Intratumoral TIGIT\(^+\) CD8\(^+\) T-cell infiltration determines poor prognosis and immune evasion in patients with muscle-invasive bladder cancer

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**ABSTRACT**

**Background** T-cell immunoglobulin and ITIM domain (TIGIT) is identified as a novel checkpoint receptor that can facilitate immune escape via mediating T-cell exhaustion in tumors. However, the clinical significance and immune contexture correlation of intratumoral TIGIT\(^+\) CD8\(^+\) T-cells remain to be further explored in muscle-invasive bladder cancer (MIBC).

**Methods** 259 patients with MIBC from two clinical centers (Zhongshan Hospital, \(n=141\); Shanghai Cancer Center, \(n=118\)) were analyzed to evaluate the prognostic value and immune contexture association of TIGIT\(^+\) CD8\(^+\) T-cells through immunohistochemistry. Fresh tumor tissue samples from 26 patients with MIBC were examined to discover the phenotype of this CD8 subpopulation by flow cytometry.

**Results** High infiltration of intratumoral TIGIT\(^+\) CD8\(^+\) T-cells predicted poor overall survival (OS) and recurrence-free survival (RFS) in MIBC. For patients with stage II MIBC with low infiltration of TIGIT\(^+\) CD8\(^+\) cells, adjuvant chemotherapy (ACT) could significantly prolong their OS and RFS. Intratumoral TIGIT\(^+\) CD8\(^+\) T-cell abundance was correlated with impaired CD8\(^+\) T-cell cytotoxicity and exhibited production of immunosuppressive cytokine IL-10. Further analysis of tumor-infiltrating immune cell landscape revealed TIGIT\(^+\) CD8\(^+\) T-cells were associated with suppressive immune contexture, including Th2 cells, regulatory T-cells, mast cells and neutrophils.

**Conclusion** Intratumoral TIGIT\(^+\) CD8\(^+\) T-cell abundance could serve as an independent prognosticator for clinical outcome and a predictive biomarker for inferior ACT responsiveness. Intratumoral TIGIT\(^+\) CD8\(^+\) T-cell abundance correlated with dampened CD8\(^+\) T-cell antitumor immunity and immunosuppressive contexture abundance, highlighting a tumor-promoting role of TIGIT\(^+\) CD8\(^+\) T-cells.

**INTRODUCTION**

Bladder cancer, a complex disease associated with high morbidity and mortality rates, is the ninth most common malignant disease worldwide.\(^1\) Approximately 25\% of patients are diagnosed as muscle-invasive bladder cancer (MIBC), an advanced urothelial tumor with inferior prognosis.\(^2\) For these patients, the systemic cisplatin-based chemotherapy offers the chance to cure but still lacks enough evidence.\(^3\)\(^\text{,}\)\(^\text{,}\)\(^\text{,}\) Immune checkpoint inhibitors (ICIs) targeting program death-1 (PD-1)/program death-ligand 1 (PD-L1) axis and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) are emerging as a viable salvage treatment for patients in whom chemotherapy cannot control the disease, while the response rates are relatively low (21\%).\(^5\)

Hence, biomarkers for predicting patient survival outcomes and efficacy of chemotherapy and ICIs are being pursued.

As we have previously reported, tumor-infiltrating immune cells, including regulatory T-cells (Tregs), macrophages, mast cells and B cells, could affect the balance between antitumor immunity and immune evasion in MIBC.\(^6\)\(^\text{,}\)\(^\text{,}\)\(^\text{,}\)\(^\text{,}\)\(^\text{,}\) CD8\(^+\) T-cells, as the main effector immune cells, are critical to tumor initiation and progression and play a significant role in antitumor effect.\(^6\) However, CD8\(^+\) T-cells can be shifted from the effector state to the dysfunction state.\(^7\) Increasing studies have reported that intratumoral CD8\(^+\) T-cells are a highly heterogeneous population.\(^8\) A more precise identification of CD8\(^+\) T-cell subtypes is necessary for predicting disease progression and understanding the intrinsic anti-tumor mechanism in patients with MIBC.

T-cell immunoglobulin and ITIM domain (TIGIT), also known as Vstm3 and VSIG9, is a novel coinhibitory receptor.\(^9\) Within the tumor microenvironment, TIGIT that is mainly expressed on NK cells, CD8\(^+\) T-cells, and Tregs can facilitate immune evasion in acute myeloid leukemia, colon cancer and
TIGIT inhibits immune responses mediated by T-cells and NK cells through triggering CD155 on dendritic cells (DCs) or tumor cells. Currently, several studies have paid close attention to the role of targeting TIGIT in antitumor immunity and facilitate the development of anti-TIGIT monoclonal antibodies (mAbs). Preclinical models indicated that anti-TIGITs have demonstrated synergy with anti-PD-1/PD-L1 treatment. Previous studies have shown that a CD8+ T-cell subset expressing high levels of TIGIT infiltrated into multiple myeloma and glioblastoma multiforme, in which the TIGIT blockade strategies rapidly enhance the CD8+ T-cell-mediated immune response. However, the TIGIT+ CD8+ T-cell subset is poorly explored in MIBC, and the clinical significance of this subset still remains ambiguous.

In this study, we evaluated that intratumoral TIGIT+ CD8+ T-cells could be applied as a prognosticator and a predictive biomarker for adjuvant cisplatin-based chemotherapy with the retrospective analysis of 259 patients with MIBC from two independent clinical centers. Furthermore, we discovered an immunosuppressive contexture and the clinical significance of this subset still remains ambiguous.

Flow cytometry
Fresh samples, including tumor tissues (n=26) and peritumor tissues (n=13), were collected from five different clinical centers (Zhongshan Hospital of Fudan University, Fudan University Shanghai Cancer Center, Ruijin Hospital, Shanghai General Hospital and Shanghai Ninth People’s Hospital). The peritumor tissues are obtained from an area of ≥2 cm from the tumor margin.

Single-cell suspension was performed as described. Then samples were stained with the indicated mAbs for 30 min at 4°C after lysing red blood cells. Cells were stimulated for 5 hours with phorbol myristate acetate (50 ng/mL) and ionomycin (1 µg/mL) in the presence of GolgiStop protein transport inhibitor (1:1000) for intracellular cytokine measurement. Cells were stained with interested surface markers, and Fixation/Permeabilization Solution Kit (BD Biosciences) was used for intracellular protein staining according to the manufacturer’s instructions. Stained cells were washed and resuspended in phosphate-buffered saline (PBS)/0.1% bovine serum albumin with 0.5% azide. Flow cytometry data were analyzed by FlowJo software (Tree Star, San Carlos, California, USA). All flow cytometry antibodies are listed in online supplementary table 1.

Statistical analysis
Descriptive statistics was used to summarize patients’ baseline characteristics and disease factors. Results are shown as mean±SD, and Mann-Whitney U test, Wilcoxon signed-rank test, χ2 test and Spearman correlation analysis were used in this study. OS and RFS were determined by the Kaplan-Meier method, which was evaluated by log-rank tests. Multivariate analyses of the Cox regression model were applied to estimate HRs and 95% CIs. A two-tailed p value of <0.05 was considered statistically significant in our study. All statistical analyses were conducted using
IBM SPSS Statistics V.25.0, R V.3.5.1, GraphPad Prism Software V.8.0 and MedCalc V.15.

RESULTS
Residency of intratumoral TIGIT⁺ CD8⁺ T-cells correlates with disease progression in MIBC
The residency of TIGIT⁺ CD8⁺ T-cells in MIBC was identified through double IHC staining and flow cytometry (figure 1A,B and online supplementary figure 1). The patient characteristics are listed in online supplementary table 3. We found the proportion of patients with high TIGIT⁺ CD8⁺ T-cell infiltration expanded with progression of pathology Tumor (pT) or pathology Node (pN) stage (figure 1C). Moreover, the infiltration of TIGIT⁺ CD8⁺ T-cells, as well as the proportion of TIGIT⁺ CD8⁺ T-cell among CD8⁺ T-cells, was positively associated with the increased tumor stages (figure 1D and online supplementary figure 2). Furthermore, compared with peritumor tissues, we found that TIGIT⁺ CD8⁺ T-cells were dramatically more infiltrated in matched tumor tissues (figure 1E). In conclusion, these results confirmed the existence of TIGIT⁺ CD8⁺ T-cells in MIBC and indicated that intratumoral TIGIT⁺ CD8⁺ T-cells were correlated with MIBC progression.

Intratumoral TIGIT⁺ CD8⁺ T-cells act as a prognosticator for survival outcome in MIBC
The prognostic ability of TIGIT⁺ CD8⁺ T-cells was next explored. We found that CD8⁺ T-cell infiltration could prolong the OS of patients with MIBC both in the ZSHS cohort and the FUSCC cohort (p=0.023 and p=0.007, figure 2A). However, the RFS showed no difference with CD8⁺ T-cell strata (p=0.859 and p=0.142, figure 2B). Interestingly, the TIGIT⁺ CD8⁺ cells high infiltration group possessed inferior OS and RFS than the TIGIT⁺ CD8⁺ cells low infiltration group in both cohorts (OS: p=0.010 and p=0.013, figure 2C; RFS: p=0.009 and p=0.047, figure 2D), which was entirely contrary to the prognosis of CD8⁺ T-cell infiltration. Additionally, multivariate Cox regression analysis showed that TIGIT⁺ CD8⁺ T-cell infiltration was an independent prognostic factor for patients with MIBC after adjustment for age, gender, grade, lymphovascular invasion, adjuvant chemotherapy (ACT), pathological T/N stage and CD8⁺ T-cell infiltration as confounders (online supplementary table 4). Therefore, TIGIT⁺ CD8⁺ T-cells proved to be an independent unfavorable factor to predict survival and recurrence in patients with MIBC.

Figure 1 Intratumoral TIGIT⁺ CD8⁺ T-cells accumulate in MIBC and correlate with tumor progression. (A) Double immunohistochemistry staining for TIGIT (blue) and CD8 (brown) in MIBC tissues. Black arrowheads indicate TIGIT⁺ CD8⁺ T-cells. (B) Comparison of TIGIT⁺ CD8⁺ T-cell infiltration in tumor and peritumor tissues of patients with MIBC. (C) Proportion of patients with high/low TIGIT⁺ CD8⁺ T-cell infiltration in pT stage and pN stage. (D) Association of TIGIT⁺ CD8⁺ T-cell infiltration with pT stage and pN stage. (E) Comparison of TIGIT⁺ CD8⁺ T-cells among CD45⁺ cells in tumor and peritumor tissues of patients with MIBC. *P<0.05, **P<0.01, ***P<0.001 by χ² test, Mann-Whitney U test and paired t-test. pT, pathology Tumor; pN, pathology Node; MIBC, muscle-invasive bladder cancer; TIGIT, T-cell immunoglobulin and ITIM domain.
Intratumoral TIGIT+ CD8+ T-cells could predict ACT effectiveness within patients with stage II MIBC

ACT has been widely used in the treatment for patients with MIBC with pT3/4 or pN+ disease. Thus, we evaluated whether intratumoral TIGIT+ CD8+ T-cell abundance was associated with ACT responsiveness. Both cohorts were combined for further investigation. In all patients or patients with stage III MIBC, intratumoral TIGIT+ CD8+ T-cell abundance failed to predict ACT responsiveness (online supplementary figure 3). No evidence suggested that ACT could improve OS or RFS in patients with stage II MIBC (OS: p=0.087, RFS: p=0.128; figure 3A,D). CD8+ T-cells still failed to predict ACT effectiveness in patients with stage II MIBC (online supplementary figure 4). However, after dividing patients into TIGIT+ CD8+ T-cells high and low infiltration subgroups, we found that ACT successfully prolonged both OS and RFS in patients with low TIGIT+ CD8+ T-cell infiltration (OS: p=0.043, RFS: p=0.013; figure 3C,F), while no survival benefit was observed in patients with high TIGIT+ CD8+ T-cell infiltration (OS: p=0.261, RFS: p=0.769; figure 3B,F). In addition, univariate Cox regression analysis was performed to assess the relationship between TIGIT+ CD8+ T-cell infiltration and ACT benefit, which suggested that patients with stage II MIBC with TIGIT+ CD8+ T-cells low infiltration could receive more benefit from ACT (OS: HR, 0.340, 95% CI, 0.114 to 0.950, p=0.049; RFS: HR, 0.239, 95% CI, 0.070 to 0.817, p=0.022; online supplementary table 5). Herein, these results suggested that TIGIT+ CD8+ T-cells high abundance predicted suboptimal ACT responsiveness within patients with stage II MIBC. Patients with low TIGIT+ CD8+ T-cell infiltration could benefit more from ACT.

Intratumoral TIGIT+ CD8+ T-cell abundance impairs CD8+ T-cell antitumor immunity in patients with MIBC

Coinhibitory receptors, also known as immune checkpoints, are often coexpressed on dysfunctional CD8+ T-cells. Compared with TIGIT− CD8+ T-cells, TIGIT+ CD8+ T-cells expressed higher levels of immune checkpoints, including PD-1, CTLA-4, Lag-3 and Tim-3 (online supplementary figure 5A). Meanwhile, TIGIT+ CD8+ T-cells had a higher level of proliferative marker (Ki-67), effector markers (interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-2) and cytolytic marker (CD107a) compared with their TIGIT− counterparts (online supplementary figure 5B–D), which inferred that TIGIT+ CD8+ T-cells could be closely related to a terminally exhausted CD8+ T-cell phenotypes as previously reported.

The global characterization of CD8+ T-cells was subsequently investigated according to TIGIT+ CD8+ T-cell infiltration. We found that CD8+ T-cells in TIGIT+ CD8+ T-cells high infiltration tumors expressed increased immune checkpoints, including PD-1, CTLA-4 and Lag-3 (figure 4A) while exhibiting more proliferative ability (Ki-67) than their counterparts in TIGIT+ CD8+ T-cells low infiltration tumors (figure 4B). Additionally, the effector cytokines (IFN-γ and TNF-α) and cytotoxicity activation...
molecules (granzyme B (GZMB)) expressed by CD8+ T-cells were decreased in TIGIT+ CD8+ T-cells high infiltration tumors (figure 4C,D). These results indicated that high TIGIT+ CD8+ T-cells were associated with impaired CD8+ T-cell antitumor immunity. In addition to CD8+ T-cells, CD45+ T-cells in TIGIT+ CD8+ T-cells high infiltration tumors expressed increased immune checkpoints, including PD-1, CTLA-4 and Tim-3, while the percentage of CD45+ T-cells expressing effector cytokines (IFN-γ and TNF-α) decreased in TIGIT+ CD8+ T-cells high infiltration tumors. (online supplementary figure 6). Interestingly, we found that TIGIT+ CD8+ T-cells could produce immunosuppressive cytokine IL-10 (online supplementary figure 5E). TIGIT+ CD8+ T-cell infiltration was also found positively correlated with protumor Th2 cells (Spearman r=0.317, p<0.001), Tregs (Spearman r=0.309, p<0.001), mast cells (Spearman r=0.334, p<0.001), neutrophils (Spearman r=0.178, p=0.035) infiltration and antitumor NK cells (Spearman r=0.183, p=0.029), and M1 macrophages (Spearman r=0.251, p=0.003) infiltration (figure 5B,C). These data indicated that intratumoral TIGIT+ CD8+ T-cell abundance indicated tumor-promoting immune microenvironment in MIBC.

**DISCUSSION**

Currently, CD8+ T-cells were regarded as a protective factor in various solid tumors, including bladder cancer.27 28 However, CD8+ T-cells remain heterogeneous, and several subtypes of intratumoral CD8+ T-cells are associated with poor clinical outcomes.29 TIGIT, a novel co-inhibitory receptor, can promote tumor growth and drive the exhaustion of tumor-infiltrating lymphocytes, including CD8+ T-cells in multiple cancer types.14 15 20 In this study, we reported that intratumoral TIGIT+ CD8+ T-cell abundance was positively correlated with tumor development and enriched in MIBC tissues. Contrary to CD8+ T-cells in MIBC, TIGIT+ CD8+ T-cells were identified as an adverse prognosticator through the retrospective analysis of a considerable population.

The application of ACT is supported by recent data while still lacking enough evidence.4 It is showed that a tumor immune microenvironment may influence the
chemotherapeutic efficacy. Our previous studies have uncovered the contribution of certain immune contexture to ACT resistance, including tumor-infiltrating masT-cells, neutrophils, B cells and an immune type A/B classification. Though no association was found between CD8+ T-cell infiltration and ACT beneficial, TIGIT+ CD8+ T-cell abundance could predict a suboptimal ACT responsiveness, which further exhibited the potential heterogeneity of intratumoral CD8+ T-cells. The identification of certain CD8+ T-cell subpopulation could assist in guiding adjuvant therapy. Of note, the usage of ACT was not randomized; there could be possible bias introduced by comparing those groups. The confounders could be the tumour, node, metastasis stage of the patients and other possible factors. Through adjusting these confounders, we found that intratumoral TIGIT+ CD8+ T-cell infiltration failed to predict ACT responsiveness in all patients or patients with stage III MIBC. However, in patients with stage II MIBC, ACT successfully prolonged both OS and RFS in patients with low TIGIT+ CD8+ T-cell infiltration. We would further validate our findings in a prospective, larger, multicentered randomized trial in the future.

Figure 4  Intratumoral TIGIT+ CD8+ T-cell infiltration impairs CD8+ T-cell antitumor immunity in patients with muscle-invasive bladder cancer. (A) Expression of coinhibitory receptors (PD-1, n=24; CTLA-4, n=16; Tim-3, n=14; Lag-3, n=17) on CD8+ T-cells in TIGIT+ CD8+ T-cells high/low infiltration group. (B) Expression of proliferation marker (Ki-67, n=16) on CD8+ T-cells in TIGIT+ CD8+ T-cells high/low infiltration group. (C) Expression of effector cytokines (IFN-γ, n=23; TNF-α, n=23; IL-2, n=19) on CD8+ T-cells in TIGIT+ CD8+ T-cells high/low infiltration group. (D) Expression of cytotoxicity activation molecules (GZMB, n=19; CD107a, n=18; PRF-1, n=8) on CD8+ T-cells in TIGIT+ CD8+ T-cells high/low infiltration group. (E) Correlation between TIGIT+ CD8+ T-cells and IL-10+ CD8+ T-cells among CD45+ cells and IL-10+ cells based on the results of flow cytometry. (F) Correlation between TIGIT+ CD8+ T-cells and IL-10+ cells based on the evaluation of immunohistochemistry staining. *P<0.05, **P<0.01 by Mann-Whitney U test. PD-1, program death-1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; IFN, interferon; IL, interleukin; GZMB, granzyme B; ns, no significance; TIGIT, T-cell immunoglobulin and ITIM domain.
that TIGIT+ CD8+ cell abundance impaired CD8+ T-cell antitumor immunity. Additionally, TIGIT+ CD8+ T-cells were associated with suppressive immune contexture, including Th2 cells, Tregs, mast cells and neutrophils,32 of which mast cells and neutrophils infiltrating into MIBC were identified as protumor immunocytes in our previous studies.7 9 A confusing point was that TIGIT+ CD8+ T-cells were positively correlated with M1 macrophages. One possible explanation could be that the elevated M1 macrophages triggered an antitumor immunity. In order to escape the antitumor immunity, the tumor microenvironment upregulated the expression of TIGIT on CD8+ T-cells. It is showed that TIGIT is highly expressed on NK cells,13 which might be the cause of the positive correlation between TIGIT+ CD8+ T-cells and NK cells. Early studies revealed that TIGIT could exert immunoregulatory function through multiple mechanisms, including promoting IL-10 by Tregs and dendritic cells,17 33 suppressing the activity of its costimulatory counterpart CD226 34 or directly inhibiting the intrinsic recruitment of Src homology domain containing tyrosine phosphatase (SHP).35 Interestingly, we found that TIGIT+ CD8+ T-cells have the capacity for IL-10 production, which could be one explanation for the CD8+ T-cell dysfunction and immunoveasive microenvironment in tumors with high TIGIT+ CD8+ T-cell infiltration. Although there were limited reports about IL-10 producing CD8+ T-cells in the tumor immune microenvironment, specific CD8+ T-cells producing IL-10 on antigen recognition could be observed in chronic infection like hepatitis B virus (HBV) and human T-cell lymphotropic virus type 1 (HTLV-I).36 37 The intrinsic mechanism of TIGIT shaping the dysfunction state of CD8+ T-cells needs further investigation.

Intriguingly, the phenotype of TIGIT+ CD8+ T-cells presented a high level expression of both immune checkpoints and effector molecules, which were identified as terminally exhausted CD8+ T-cells with cytotoxicity but short-lived in tumor immunity.26 Thus, these subpopulations presented inferior tumor control compared with the poorly cytotoxic but long-lived counterpart (progenitor exhausted CD8+ T-cells). Immune checkpoint blockade therapy, such as nivolumab (anti-PD-1) and atezolizumab (anti-PD-L1), presented a confirmed but relatively low response rate on advanced cancer of urinary bladder (19.6% and 13.4%).38 39 It is reported that PD-1 blockade shows no act on terminally exhausted CD8+ T-cells.26 Therefore, the existence of TIGIT+ CD8+ T-cells in MIBC may be one explanation for the low response rate of anti-PD-1 therapy. On the other hand, whether TIGIT+ CD8+ T-cells could predict traditional immune checkpoint blockade efficacy should be investigated. Moreover, the combination of anti-PD-1 and anti-TIGIT presented a considerable efficacy in glioblastoma multiforme.21 The immunotherapeutic potential

Figure 5 Intratumoral TIGIT+ CD8+ T-cell infiltration shapes immunosuppressive contexture in patients with muscle-invasive bladder cancer. (A) Heatmap displaying scaled expression of various immune cell types between high/low percentage of TIGIT+ CD8+ T-cells. (B,C) Correlation between TIGIT+ CD8+ T-cell infiltration and CD8+ T-cells, CD4+ T-cells, Th1 cells, Th2 cells, regulatory T-cells, natural killer cells, M1 macrophages, M2 macrophages, mast cells and neutrophil infiltration based on the evaluation of immunohistochemistry staining. P<0.05 by Spearman rank correlation test. TIGIT, T-cell immunoglobulin and ITIM domain.
of TIGIT and the synergetic effect of double immune checkpoint blockade in MIBC are worthy of further study.

CONCLUSION

In summary, the current investigation identified intratumoral TIGIT+ CD8+ T-cell abundance as an adverse prognostic factor for clinical outcome and a predictive biomarker for suboptimal ACT responsiveness in MIBC. Furthermore, intratumoral TIGIT+ CD8+ T-cell abundance correlated with impaired CD8+ T-cell antitumor immunity and shaped immunosuppressive contexture, highlighting its tumorigenic role. These findings indicated that TIGIT might be a potential immunotherapeutic target in MIBC.

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Contributors
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Disclaimer
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Competing interests
None declared.

Patient consent for publication
Not required.

Ethics approval
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Data availability statement
Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. All data generated that are relevant to the results presented in this article are included in this article. Other data that were not relevant for the results presented here are available from the corresponding author, WZ, upon reasonable request.

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REFERENCES


