

## M2 subtype tumor associated macrophages (M2-TAMs) infiltration predicts poor response rate of immune checkpoint inhibitors treatment for prostate cancer

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### ABSTRACT

**Background:** Prostate cancer (PCa) is poor response to the immunotherapy for its high heterogeneity of immune microenvironment. In this study, we aim to introduce a new immune subtype for PCa involving M2 tumour associated macrophages (M2-TAMs).

**Methods:** Three hundred and sixty-two PCa patients and matched normal prostate tissues were selected from the Cancer Genome Atlas and Gene Expression Omnibus databases. Patients' immune infiltration characters were then analyzed based on the gene expressions. The immune subtypes were identified by the method of unsupervised hierarchical clustering. Finally, the relationship between the M2-TAMs infiltration and anti-programmed death-ligand-1 (PD-L1) therapy was investigated in the IMvigor210 cohort.

**Results:** PCa expressed lower immune-related genes levels compared with the adjacent normal tissues. Based on the proved immunosuppressive mechanisms in PCa, tumour patients were classified into three independent subclasses with high infiltrated cytolytic activity (CYT), M2-TAMs and regulatory T cell (Tregs), respectively. Among these subtypes, M2-TAMs infiltration subtype showed the worst clinicopathological features and prognosis compared with the other two subtypes. The results of the IMvigor210 cohort demonstrated poor response of anti-PD-L1 therapy for patients with high M2-TAMs infiltration.

**Conclusion:** Prostate tumours involved in significant immunosuppression, and high infiltration of M2-TAMs can be applied to predict the effect of anti-PD-L1 therapy.

### KEY MESSAGES

- PCa patients can be classified into three immunotypes of high infiltrated CYT, M2-TAMS, and Tregs according to the immunosuppressive mechanisms.
- High M2-TAMs infiltration subtype reflected the worst clinical characters, immune infiltration, and lowest expression of immune checkpoint inhibitors among the three subclasses in PCa.
- High M2-TAMs infiltration predicts the low response rate of anti-PD-L1 therapy.

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### KEYWORDS

Prostate cancer; tumour microenvironment; immune subtype; M2-TAMs; anti-PD-L1


## Introduction

Prostate cancer (PCa) is one of the most common malignant tumours in the world and the second leading cause of cancer death in men. Until 2018, there were about 359,000 cases of prostate cancer in the world, accounting for 6.7% of the total deaths in men [1]. However, PCa patients have limited benefits from the current treatments. Nearly 20–35% of patients

with localized prostate cancer are reported to experience recurrence after the radical prostatectomy [2]. With the use of androgen deprivation therapy (ADT) for early-stage patients, most patients have not a durable response and eventually the disease progresses [3]. Thus, new immunotherapies are required to treat PCa patients in recent years, however, some immunotherapeutic agents, like anti-programmed

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death-ligand-1 (PD-L1) or anti-programmed death-1 (PD-1), are applied to treat a fraction of advanced PCa patients with poor response rate [4]. The interpatient clinical heterogeneities among these patients have not been resolved.

Tumour microenvironment (TME) is a highly heterogeneous ecosystem of tumour cells, immune cells and other stromal cells [5]. The infiltrated immune cells in the TME have been shown to be significantly associated with the development and invasion in tumour patients. It was proved that high infiltrated immunosuppressive cells, such as M2 tumour associated macrophages (M2-TAMs) and regulatory T cell (Tregs), as important causes of treatment failure and shorter survival times in PCa patients [6–8]. Of note, some defined as anti-tumour immune cells, like CD8+ T cells, were shown to be associated with lymph node metastasis in PCa [9]. This seems to indicate a high degree of immunosuppression in PCa. Given this, deeply analyze the specific mechanisms of PCa immune microenvironment may help us to understand different treating prognoses. In recent years, new computer-based technologies are applied to estimate the infiltrated immune cells density in TME of bulk tumours by using gene expression profiles [10–12]. By assigning values to each type of immune cell infiltration density, many tumour patients can be classified into different immune subtypes to predict the treatment and prognosis [13–15]. This demonstrates that the clinical diagnosis guided by immune infiltration is available. However, to date, there have been few studies to systematically depict the characters of immune microenvironment and immunotypes in PCa based on genome-sequencing data. One study explored a classification based on immune related genes to predict the clinical prognosis of PCa, but this has limitations as it ignores the role of immune cells in tumour development [16]. Besides, the precise relationship between immune cells and immunotherapy is still unclear. Hence, to explore the mechanisms of tumour microenvironments, and develop efficient predictors for clinical prognosis and therapy response in PCa is of great importance.

In this study, we performed the immunosuppressive characteristics of 362 PCa patients and their matched normal prostate tissues from TCGA and GEO databases based on the immunological profiles. According to the proved immunosuppressive mechanisms in PCa, tumour patients were classified into three independent subclasses with highly infiltrated cytolytic activity (CYT), M2-TAMs and Tregs, respectively. Patients with M2-TAMs subtype had the most immunosuppressive

performance compared with the other two subtypes. Furthermore, analysis of the IMvigor210 cohort reflected that high M2-TAMs infiltrated patients showed poor response to anti-PD-L1 therapy. Our results demonstrated the unique immune characteristics of PCa and provide a more accurate theoretical basis for immunotherapy of prostate cancer.

## Methods and materials

### *Patients and gene expression data*

The gene expression profiles were obtained from the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database, respectively as the discovery cohort and validation cohort. The patients with incomplete clinical, follow-up and pathological information were excluded. Finally, the FPKM RNA-Seq data and clinical information of 225 PCa patients and matched 51 normal prostate tissues were included from the TCGA database (<https://portal.gdc.cancer.gov/>), normalized gene expression data of 137 PCa patients and matched 70 normal prostate tissues were from the GSE70770 cohort (<https://www.ncbi.nlm.nih.gov/geo/>), respectively. The clinical information of GSE70770 cohort was further retrieved from a previous study [17]. Gene symbol names of GSE70770 were annotated based on the annotation platforms GPL10588 by R language and data normalization were performed with the  $\log_2^{(x+1)}$  expression, respectively.

The clinical information of the patients was summarized in [Supplementary Table 1](#), As a previous study [18], the D'Amico risk grade of PCa patients was identified based on the value of PSA, clinical T stage, and Gleason score.

### *Immune signatures*

As a previous study [19], CYT was computed as the geometric mean between the gene expression of PRF1 and GZMA. The prediction of immune cell infiltration of B cells, central memory CD4 T cell (CD4 + Tcm), effector memory CD4 T cell (CD4 + Tem), CD8 + T-cells and natural killer (NK) cells were performed using single-sample gene set enrichment analysis (ssGSEA) in the web analysis tool XCELL (<https://xcell.ucsf.edu/>). The signatures of interferon (IFN)-alpha response and IFN-gamma response pathway, innate immune cells and adaptive immune cells were obtained from two previous studies respectively [19,20]. The member genes of these signatures were listed in [Supplementary Table S2](#). Gene list of immune-related genes were obtained from the IMMPORT database

additionally (<https://www.immport.org/home>). Besides, we used ESTIMATE R package for calculating ESTIMATE immune score and stroma score of each sample. The prediction of M2 macrophage and Tregs fractions were computed by the web tool CIBERSORT (<https://cibersort.stanford.edu/>) when required. All signature values were normalized by limma package when required.

### **Identification of PCa immune subtypes**

Three immune gene signatures were selected for classifying the PCa: CYT, M2-TAMs and Tregs. The infiltration of M2-TAMs and Tregs were estimated using the CIBERSORT by submitting FPKM RNA-Seq of TCGA data and  $\log_2^{(x+1)}$  normalized gene expression matrix of GSE70770 data, respectively. The value of CYT was transformed using a logarithm. The cluster distance between each sample was defined Euclidean. Finally, the hierarchical cluster of 225 cases of tumour patients from the TCGA cohort and 137 cases from the GEO cohort was performed using the “Ward D2”, respectively.

For analysis of biological progress of M2-TAMs subtype, the top 10 upregulated hub genes of M2-TAMs subclass were selected from the two-fold differential expressed upregulated genes in M2-TAMs subclass compared to CYT and Tregs subclasses. The results of Go analysis were obtained from the DAVID online server by submitting gene symbols (<https://david.ncifcrf.gov/>). GSEA analysis was performed by uploading the transformed TPM RNA-Seq of the TCGA cohort to the GSEA server 3.0.

### **Analysis of a correlation between M2-TAMs subclass and response to immune checkpoint inhibitors (ICIs)**

Two hundred and ninety-eight patients with urothelial cancer who received anti-PD-L1 blocker (pembrolizumab) therapy was obtained from the IMvigor210 cohort [21]. The count RNA-Seq data and clinical information were collected from <http://research-pub.gene.com/IMvigor210CoreBiologies>. The DEseq2 R package was used to perform normalization, and the count RNA-Seq value was transformed into the TPM value for CIBERSORT analysis when required.

### **Statistical analysis**

All statistical analyses and visualization were conducted using R software (San Francisco, CA, Version

3.5.1) and GraphPad Prism (GraphPad Software, La Jolla, CA, Version 7.0). A log-rank test was used to estimate an association between different groups and survival prognosis of PCa patients. The Mann-Whitney test and Chi-squared test were used to evaluate differences between groups of continuous and categorical variables, respectively. Correlation tests were based on the person and distance analysis. The univariate and multivariate Cox regression analyses were performed to detect the prognostic factors. All statistical  $p$  value were two-side, with  $p < .05$  as statistically significant.

## **Result**

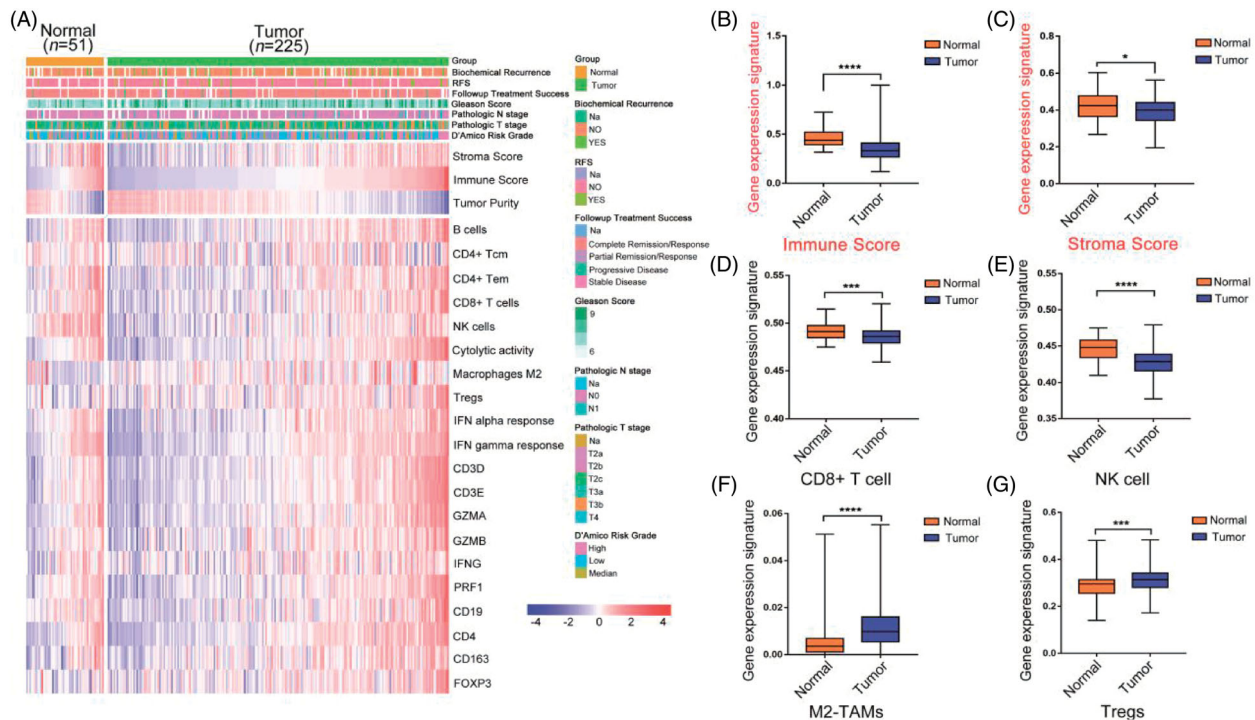
### **Immune characteristics of PCa**

To estimate immune cell infiltration in PCa, we firstly compared the immune infiltration of tumour and non-tumour prostate tissues respectively using ssGSEA. The multiple gene expression enrichment scores of seven immune cells and two inflammatory responses signal pathways were depicted in the TCGA cohort (Figure 1(A)). The results showed that significant lower immune score and stroma score in the tumour tissues compared with the normal tissues (Figure 1(B,C)). Several immune cells proportion between tumour and non-tumour prostate tissues were further analyzed. The infiltration of CD8+ T cell and NK cell in tumour lesion was significantly reduced compared with normal adjacent tissues (Figure 1(D,E)). In contrast, M2-TAMs and Tregs were highly infiltrated in prostate tumours than normal tissues (Figure 1(F,G)).

To validate the findings, we subsequently evaluated the difference of the immune infiltration between tumour and normal tissues of the prostate in the GEO cohort (Figure S1(A)). Likewise, lower immune score and stroma score were observed in tumours compared with normal tissues, although there was no significance of immune score between tumour and normal in the GEO cohort (Figure S1(B, C)). The changes of immune infiltration in prostate tumours showed significantly lower CD8+ T cells and NK cells compared with that in normal tissues (Figure S1(D, E)). While the infiltration of M2-TAMs and Tregs in tumours showed higher than that in normal tissues comparatively (Figure S1(F, G)). These results suggest that prostate cancer was immunosuppressed to some extends.

### **Immunosuppressive subtypes of PCa**

Previous studies have proved that the immune resistance of PCa related to the infiltrated immunosuppressive cells in TME. It was reported that high infiltration



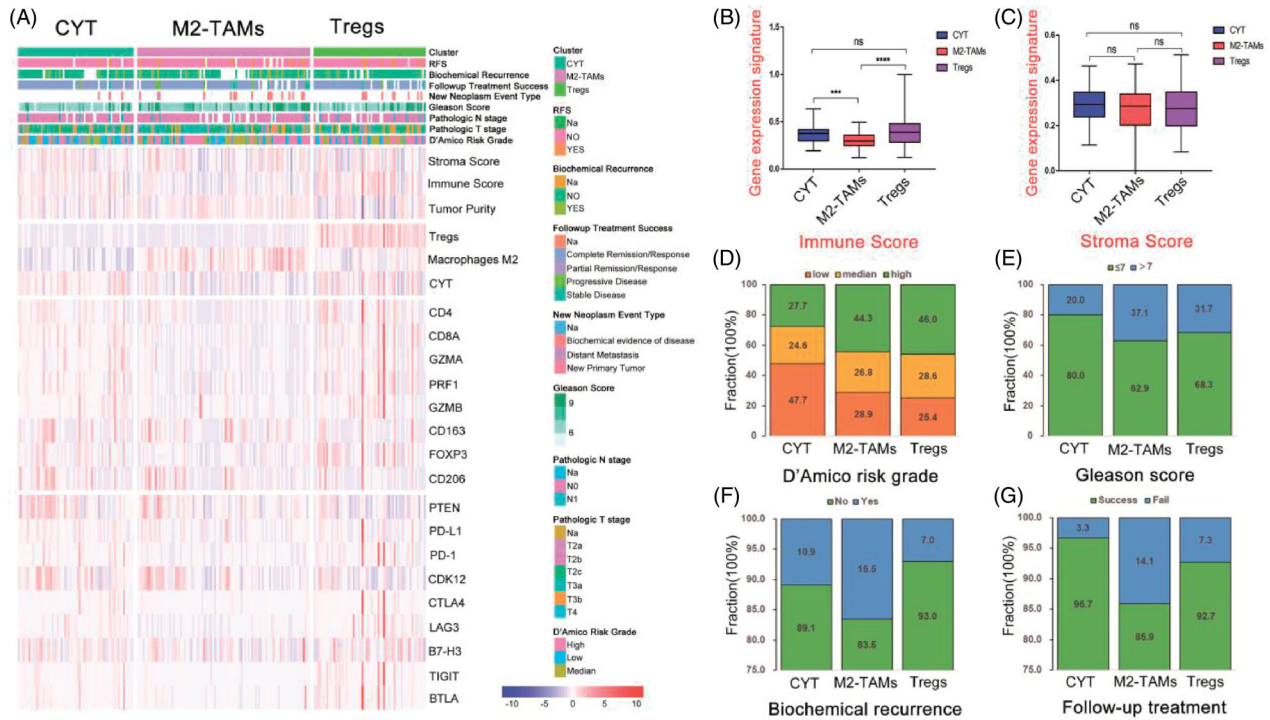
**Figure 1.** Prostate cancers showed immunosuppression in TCGA cohort. (A) The expression levels of immune-related genes and multigene signatures in tumour and non-tumorous prostate. Patients were ordered by the immune scores of each sample. RFS: recurrence free survival; CD4+ Tcm: central memory CD4 T cell; CD4 + Tem: effector memory CD4 T cell. (B, C) The difference of immune score and stroma score between normal and tumour prostate samples.  $p$  Values were computed using Mann-Whitney test (\*\*\*\* $p < .0001$ ; \* $p < .05$ ). (D–G) The difference expression of immune cell gene signature in normal and tumour prostate samples.  $p$  Values were computed using Mann-Whitney test (\*\*\* $p < .0001$ ; \*\* $p < .001$ ). M2-TAMs, M2 subtype tumour associated macrophages.

of M2-TAMs [22] promoted drug resistance in Castrate-resistant prostate cancer (CRPC) patients, and the Tregs were required to established and maintain the immune resistance. The CYT that represented immune activity of CD8 + T and NK cells had recently been used to predict the effect of immunosuppression in tumours [23]. Notably, our study showed that higher infiltrated M2-TAMs significantly related to the lower CYT infiltrations in both data of the TCGA and GEO cohort (Figure S2(A, D)). We hypothesized that PCa patients can be classified into different individual immune subtypes because of the immune divergence of these factors.

To investigate whether these immune mechanisms work jointly or separately in clinical samples in PCa, we further conducted an unsupervised hierarchical cluster of tumour samples by immune-related gene signatures within the TCGA cohort, the patients have finally identified three subtypes with highly infiltrated CYT, M2-TAMs, and Tregs (Figure 2(A)). Among these subtypes, the signature of CYT represented anti-tumour immunity, and other two clusters (M2-TAMs and Tregs) represented immunosuppressive components. Patients' numbers of these clusters were 65, 97, 63 (28.9, 43.1 and 28.0%), respectively. Based on the

immune characters, M2-TAMs cluster showed the lowest immune score among three clusters, which indicated significantly low immune infiltration in M2-TAMs subtype (Figure 2(B)), but the prediction of stroma score among three clusters showed no difference (Figure 2(C)). Differential clinical features among three clusters were then analyzed. Patients with M2-TAMs subclass showed the worst outcome compared with CYT and Tregs subtypes (Figure 2(D–G)), the results showed that tumours with high D'Amico risk, high Gleason score ( $>7$ ), and biochemical recurrence were concentrated in this subtype, which predicted a higher risk of recurrence with M2-TAMs infiltrated patients following localized treatment (Figure 2(D–F)). In contrast, patients in CYT cluster were enriched in the lowest D'Amico risk and Gleason score compared with M2-TAMs and Tregs subtype, which indicated that cluster CYT had better clinical outcome. The rate of following-up treatment success showed poorly respond to the treatment in M2-TAMs subtype compared with the other two subtypes (Figure 2(G)).

We further assessed the immune features of 137 PCa patients in GEO cohort (Figure S3A). The results showed that GEO cohort was also classified into the



**Figure 2.** Classification of prostate cancer based on immunosuppression mechanisms. (A) Unsupervised clustering of TCGA cohort ( $n=225$ ) prostate cancer by three immune-gene expression signatures (cytolytic activity, M2-TAMs and Tregs); expression levels of marker for T cells (CD4 and CD8A); Tregs (FOXP3); M2-TAMs (CD163 and CD206) and primary immune check points were also shown. (B, C) The differential expression of immune score and stroma score value among three immune subclasses (\*\*\*\* $p < .0001$ ; \*\*\* $p < .001$ ; ns: negative significance, Mann-Whitney test). (D–G) The differential proportions of tumour patients classified by clinical characters within three immune subclasses.

three clusters (CYT, 55.5%; M2-TAMs, 24.8% and Tregs, 19.7%), which indicated the stability of our immune classification. Consistent with the TCGA cohort, M2-TAMs subclass in the GEO cohort showed lower immune infiltration compared with the other two subclasses (Figure S3(B)), but with no difference of stroma component among these subclasses (Figure S3(C)). Besides, the difference of clinical features among three subtypes showed that patients in M2-TAMs subtype had the highest proportion for poor outcome compared with the other two clusters (Figure S3(D–F)).

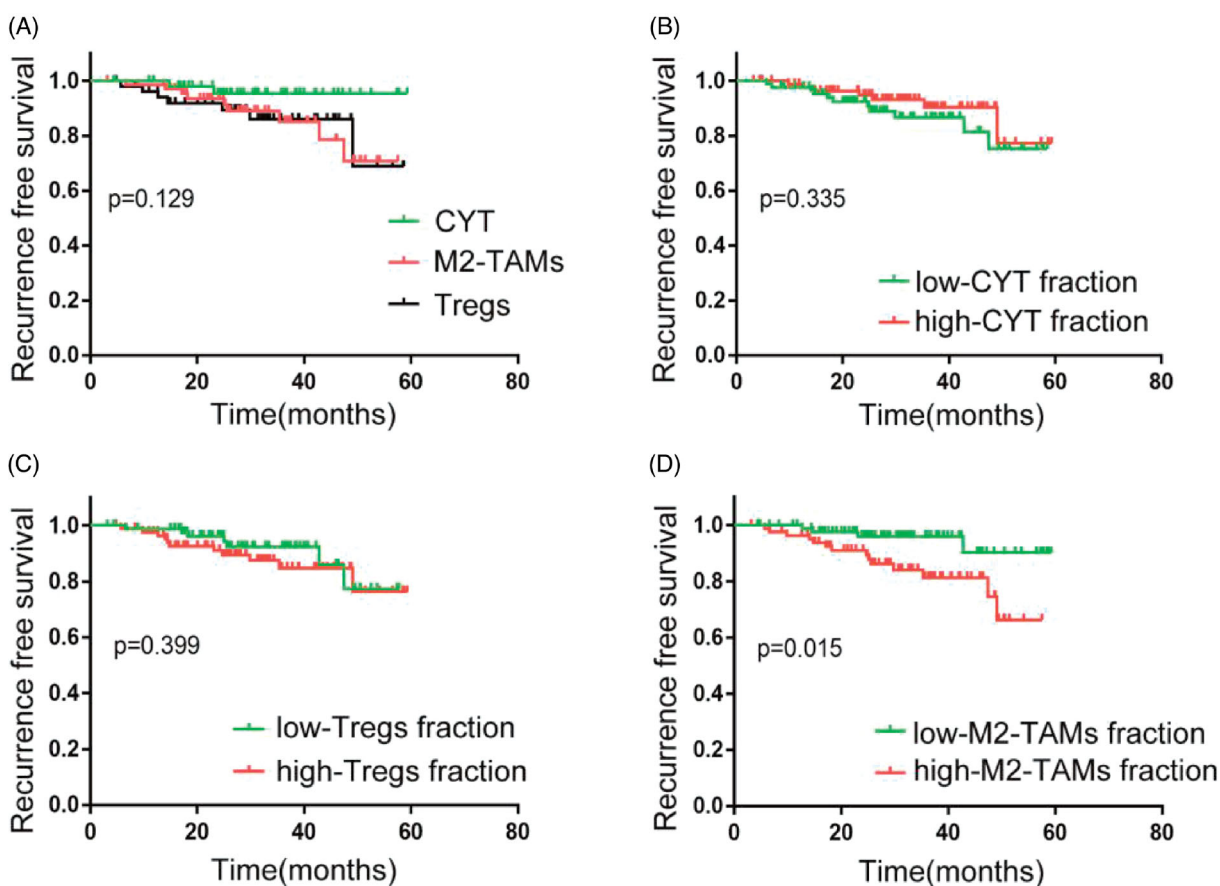
### M2-TAMs infiltration subtype indicate poor prognosis

To investigate the prognostic difference of three immune subtype PCa patients, biochemical recurrence-free survival (RFS) was conducted using Kaplan-Meier survival curve in the TCGA cohort (Figure 3(A)). The results showed that patients with M2-TAMs and Tregs subclass had a shorter survival time compared with the CYT subclass. Notably, within the classification of three immune clusters for predicting RFS, patients with high M2-TAMs group were significantly associated with poor prognosis compared with that in

low M2-TAMs group, which indicated that high M2-TAMs infiltration might effectively impact patients' outcome. However, the Tregs or CYT group had no significance on RFS (Figure 3(B–D)). Consistent with this, the cox proportion hazards model showed that M2-TAMs infiltration was one of the risk factors for predicting RFS ( $p = .023$ , Table 1). And the effect of three subtype's classification on biochemical recurrence survival in the GEO cohort obtained similar results (Figure S4). Of note, patients in high CYT group were significantly associated with better outcomes compared with those in low CYT group in the GEO cohort (Figure S4(B), Table S3), which indicated that CYT value may be one protector in PCa progression.

### Immunosuppressive performance of M2-TAMs infiltration in PCa

The infiltration of immune cells in TME had been shown to promote tumour immune escape by cross-talking with each other, thereby altering the proportion of immune cells in the TME of tumour patients [24,25]. Given this, we analyzed the infiltration abundance of innate and adaptive immune cells in three subtypes additionally. The results showed both innate



**Figure 3.** Association between three subtypes and prognosis in PCa in TCGA cohort. (A) Kaplan-Meier analysis of immune subtypes based on five-year recurrence free survival in TCGA cohort.  $p$  Value was calculated by the log-rank test among subtypes. (B–D) Kaplan-Meier analyses of tumours stratified by cytolytic activity, M2-TAMs, and Tregs infiltration score.  $p$  Value was calculated by the log-rank test.

immune cells and adaptive immune cells in M2-TAMs subclass had significantly reduced infiltration compared with CYT and Tregs subtypes (Figure S5(A)). Furthermore, the differential expression of immunomodulatory factors in three clusters showed that cluster M2-TAMs had poorly inflammatory induction compared with the other two subclasses (Figure 4(A)). It was observed that several genes relatively highly expressed in M2-TAMs subclass. Interleukin (IL)-4 and protein kinase B (AKT1) expression were related to the infiltration and polarisation of tumour-associated macrophages in TME of PCa, and BMP6 and GDF15 were reported to be related to the development of CRPC [26] and docetaxel resistance [27] respectively in PCa patients, these may explain the reason why patients in M2-TAMs patients showed poor outcomes.

To uncover the immune resistance among three clusters, the differential expression of the immune checkpoint in these subtypes was further investigated from TCGA and GEO cohort respectively (Figure 4(B, C); S5(B AKT1)). Significantly lower expression of immune checkpoints was observed in M2-TAMs subclass compared

with the other two clusters. Of note, patients in the M2-TAMs cluster showed lower expression of PD-L1 and PD-1 compared with the CYT or Tregs subclass, which indicated that M2-TAMs infiltration was associated with stronger resistance to the immune treatment.

We further interrogated the molecular biology progress of the M2-TAMs subclass (Figure 4(D)). In terms of biological progress function, M2-TAMs subclass showed significantly negative regulations on inflammatory response and apoptosis, which might explain the reasons why M2-TAMs subclass with lower immune infiltration. Besides, the results of GSEA analysis showed that highly expressed genes in M2-TAMs subclass related to the Wnt pathway compared with the other two subclasses, the Wnt pathway involved in immunosuppression in tumours (Figure 4(E)).

#### **Association between the M2-TAMs infiltration and response to ICIs**

We further evaluated whether or not M2-TAMs infiltration had the ability to predict immunotherapy

**Table 1.** Univariate and multivariate cox analysis for predicting RFS in the TCGA cohort.

	Univariate		Multivariate	
	HR (95% CI)	<i>p</i> Value	HR (95% CI)	<i>p</i> Value
Age	1.001 (0.935–1.072)	.975		
PSA	1.101 (0.969–1.252)	.141		
Gleason score (>7 vs. ≤7)	5.103 (1.908–13.647)	.001 <sup>a</sup>	4.104 (1.493–11.277)	.006 <sup>b</sup>
Pathological T stage (T3 + T4 vs. T1 + T2)	2.547 (0.906–7.164)	.076		
Pathological N stage (N1 vs. N0)	2.396 (0.687–8.354)	.170		
CYT (high vs. low)	0.647 (0.250–1.672)	.369		
Tregs (high vs. low)	1.500 (0.581–3.876)	.402		
M2-TAMs (high vs. low)	3.625 (1.193–11.016)	.023 <sup>a</sup>	2.550 (0.814–7.984)	.108

RFS, recurrence free survival; PSA, prostate-specific antigen.

<sup>a</sup>By univariate analysis, *p* < .05.

<sup>b</sup>By multivariate analysis, *p* < .05.

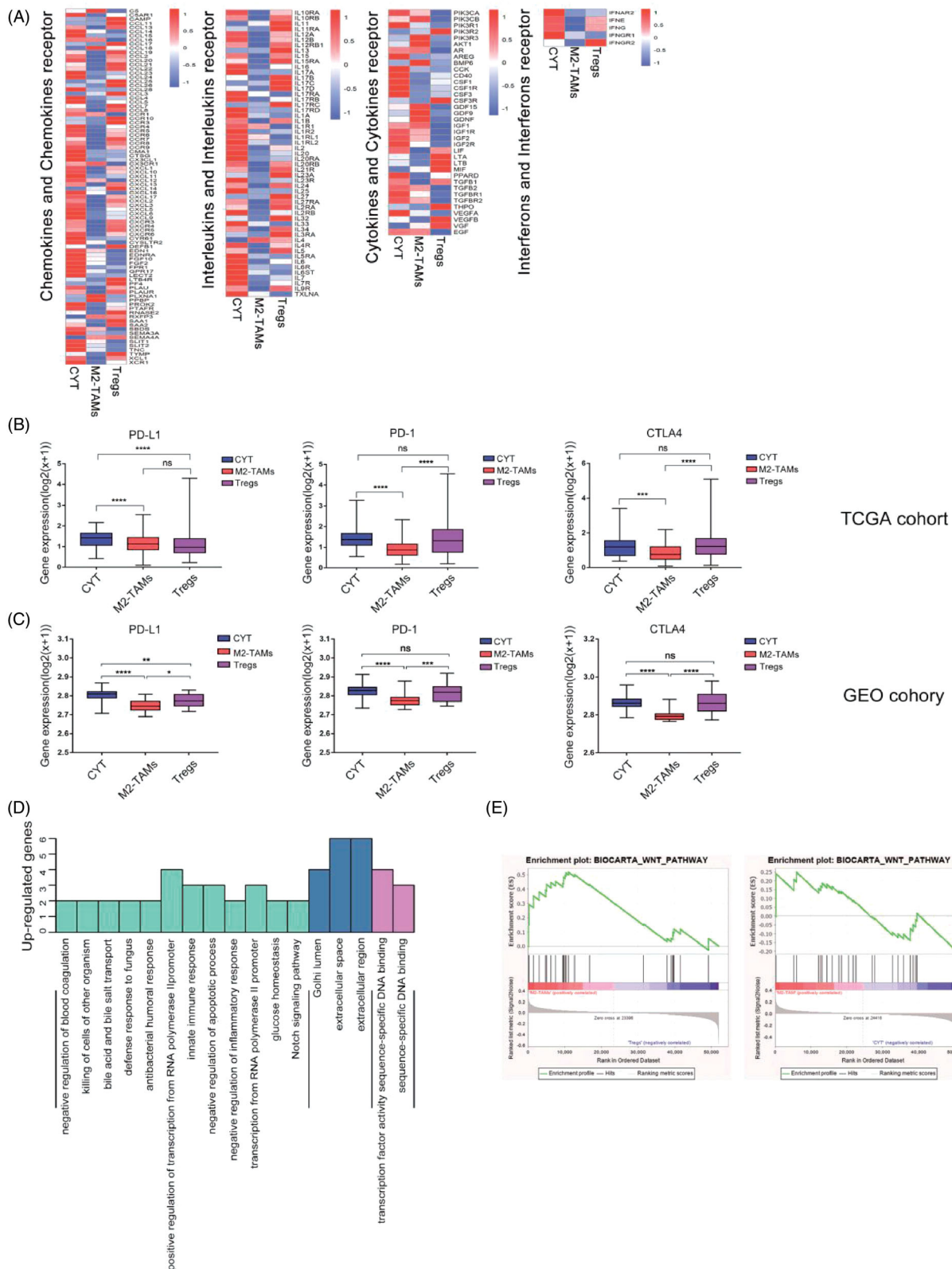
response. Two hundred and ninety-eight urothelial carcinoma patients who received anti-PD-L1 therapy in IMvigor210 cohort were selected to evaluate the relationship between the infiltration of M2-TAMs and response to ICIs. The results showed that the patients with progress response and stable response exhibited higher M2-TAMs to CYT ratios (Figure 5(A)). We classified patients into three groups based on their M2-TAMs to CYT ratios, the significantly reduced PD-L1+ immune cells were seen in high ratios group while low ratios group comprised of increased infiltration of PD-L1+ immune cells, respectively (*p* < .0001, Figure 5(B)), which indicated that higher M2-TAMs infiltration showed lower effect treated with anti-PD-L1 blocker. At the same time, we found patients in high M2-TAMs/CYT ratios group showed a relatively poor clinical response to PD-L1 blockade therapy than those in low M2-TAMs/CYT ratios group comparatively (18.4 vs. 28.8%; Figure 5(C)). These results indicated that high M2-TAMs infiltration in TME might reflect poor effective therapy with an inhibitor of anti-PD-L1. To some extent provided a valuable target for immune treatment in PCa.

## Discussion

In prostate cancer, unsatisfactory effect of anti-PD-L1/PD-1 therapy has become a public problem because of the unstable immune check-points expression in PCa tissues [28–30]. The poor prognosis of immunotherapy for PCa patients was thought to be related with microenvironment immunosuppression [31,32]. Some infiltrating immune cells, such as M2-TAMs [22], Tregs [33] were reported highly infiltrated in PCa and proved to be the performance of immunosuppression. However, whether these mechanisms interact or related to immunotherapy in PCa remains unclear. Based on the immunological profiles, we analyzed the different immune characters of 362 PCa patients and matched normal prostate tissues in TCGA and GEO

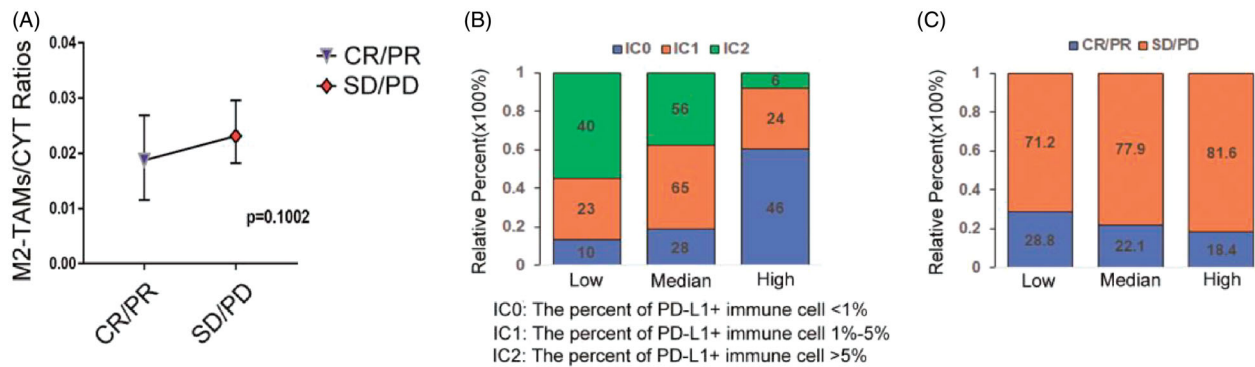
cohort, respectively. We introduced a new immune subtype for PCa patients with high infiltration of CYT, M2-TAMs, and Tregs, our results showed that patients in high M2-TAMs infiltration subtype showed not only the worst clinical prognosis among the three subtypes, but the more significant is that patients with high M2-TAMs infiltration showed lower immune check-points expression compared with the other two subgroups, especially for expression of PD-L1/PD-1. Meanwhile, high M2-TAMs infiltration predicted poorly respond to the anti-PD-L1 treatment in the IMvigor210 cohort, suggesting that M2-TAMs infiltration may be an important factor to attenuated the remission rate of anti-PD-L1 therapy.

In fact, pieces of research have been proved the immunosuppression of M2-TAMs [22,34,35]. Xu L et al. [22] reported that *in vitro*-induced M2-TAMs induced the resistance of PCa cells to cytotoxic action of NK cell. Macrophages in lung squamous-cell carcinoma matrix can prevent T cells from migrating in TME and their interaction with stimulation of antigen-presenting cells populations [35], which indicated that M2-TAMs infiltration can affect the activity of CD8+ T cells and NK cells respectively. In our study, the infiltration of CD8+ T cells and NK cells involving in anti-tumour immunity was significantly decreased in the tumour samples while with higher M2-TAMs infiltration comparatively (Figure 1(D–F)), which suggested that high infiltration of M2-TAMs may be one important reason for destroying the immune microenvironment homeostasis in PCa. On the other hand, we also found that IL-4 and AKT1 involved in M2 polarization were expressed highly in M2-TAMs subclass (Figure 4(A)). The higher expression of BMP6 and GDF15 in M2-TAMs subtype has been proved to be related to the development of CRPC [26] and docetaxel resistance [27], respectively in our study. These findings may explain the unique immunosuppression of M2-TAMs subclass.



**Figure 4.** The immunopressive mechanisms of M2-TAMs subclass. (A) The median expression level of the immune-related genes in three subclasses of prostate cancer (Gene list: chemokines and chemokines receptor; interleukins and interleukins receptor; cytokines and cytokines receptor and interferons and interferons receptor). (B, C) Gene expression levels of PD-L1/PD-1 of three immune subclasses in TCGA cohort and GEO cohort prostate cancer samples. *p* Values were computed using Mann-Whitney test (\*\*\*\**p* < .0001; \*\*\**p* < .001; \*\**p* < .01; \**p* < .05; ns, *p* > .05). (D) Go analysis plots were shown the hub genes that up-regulated in the M2-TAMs subclass in prostate cancer samples. BP: biological process; CC: cellular component; MF: molecular function. (E) Gene set enrichment plots were shown the up-regulated genes in the M2-TAMs subclass compared to the CYT or Tregs subclass.





**Figure 5.** M2-TAMs subclass showed poorly respond to ICIs. (A) The ratios of infiltration fractions of M2-TAMs to CYT value in the effective and ineffective anti-PD-L1 clinical therapy groups of the IMvigor210 cohort patients ( $n = 298$ ). CR: complete response; PR: partial response; SD: stable response; PD: progress response ( $p = .1002$ , Mann-Whitney test; median (95% confidence interval)). (B) The proportions of patients with different PD-L1+ immune cells infiltrating fractions in three groups of low, medium and high M2-TAMs/CYT values (low vs. high:  $p < .0001$ ; Chi-square test). (C) The proportions of patients with response to PD-L1 blockade therapy in three groups of low, medium and high M2-TAMs/CYT values (low vs. high:  $p = .136$ ; Chi-square test).

Different from the M2-TAMs subtype, CYT and Treg subtypes showed higher immune infiltration (Figure 2(B)). Previous studies showed that the infiltration of Treg cells was related to the poor prognosis of PCa [33]. In a study of PCa patients treated with GM-CSF-secreting allogeneic cellular vaccine, the CD8+ T-cell infiltrate was accompanied by a proportional increase in Tregs cells, suggesting that adaptive Treg resistance may dampen the immunogenicity of PCa [8]. This may be the difference of immunosuppression between the Treg and M2-TAMs subclass.

Appearance of immune check-point blocker provides a new idea for the treatment of refractory PCa in recent years. However, series clinical trials showed a poorly respond to the treatment with anti-PD-L1 and anti-PD-1 blocker in PCa [36–38]. In keynote-028, the total effective rate of patients treated with pembrolizumab (anti-PD-1 blocker) was only 13% [36]. Another study of patients with recurrent or advanced PCa showed a response rate of 19% with pembrolizumab treatment [37]. Thus, the reason why PCa patients treated with immune check-point blocker received poorly response remains unclear. Several evidence suggested that some immune cells infiltrated in TME can predict the response of immune checkpoint [39–41]. In our study, the expression of PD-L1 and PD-1 were significantly lower in M2-TAMs subclass among three subclasses (Figure 4(B, C)), this suggests that M2-TAMs patients may have poor efficacy with treated of PD-L1 blockers. More important, another data analysis, the patients with urothelial carcinoma received anti-PD-L1 treatment in IMvigor210 cohort showed higher M2-TAMs infiltration was related to poor effective therapy (Figure 5(C)). Our results demonstrated

that M2-TAMs subclass PCa patients may not be suitable for anti-PD-L1 or PD-1 treatment.

This study had a few limitations which should be addressed in the future. First, the sample size of this study was limited, there were only two cohorts, including 362 PCa patients. Second, this study lacked direct evidence to investigate the relationship between M2-TAMs infiltration and response rate of ICIs treatment for prostate cancer. Further confirmation of the mechanism of influence between M2-TAMs infiltration and anti-PD-L1 immune response will be the focus of future studies.

## Conclusion

In conclusion, our study established a new immune subtype for PCa patients based on the immunosuppressive cell infiltration. High M2-TAMs infiltration subtype showed the lower expression of immune check-points in PCa patients. For prognosis of immune therapy, the levels of M2-TAMs infiltration could be as one potential maker for assessing the effective remission of PCa patients receiving immunotherapy.

## Author contributions

MKC and SCZ designed the study and revised the manuscript. JWZ, CXD, CDL, YL collected, analyzed and interpreted the TCGA, GEO and IMvigor210 cohort data. JWZ, CXD, YL wrote and revised the manuscript. JKY, HFD, CY, ZPH and HYW participated in the data collections. DYL, ZJL, XX, QZZ, KYX, WBG and MX participated in the data analysis. JHZ and JMB participated in the language editing of the manuscript. All the listed

authors have participated actively in the study. All authors read and approved the final manuscript.

## Disclosure statement

The authors report no conflicts of interest.

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## Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its [supplementary materials](#).

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