and HGF were among the top upregulated proteins in TCTP-high tumors. Furthermore, positive correlations were observed between their expression and plasma TCTP levels (figure 2B and table 2). Online supplemental figure 4 presents the detailed expression levels of these proteins. Pathway analysis revealed that proteins upregulated in TCTP-high tumors were linked to inflammatory response, vascular endothelial response, and monocyte chemotaxis (figure 2D).

In contrast, the TCTP-low group exhibited prominent upregulation of proteins involved in T-cell activation/exhaustion (ie, PD-1, LAG-3, GZMA, CD244, and ICOSLG) (figure 2A,C, table 2). This finding was supported by their negative correlation with plasma TCTP levels (figure 2B) and pathway analysis linking their association with T-cell activation-related pathways (figure 2E).

Upregulation of TPT1 in immunosuppressive myeloid cells and inhibition of effector T-cell function

To further investigate the link between TCTP and immunosuppressive properties in the TME, we analyzed publicly available scRNA-seq data sets of gastric cancer. Unsupervised clustering identified 161,967 cells grouped into 10 distinct cellular subsets (online supplemental figure 5A). *TPT1* was expressed across epithelial (including tumor cells), myeloid, and T/NK cells (online supplemental figure 5B). Myeloid cells preferentially expressed inflammation-related molecules and/or TCTP pathways, including *TLR2, CXCR2, CXCL1, CXCL2, CXCL3,* and *CXCL8* (online supplemental figure 5C).

Subsequently, we focused on myeloid cells (n=8,879), where unsupervised clustering identified eight distinct subsets (figure 3A,B). Among these, the Macrophage-chemokine subset, characterized by high *IL-1B, CCL3, CCL20,* and *VEGFA* expression (figure 3B), exhibited the highest *TPT1* levels (figure 3C) with the highest proportion of cells in the top 25th percentile of *TPT1* expression (figure 3D). The Macrophage-chemokine subset also exhibited the highest expression levels of TCTP-related molecules such as *TLR2* (a receptor for TCTP in myeloid MDSCs), *CXCL1,* and *CXCL2* (chemokines secreted by myeloid MDSCs that activate the immunosuppressive function of PMN-MDSCs through CXCR2) (figure 3C). Additionally, Macrophage-profibrotic and PMN-MDSC subsets showed relatively high expression levels of *TPT1* and TCTP-related molecules (figure 3C,D).

Functionally, these *TPT1*-high subsets (Macrophage-chemokine subset, Macrophage-profibrotic, and PMN-MDSC) were enriched in gene signatures related to immunosuppressive characteristics of myeloid cells (figure 3E). Given the highest expression of *TPT1* and TCTP-related molecules in the Macrophage-chemokine subset, alongside its inhibitory features, interactome analyses were performed to assess its functional relevance. The Macrophage-chemokine subset interacted with the Macrophage-profibrotic and PMN-MDSC subsets through CXCL2-CXCR2, CXCL3-CXCR2, and CXCL8-CXCR2 pathways (online supplemental figure 6A), promoting

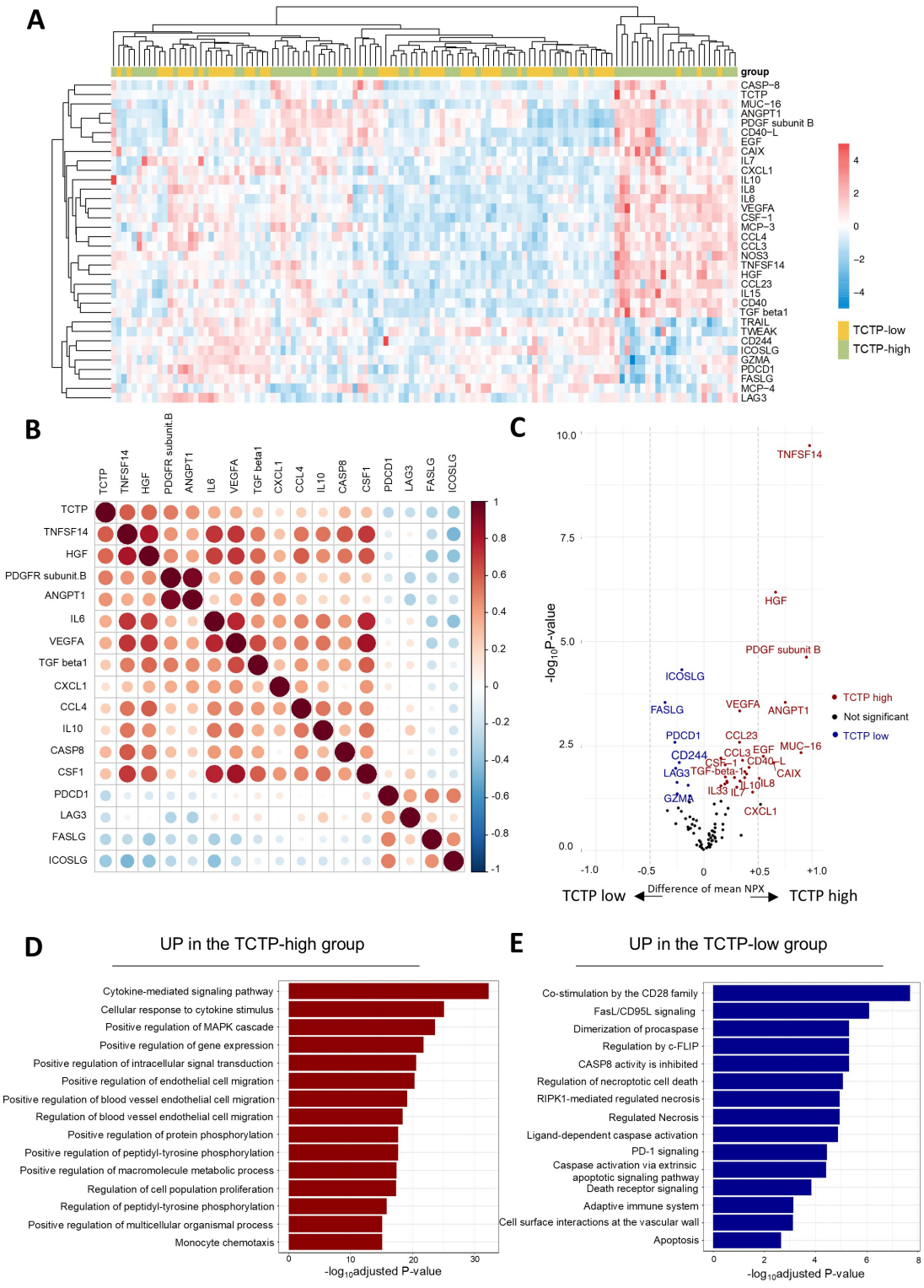


Figure 2 Proteomic features of blood based on plasma TCTP levels. (A) Heatmap illustrating clustering of protein expression levels in plasma. (B) Spearman's rank correlation plot of protein expression levels. Circle size reflects the magnitude of the correlation coefficients, and circle colors indicate the correlation coefficient (red: positive correlation; blue: negative correlation). (C) Volcano plot depicting the differential expression of proteins between the TCTP-high and TCTP-low subgroups. The X-axis shows the difference in mean NPX (mean NTX of the TCTP-high group—mean NPX of the TCTP-low group). The Y-axis represents the –log p value, which indicates the statistical significance of the differences between the two groups (ie, TCTP high vs TCTP low). Proteins with significant differential expression are situated at the extremes of the plot. Upregulated proteins in the TCTP-high group are shown as red dots on the right, while downregulated proteins in the TCTP-low group are shown as blue dots on the left. (D–E) Pathway analysis of proteins enriched in TCTP-high (D) and TCTP-low (E) subgroups. P values are adjusted using the Benjamini-Hochberg method. NPX, normalized protein expression; TCTP, translationally controlled tumor protein; UP, upregulated.

Table 2 Proteins significantly correlated with plasma translationally controlled tumor protein levels

Protein	Spearman's correlation coefficient (ρ)	P value
TNFSF14	0.607	<0.001
HGF	0.578	<0.001
PDGF subunit B	0.512	<0.001
ANGPT1	0.450	<0.001
IL-6	0.363	<0.001
VEGFA	0.357	<0.001
EGF	0.346	<0.001
CD40-L	0.343	<0.001
CASP-8	0.326	<0.001
MUC-16	0.303	<0.001
MCP-3	0.289	<0.001
CSF-1	0.285	<0.001
NOS3	0.281	0.002
CCL3	0.263	0.003
TGF-beta-1	0.256	0.004
CCL23	0.243	0.007
IL-15	0.243	0.007
CCL17	0.240	0.008
CCL4	0.238	0.008
CD40	0.219	0.015
IL-7	0.212	0.019
TIE2	0.200	0.027
IL-10	0.200	0.027
Gal-9	0.185	0.042
CXCL13	0.180	0.047
PD-L1	0.179	0.048
MCP-4	−0.179	0.049
TWEAK	−0.199	0.028
CD244	−0.225	0.013
LAG3	−0.233	0.010
PDCD1	−0.265	0.003
FASLG	−0.291	0.001
ICOSLG	−0.365	<0.001

TCTP-related immunosuppressive functions. Additionally, interactome analyses between the Macrophage-chemokine and T/NK cell subsets (online supplemental figures 6B,C) revealed inhibitory signaling from the Macrophage-chemokine subset to T-cell subsets through CCL20-CCR6[29] and THBS1-CD47[30] pathways (figure 3F).

Spatial distribution of tumor and immunosuppressive myeloid cells

To further investigate the functional relevance of immunosuppressive *TPT1*-high myeloid cells, we analyzed a publicly available spatial transcriptomics dataset of gastric cancer. We performed Robust Cell Type Decomposition to deconvolute spatial transcriptomic data with gene expressions from each cell type of previously analyzed scRNA-seq data. Unsupervised clustering (figure 4A and online supplemental figure 7A) identified a spot cluster enriched in tumor cells alongside *TPT1*-high Macrophage-chemokine and Macrophage-profibrotic (Tumor_MDSC spot cluster) (figure 4B). This subset exhibited the highest *TPT1* expression levels among all clusters (figure 4C), with the proportion of this spot positively correlating with average *TPT1* expression levels (figure 4D). High *TPT1* expression in the tumor spatially coincided with the distribution of the tumor_MDSC spot and *TPT1*-high myeloid subsets such as Macrophage-chemokine and Macrophage-profibrotic (figure 4E). Conversely, high *TPT1* expression was spatially exclusive of the T_B myeloid spot, enriched for CD8+ and CD4+ T cell and dendritic cell subsets (figure 4E), alongside T-cell subclusters (online supplemental figure 7B).

Overall, *TPT1* is highly expressed, specifically in an immunosuppressive macrophage subset that transmits inhibitory signals to T/NK subsets. The spatial co-localization of tumor cells and immunosuppressive *TPT1*-high myeloid cells in the gastric cancer microenvironment, with their abundance positively correlating with tumor *TPT1* expression level, suggests a coordinated role in establishing an immunosuppressive gradient and limiting effector T-cell infiltration.

Survival outcomes with nivolumab based on TCTP levels

Given the immunosuppressive features associated with high TCTP expression levels, we attempted to validate the clinical relevance of elevated TCTP levels in the context of ICI. Therefore, we correlated the clinical outcomes of patients with gastric cancer treated with nivolumab as third or later line of treatment (n=165) (cohort 2) (online supplemental table 4). The proportion of patients with a PD-L1 CPS of ≥1 and ≥5 was similar between high and low plasma TCTP groups (online supplemental table 4).

Using the same cut-off level as in cohort 1, patients with high plasma TCTP levels exhibited significantly poor PFS (median PFS 1.2 vs 1.6 months, log-rank p=0.048) and OS (median OS 2.6 vs 4.7 months, log-rank p=0.015) with nivolumab treatment (figure 5A). Among patients with measurable lesions (n=111), ORR was 6.8% and 7.5% in the TCTP-high and low groups (p=0.999), respectively. Multivariate analysis revealed that, whereas PD-L1 CPS≥5 was independently associated with improved PFS (HR 0.30, 95% CI: 0.16 to 0.75, p=0.002) and OS (HR 0.21, 95% CI: 0.10 to 0.44, p<0.001), high TCTP levels independently predicted shorter PFS (HR 1.64, 95% CI: 1.08 to 2.48, p=0.021) and were marginally linked to poor OS (HR 1.46, 95% CI: 0.94 to 2.28, p=0.095) (online supplemental table 5).

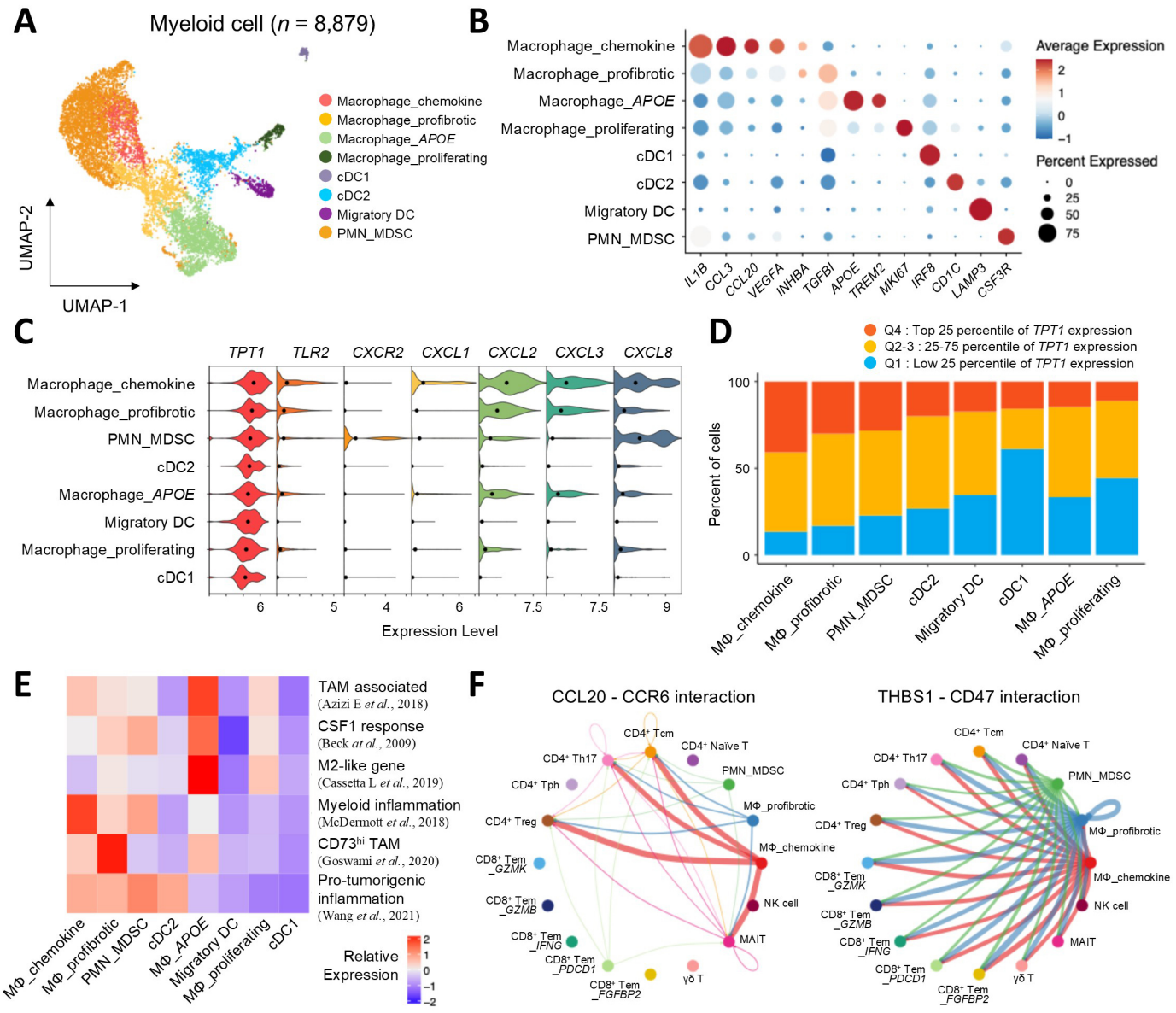


Figure 3 Single-cell RNA sequencing analysis of myeloid cells focusing on TCTP-related molecules. (A) Unsupervised clustering of myeloid cells revealing eight distinct subsets visualized using UMAP embedding. (B) Dot plots showing the average normalized expressions of marker genes in each myeloid cell cluster. (C) Violin plots displaying expression levels of *TPT1* and TCTP-related genes. (D) Proportion of cells with varying *TPT1* expression levels across myeloid subsets. (E) Heatmap illustrating the expression levels of gene signatures related to immunosuppressive features in myeloid cells. (F) Dot plots showing the relationship between latent patterns and cell groups in the CCL20-CCR6 and THBS1-CD47 pathway between *TPT1*-high myeloid cells (ie, Macrophage-chemokine, Macrophage-profibrotic and PMN-MDSC) and T/NK cells. MDSC, myeloid-derived suppressor cell; NK, natural killer; PMN, polymorphonuclear; TAM, tumor-associated macrophage; TCTP, translationally controlled tumor protein; UMAP, Uniform Manifold Approximation and Projection.

DISCUSSION/CONCLUSION

In this study, we examined the potential of TCTP as a marker for treatment efficacy and prognosis in gastric cancer. In this multimodal biomarker study, we employed prospectively collected clinical samples and analyzed external scRNA-seq and spatial transcriptomics datasets of gastric cancer to investigate the prognostic and immunological relevance of TCTP in patients with advanced gastric cancer. Our findings showed that high plasma TCTP levels were associated with poor survival outcomes in patients treated with first-line FP doublet chemotherapy. Multiplex proteomic analysis revealed that high plasma TCTP levels were associated with the upregulation of proteins involved in immunosuppressive myeloid cell inflammation, angiogenesis, and immune exclusion and/or suppression of T/NK cell function. scRNA-seq and spatial transcriptomics data also revealed high levels of *TPT1* expression in myeloid cells with immunosuppressive characteristics. Finally, high plasma TCTP levels were associated with poor survival outcomes

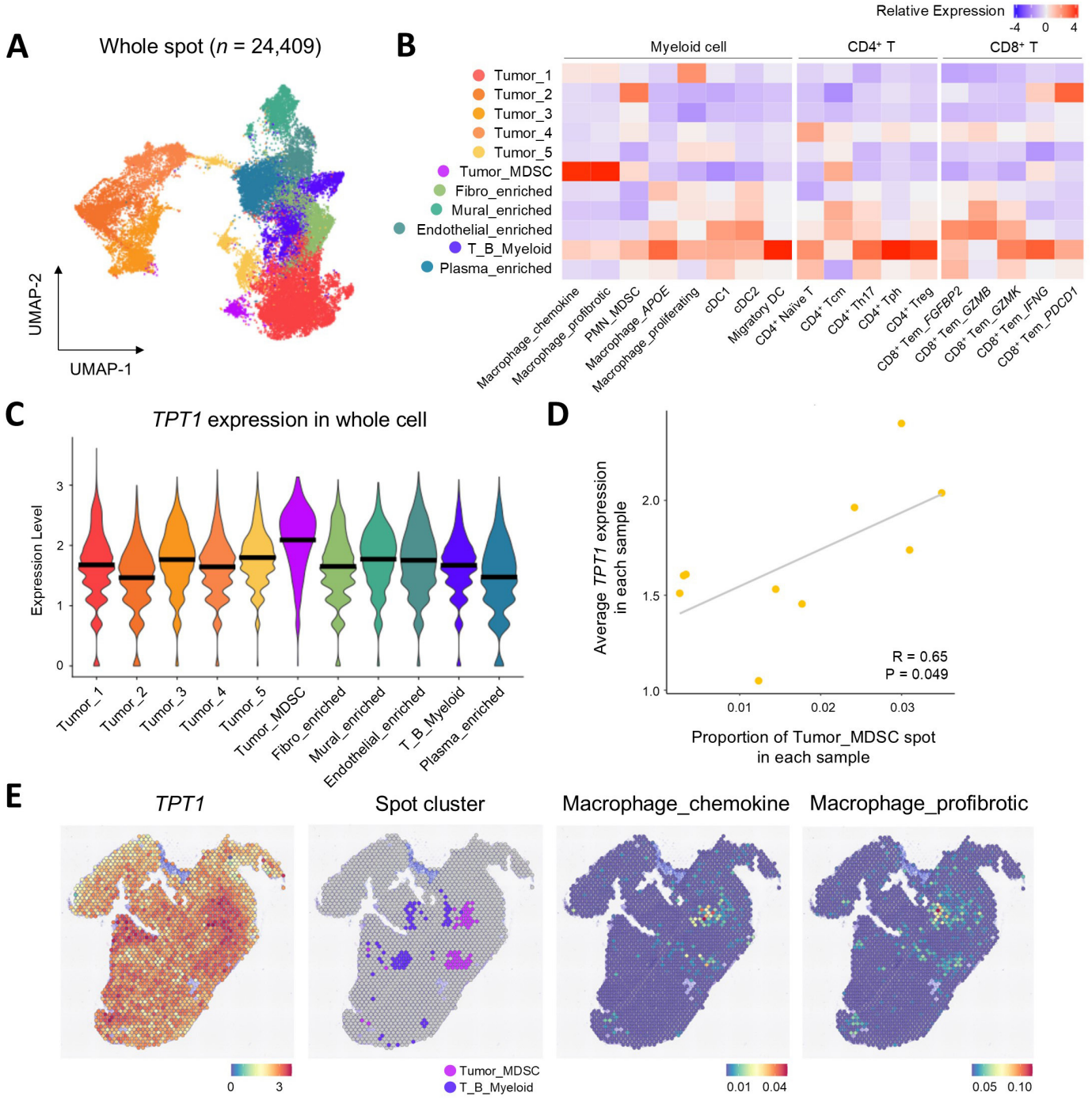


Figure 4 Spatial transcriptomic analysis with regards to *TPT1* expression. (A) Unsupervised clustering of spot clusters revealing 11 distinct clusters as visualized using UMAP embedding. (B) Heatmap depicting the abundance of myeloid and T-cell subsets across each spot cluster. (C) Violin plot showing *TPT1* expression levels in each spot cluster. (D) Correlation between the proportion of the tumor_MDSC spot and average TPT1 expression in each sample. (E) Spatial distribution of TPT1 expression, the tumor_MDSC and T_B_myeloid spot cluster, and abundance of *TPT1*-high myeloid cells (ie, Macrophage-chemokine and Macrophage-profibrotic). MDSC, myeloid-derived suppressor cell; UMAP, Uniform Manifold Approximation and Projection.

in patients with gastric cancer treated with nivolumab. Our findings suggest plasma TCTP as a prognostic biomarker that reflects clinically relevant immunosuppressive signals in patients with gastric cancer. To our knowledge, this study is the first to systematically delineate the clinical relevance of a blood-based biomarker and its immunological implications in patients with gastric cancer treated with ICI and cytotoxic chemotherapy. Moreover, our findings provide a rationale for incorporating TCTP-targeted therapy into chemotherapy and/or ICI to reverse the immunosuppressive gradient in the gastric cancer microenvironment and

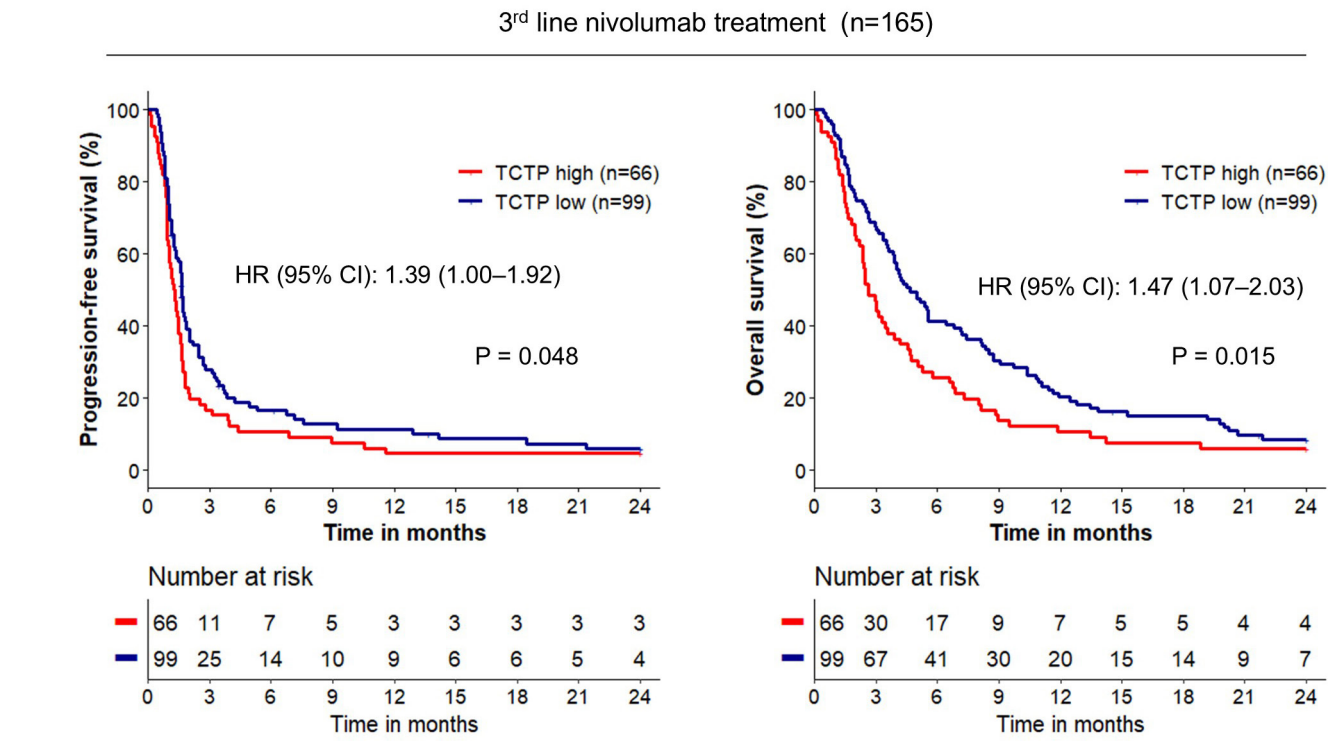


Figure 5 Survival outcomes of patients with gastric cancer treated with nivolumab monotherapy based on plasma TCTP levels. Survival outcomes of patients with gastric cancer treated with nivolumab (n=165), categorized by plasma TCTP levels (A). TCTP, translationally controlled tumor protein.

ultimately improve clinical outcomes of patients with gastric cancer.

Our study has clinical implications to demonstrate the unfavorable prognostic value of TCTP in independent cohorts of gastric cancer treated with ICI and cytotoxic chemotherapy. Our findings align with those of previous preclinical studies showing that TCTP is associated with resistance to cytotoxic chemotherapy[31] and ICIs.[15] Myeloid cells have been implicated as key drivers of resistance to chemotherapy and immunotherapy, among factors contributing to rapid disease progression and/or resistance to chemotherapy and ICI.[8 9] However, no specific biomarker represents the immunosuppressive gradient of these myeloid cells. Our analysis links high plasma TCTP levels with proteins involved in immunosuppressive myeloid cell inflammation, angiogenesis, and immune exclusion and/or suppression of T/NK cell function. These findings align with the concept that proangiogenic signals collaborate with myeloid cells to suppress antitumor T-cell responses.[8 25 32] In contrast, patients with low plasma TCTP levels exhibit enriched signals indicative of active antitumor T-cell responses and subsequent T-cell exhaustion. This suggests that TCTP may inhibit antitumor T-cell responses, consistent with the findings of previous studies.[14 15] Future studies should evaluate the relationship between TCTP expression and molecular subtypes, such as the mesenchymal subtype, which is linked to angiogenesis and poor prognosis.[33 34]

To further validate our results, we analyzed publicly available scRNA-seq and spatial transcriptomics data sets of gastric cancer. *TPT1* is highly expressed specifically in the immunosuppressive Macrophage-chemokine subset, which inhibits T/NK subsets through immunosuppressive pathways,[29 30] suggesting its role in suppressing effector T-cell responses. Furthermore, the *TPT1*-high Macrophage-chemokine subset interacts with other immunosuppressive myeloid cells through CXCL2-CXCR2, CXCL3-CXCR2, and CXCL8-CXCR2 pathways, validating known mechanisms of *TPT1* in gastric cancer myeloid cells.[14] The spatial coexistence of tumor cells and immunosuppressive *TPT1*-high myeloid cells in the gastric cancer microenvironment, with their abundance positively correlating with *TPT1* expression levels in the tumor, suggests that these cells may jointly drive an immunosuppressive gradient and impede effector T-cell infiltration. These findings suggest that differential *TPT1* expression in the gastric cancer microenvironment is linked to the abundance and potential interactions of immunosuppressive *TPT1*-high myeloid cells with tumor cells. These results also demonstrate that tumor cells and immunosuppressive myeloid cells are potentially major sources of *TPT1* in the gastric cancer microenvironment. On the other hand, *TPT1* expression in other cell types such as T/NK cells suggests the need for future studies to explore its role in driving immunosuppressive gradients in these cell types. Overall, TCTP expression is related

to the immunosuppressive features of a specific myeloid subset, contributing to the suppression of antitumor response in gastric cancer, which is in line with our findings with Olink analyses. Therefore, our data support the immunosuppressive role of TCTP in myeloid cells, which may serve as a critical factor in immune evasion within the gastric cancer microenvironment.

Compared with previous studies that assessed the prognostic value of TCTP in other cancer types or the immunological effect of TCTP on immune response using mouse models, our study uniquely explores the detailed immunological implications of TCTP levels, identifying a specific immunosuppressive myeloid subset and its functional role in immune evasion. Moreover, while previous studies focused on evaluating TCTP expression in tumor tissue,[14–16] our study emphasizes the clinical relevance of measuring plasma TCTP levels in patients with cancer. Given the diagnostic and therapeutic challenges posed by substantial intratumoral heterogeneity in gastric cancer,[35] plasma TCTP levels may serve as a non-invasive, measurable, practical biomarker that can comprehensively reflect immunosuppressive signals in the TME, and it could be readily employed in clinical practice. No established consensus exists on the TCTP cut-off point. While we primarily adopted a cut-off with prognostic relevance in the first-line setting for conceptual comparison, sensitivity analysis revealed that cut-off levels determined by different methods showed similar values with prognostic significance, supporting their use in our study. Future studies should validate and refine the clinically relevant TCTP plasma cut-off point for patients with gastric cancer.

Interpretation of plasma TCTP levels as predictive of therapeutic response cannot be drawn from our results, as evaluation of biomarkers for their "predictive" capacity requires a control arm. Nevertheless, our finding that baseline TCTP levels are prognostically meaningful in the context of ICI treatment is clinically relevant. While TCTP alone may not compete with various predictive biomarkers such as PD-L1 CPS and MMR/MSI status, these biomarkers also have limitations.[36 37] Consequently, our data suggest that the functional relevance of plasma TCTP warrants further investigation in future studies, especially in the clinical context of currently available biomarkers, as in the cases of various biomarkers under active investigation. Our multivariate analysis in patients treated with nivolumab revealed TCTP as an independent factor for PFS alongside PD-L1 CPS and MMR status. From the exploratory analysis of the CheckMate-649 study, subgroups with a high PD-L1 CPS showed limited benefit from adding nivolumab to chemotherapy, whereas subgroups with a low PD-L1 CPS derived survival benefit from nivolumab addition.[37] Therefore, our findings, which reveal that TCTP was associated with survival outcomes independent of PD-L1 CPS, indicate that blood TCTP may complement PD-L1 CPS in predicting outcomes with ICI-based treatments in gastric cancer.

The specific high levels of *TPT1* expression in immunosuppressive myeloid cells and upregulation of TCTP-related signals in immunosuppressive myeloid cells suggest that TCTP could be a valuable therapeutic target for overcoming resistance to ICI and/or chemotherapy. Blocking TCTP reduces PMN-MDSC in the tumor and subsequently halts tumor progression in preclinical models.[14] Overall, these findings support targeting TCTP as a strategy for anticancer treatment. Efforts should focus on developing anti-TCTP treatments to improve survival outcomes with chemotherapy and/or ICIs. Several TCTP inhibitors are currently under investigation and warrant further study.[38 39] Given its mechanistic implications, blood TCTP could serve as a biomarker for selecting patients for anti-TCTP-based or anti-myeloid treatments.

The degree of survival difference between patients with high-TCTP and low-TCTP was similar in both ICI and chemotherapy-treated patients, suggesting that differential TCTP levels may not be specifically linked to ICI resistance. However, studies show that immunosuppressive myeloid features such as MDSCs and tumor-associated macrophages could affect outcomes in both ICI and chemotherapy treatments across various cancer types, including gastric cancer.[40–44] Moreover, cytotoxic chemotherapy not only exerts direct cytotoxic effects on tumor cells but also modulates immunosuppressive myeloid cells. For example, chemotherapeutic agents such as gemcitabine deplete specific subsets of immunosuppressive myeloid cells[45] and convert the phenotype of myeloid cells to an antitumor phenotype,[46] whereas others, including doxorubicin and paclitaxel, contribute to an increased accumulation of immunosuppressive myeloid cells.[47] On the other hand, chemotherapy-induced tumor cell death promotes antigen presentation and activates dendritic cells, promoting an immunostimulatory response.[48] However, recent studies indicate that the clearance of dying tumor cells by macrophages through pathways such as efferocytosis and autophagy may contribute to an immunosuppressive TME.[49 50] This interplay between cytotoxic chemotherapy and the plasticity of immunosuppressive myeloid cells supports the rationale for combining anti-myeloid cell targeting agents and cytotoxic chemotherapy (even without an ICI). Indeed, clinical trials have been designed to combine various anti-myeloid cell agents with chemotherapy (ie, NCT03177187, NCT02637531, NCT03336216, and NCT03719326). Therefore, similar survival differences with ICI and chemotherapy do not negate the functional role of TCTP in immunotherapy. Moreover, our findings suggest the association between immunosuppressive signals relevant to TCTP, chemotherapy, and nivolumab provides evidence for combining TCTP-targeted agents with chemotherapy and/or ICI in gastric cancer.

This study primarily focused on gastric cancer; however, given the prognostic effect of high TCTP expression levels in other cancer types,[14–16] our findings may be applicable to other cancer types. TCTP expression in tumor tissue is elevated in colorectal cancer and inversely correlates with an antitumor immune signature.[14] In addition, high *TPT1* expression is linked to poor survival outcomes in patients

with metastatic urothelial cancer treated with anti-PD-L1 treatment.[15] Future studies of other cancer types should validate our findings for subsequent application.

This study has some limitations. Our results of cohort 1 did not include patients with HER2-positive tumors, which may limit the interpretation of our findings in this subset. Additionally, the lack of matched blood and tumor tissue samples prevented an evaluation of the correlation between TCTP expressions in these two sources. Lastly, the prognostic value of plasma TCTP in other therapeutic settings remains unclear and warrants further investigation.

In conclusion, plasma TCTP is a readily measurable unfavorable prognostic biomarker that reflects clinically significant immunosuppressive signals from myeloid cells in patients with gastric cancer. Our findings provide a rationale for developing novel immunotherapeutic strategies targeting TCTP and underscore the need for validation in other cancer types.

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Patient consent for publication Not applicable.

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