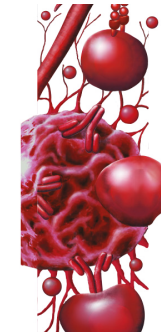
Short Communication

Immunotherapy



The effect of low-dose chemotherapy on the tumor microenvironment and its antitumor activity combined with anti-PD-1 antibody

Fangyu Lin[1], Hao Chen[1], Tao Jiang[1], Jianwei Zheng[1], Qin Liu[1], Baoyu Yang[1], Xinli Wang[1] & Xiaoyan Lin*[,1]

[1]Department of Oncology, Fujian Medical University Union Hospital, 29 Xin-Quan Road, Fuzhou, 350001, People's Republic of China

*Author for correspondence: Tel.: +86 0591 8337 3933; xiaoyanlin@yahoo.com

Aim: This study aimed to explore the effects of low-dose chemotherapy in the tumor microenvironment (TME) on a gastric cancer xenograft and its antitumor activity combined with the anti-PD-1 antibody. **Materials & methods:** Mice with gastric cancer were divided into four groups. The body weight and tumor volume of the mice were recorded. The TME was analyzed using flow cytometry. **Results:** Low-dose paclitaxel increased the PD-L1 expression level and the number of $CD8^+$ T cells, but not the $CD4^+$ T and myeloid-derived suppressor cells or PD-1^+ $CD8^+$ T cells in the TME. Low-dose 5-fluorouracil reduced the number of myeloid-derived suppressor cells and PD-1^+ $CD8^+$ T cells, but the PD-L1 expression level and the number of $CD4^+$ T and $CD8^+$ T cells did not change in the TME. The anti-PD-1 antibody inhibited tumor growth, but the combination therapy did not show superior antitumor activity. **Conclusion:** Low-dose chemotherapy altered the TME but failed to improve the responses to the anti-PD-1 antibody.

Plain language summary: The anti-PD-1 antibody shows potential as an anticancer therapy for tumors, including gastric cancer. However, the antitumor effect of the anti-PD-1 antibody alone is unsatisfactory. The tumor microenvironment (TME) is an environment in which a tumor develops and survives. The TME comprises heterogeneous molecules and cell types, including immune cells, endothelial cells and fibroblasts, besides cancer cells. This study aimed to explore the effects of low-dose chemotherapy on the TME and its antitumor effect when combined with anti-PD-1 antibody. The TME was analyzed using the flow cytometry method. Although low-dose paclitaxel and low-dose 5-fluorouracil changed the TME, both failed to enhance the antitumor activity when combined with the anti-PD-1 antibody.

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Keywords: 5-fluorouracil • combination therapy • gastric cancer • immunotherapy • low-dose chemotherapy • mice • paclitaxel • PD-1 • PD-L1 • tumor microenvironment

Gastric cancer (GC) is one of the most common tumors globally. The high morbidity and mortality of GC make it the second most common cause of cancer-related deaths. Most patients with GC are in advanced stages when diagnosed, making radical operations unfeasible. Chemotherapy, radiotherapy and targeted therapy can improve the survival of patients with GC, but the disease prognosis remains poor. The 5-year overall survival (OS) rates of advanced GC are 10%–30% [1]. More effective treatments to improve the prognosis of patients with advanced GC are therefore urgently required.

The anti-PD-1 antibody represents the most important tumor immunotherapy, showing efficacy in multiple types of solid tumors, including GC. However, the objective response rate (ORR) of the therapies remains low. PD-1 and PD-L1 are the immunosuppressive molecules belonging to the B7 family. PD-1 is expressed on the surface of T cells. The PD-L1 and PD-L2 ligands are expressed on leukocytes, macrophages and dendritic cells. PD-L1 has been found on many different tumor types. PD-1 regulates T-cell activation by binding to ligands PD-L1 and PD-L2; for example, it induces the apoptosis of activated T cells [2], causes the exhaustion of T cells or even inhibits the proliferation and activation of T cells due to the immune escape of tumor cells [3]. PD-L1 also

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plays a pivotal role in various tumors in that it can attenuate the host immune response to tumor cells. Based on these perspectives, the PD-1/PD-L1 axis is responsible for cancer immune escape and has a huge effect on cancer therapy. The anti-PD-1 antibody represents the most important of the tumor immunotherapies that have shown efficacy in multiple types of solid tumors. However, a limited number of patients have achieved clinical benefits, highlighting the importance of a better selection of patients or additional treatment to improve the responses to anti-PD-1 antibody therapy.

Low-dose chemotherapy means a lower dose than the maximum-tolerated dose and a lower dose than the systemic dose of chemotherapy used in humans. Low-dose chemotherapy not only reduces the side effects but also provides advantages, including a decrease in tumor vascularization, lowered therapeutic resistance and, most importantly, enhancement of antitumor immune responses [4]. Previous studies have shown that low-dose chemotherapy increased the number of $CD8^+$ T cells selectively [5–7]. As both low-dose chemotherapy and the anti-PD-1 antibody promote the activation of immune responses, it was hypothesized that low-dose chemotherapy combined with the anti-PD-1 antibody might lead to synergistic antitumor effects. The effects of low-dose chemotherapy on the tumor microenvironment (TME) and its antitumor activity combined with the anti-PD-1 antibody on GC xenograft mice have not been reported yet. The present study aimed to find a more reasonable way to combine low-dose chemotherapy with the anti-PD-1 antibody to improve the efficacy and safety of the combination therapy, as well as to find a therapy for patients resistant to or too weak to tolerate the maximum-tolerated dose chemotherapy. The GC xenograft models were established to explore the effects of low-dose paclitaxel and 5-fluorouracil on the TME and their antitumor effects when combined with the anti-PD-1 antibody. The mice were divided into four groups based on different treatment regimens: anti-PD-1 antibody, low-dose chemotherapy, anti-PD-1 antibody and low-dose chemotherapy and normal saline treatment groups. The TME was analyzed by flow cytometry. This study provided novel insights into combined therapy with the anti-PD-1 antibody, thus helping find a better combination therapy with the anti-PD-1 antibody in clinical practice.

Materials & methods

Reagents

Paclitaxel was purchased from Hospira Australia Pty Ltd (Mulgrave, Australia). 5-Fluorouracil was purchased from Qilu Pharmaceuticals (Jinan, China). The therapeutic anti-mouse PD-1 monoclonal antibody (20170207) was kindly provided by Dr Bingliang Chen.

Animal source

A total of 615 male mice aged 4–5 weeks were purchased from Tianjin Institute of Hematology, the Chinese Academy of Medical Science.

Tumor cell lines

MFC, a mouse GC cell line, was obtained from the Fuheng Cell Bank (Shanghai, China [FH1072]). The cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C with 5% CO_2 in a humidified atmosphere. The cell lines were stored in liquid nitrogen.

Mouse models

All mice were maintained under specific-pathogen-free conditions in the animal center of Fujian Medical University (Fuzhou, China).

MFC gastric tumor models & treatments

The mice were quarantined for 1 week and subcutaneously inoculated with 2×10^5 MFC cells. When the tumors reached 5–8 mm in diameter, the mice were divided into four groups. Five to eight mice were randomly assigned to each group: control group (intraperitoneal injection of normal saline), anti-PD-1 group (intraperitoneal injection of anti-PD-1 antibody), low-dose chemotherapy group (intraperitoneal injection of low-dose chemotherapy) and low-dose chemotherapy + PD-1 group (intraperitoneal injection of low-dose chemotherapy and anti-PD-1 antibody). The drugs were delivered every 3 days on three occasions. The body weight and tumor volume in each group were monitored every 2–3 days using a caliper. Subcutaneous tumor volumes were calculated using the following

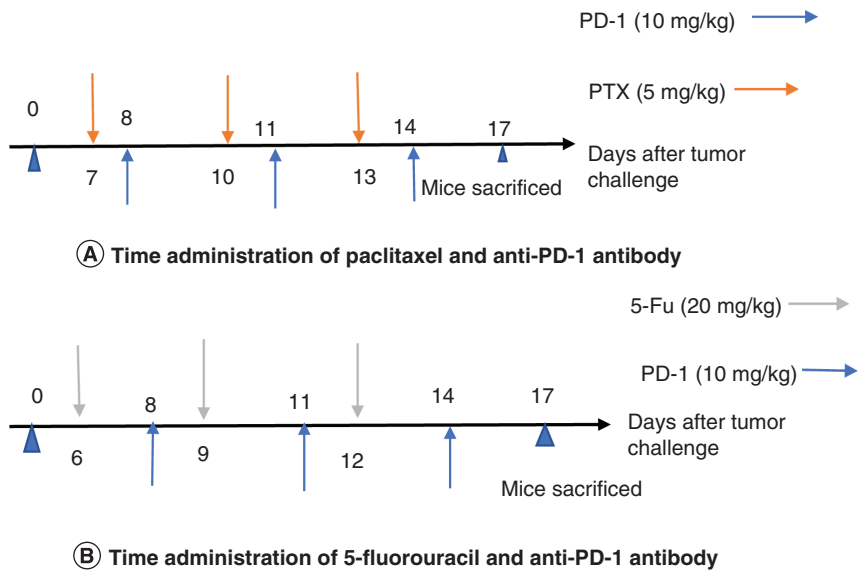

Figure 1. Time of administration of drugs. (A) Paclitaxel and anti-PD-1 antibody. **(B)** 5-fluorouracil and anti-PD-1 antibody.

formula: tumor volume (mm^3) = $a \times b^2/2$ (mm^3), where a is the longest diameter and b is the shortest diameter of the tumor. The mice were sacrificed on the third day following anti-PD-1 antibody injection (Figure 1).

Flow cytometry

The tumors were extracted from sacrificed mice and sectioned using a scalpel. The sections were added to Petri dishes containing 2–3 ml of digestion buffer (100 U/ml collagenase types II and IV [Basal Media, Shanghai, China] + 50 U/ml hyaluronidase [Solarbio, Beijing, China] + 0.5 mg/ml DNase [Macklin, Shanghai, China] in RPMI 1640) and incubated at 37°C for 45 min. Suspensions were filter strained into a new tube through a 70-µm nylon filter. Tumor-infiltrating lymphocytes (TILs) were isolated using mouse tumor tissue lymphocyte isolation kits (Hao Yang, Tianjin, China). All cells were preincubated with anti-CD16/32 monoclonal antibodies (Fc block, clone 2.4G2; BD Biosciences, NJ, USA) prior to staining to block nonspecific binding. The cells were washed and stained with antibodies for surface markers: CD3e fluorescein isothiocyanate (FITC) 145-2C11, CD8a PE 53-6.7, CD4 PE RM4-5, CD279 APC J43, CD274 APC, CD11b FITC M1/70 and Ly-6G/Ly-6C PE RB-8C5 for 30 min at 4°C. All antibodies were purchased from BD Biosciences. The samples were analyzed by a fluorescence activated cell sorter (Accuri C6, BD Biosciences). The data were analyzed using FlowJo software version 7.

Statistical analysis

All data were expressed as means ± standard error. A two-tailed Student's t-test was used to compare the two groups. A p-value < 0.05 indicated a significant difference. Statistical analyses were performed using GraphPad Prism 5 software.

Results

Effects of low-dose chemotherapy on PD-L1 expression in the TME of GC xenografts

Low-dose paclitaxel increased PD-L1 expression (a proportion of all tumor and immune cells present in the sample) in the TME of GC xenografts (10.648 ± 1.32% vs 6.406 ± 0.52%; $p < 0.05$; Figure 2A & C). However, low-dose 5-fluorouracil had no effect on PD-L1 expression in the TME of GC xenografts (3.40 ± 0.52% vs 4.60 ± 1.60%; $p > 0.05$; Figure 2B & D).

Effects of low-dose chemotherapy on myeloid-derived suppressor cells in the TME of GC xenografts

Low-dose paclitaxel had no effect on myeloid-derived suppressor cells (MDSCs) in the TME of GC xenografts (27.68% ± 1.29% vs 39.80% ± 1.17%; $p > 0.05$; Figure 3A & C). Low-dose 5-fluorouracil reduced the percentage of MDSCs in the TME of GC xenografts (10.69% ± 2.89% vs 20.34% ± 1.11%; $p < 0.01$; Figure 3B & D).

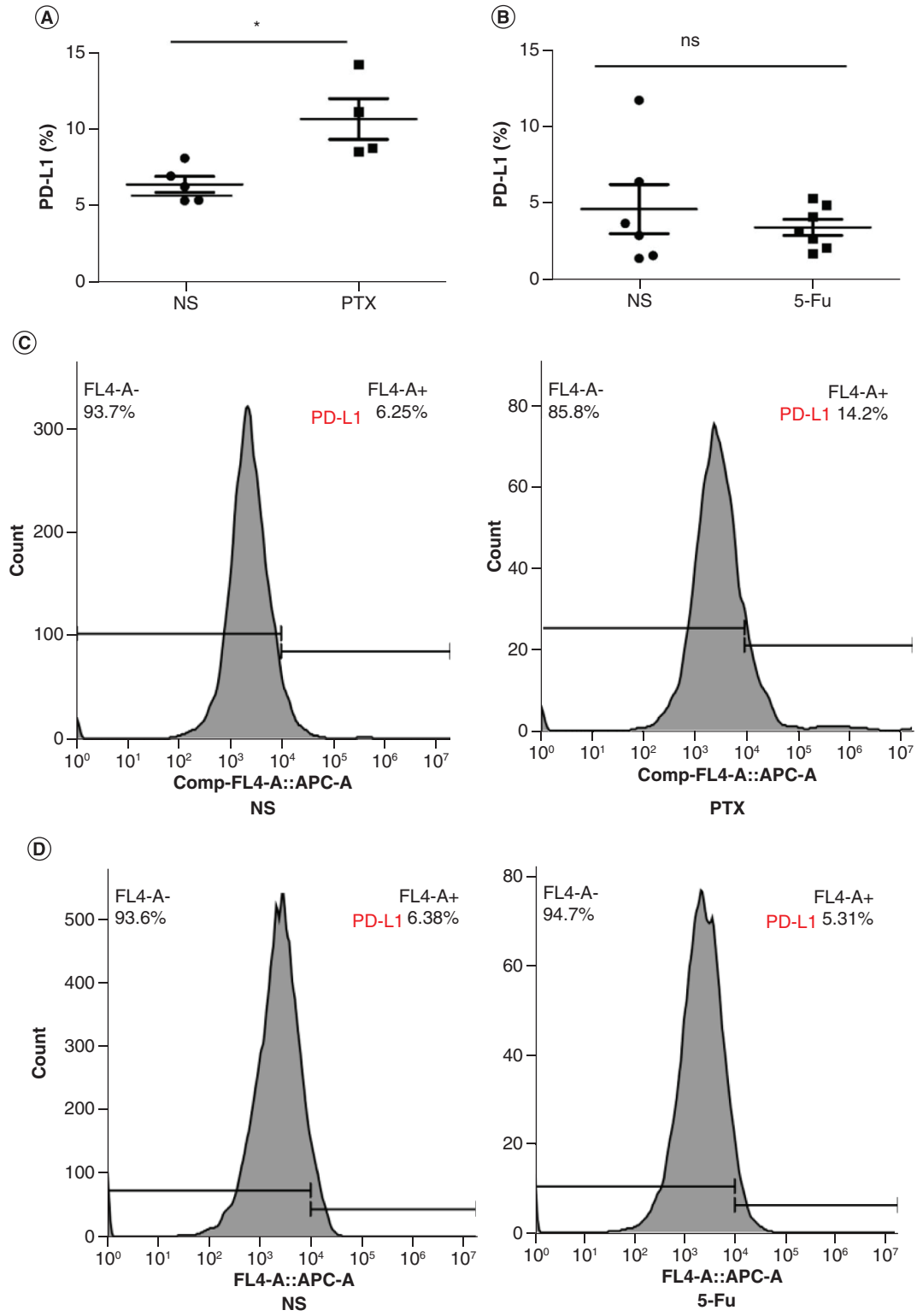

Figure 2. Effects of low-dose chemotherapy on PD-L1 expression in the tumor microenvironment of gastric cancer xenografts. (A & C) Effect of low-dose PTX on PD-L1 expression in the tumor microenvironment of gastric cancer xenografts. **(B & D)** Effect of low-dose 5-Fu on PD-L1 expression in the tumor microenvironment of gastric cancer xenografts, $n \geq 5$.
*$p < 0.05$.
5-Fu: 5-fluorouracil; PTX: Paclitaxel.

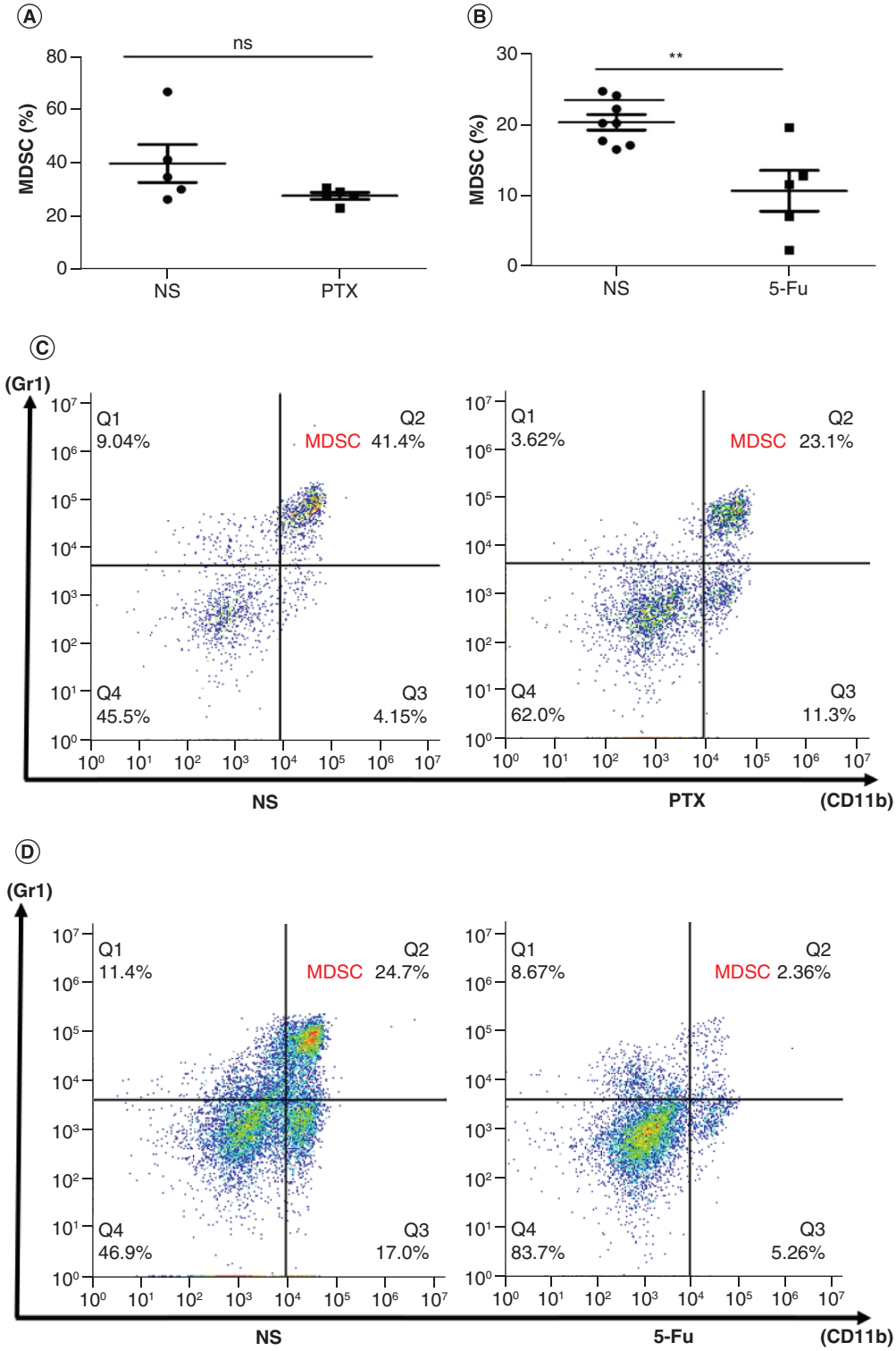

Figure 3. Effects of low-dose chemotherapy on myeloid-derived suppressor cells in the tumor microenvironment of gastric cancer xenografts. (A & C) Effect of low-dose PTX on myeloid-derived suppressor cells in the tumor microenvironment of gastric cancer xenografts, n = 5. **(B & D)** Effect of low-dose 5-Fu on myeloid-derived suppressor cells in the tumor microenvironment of gastric cancer xenografts, n ≥ 5.
*$p < 0.05$; **$p < 0.01$.
5-Fu: 5-fluorouracil; PTX: Paclitaxel.

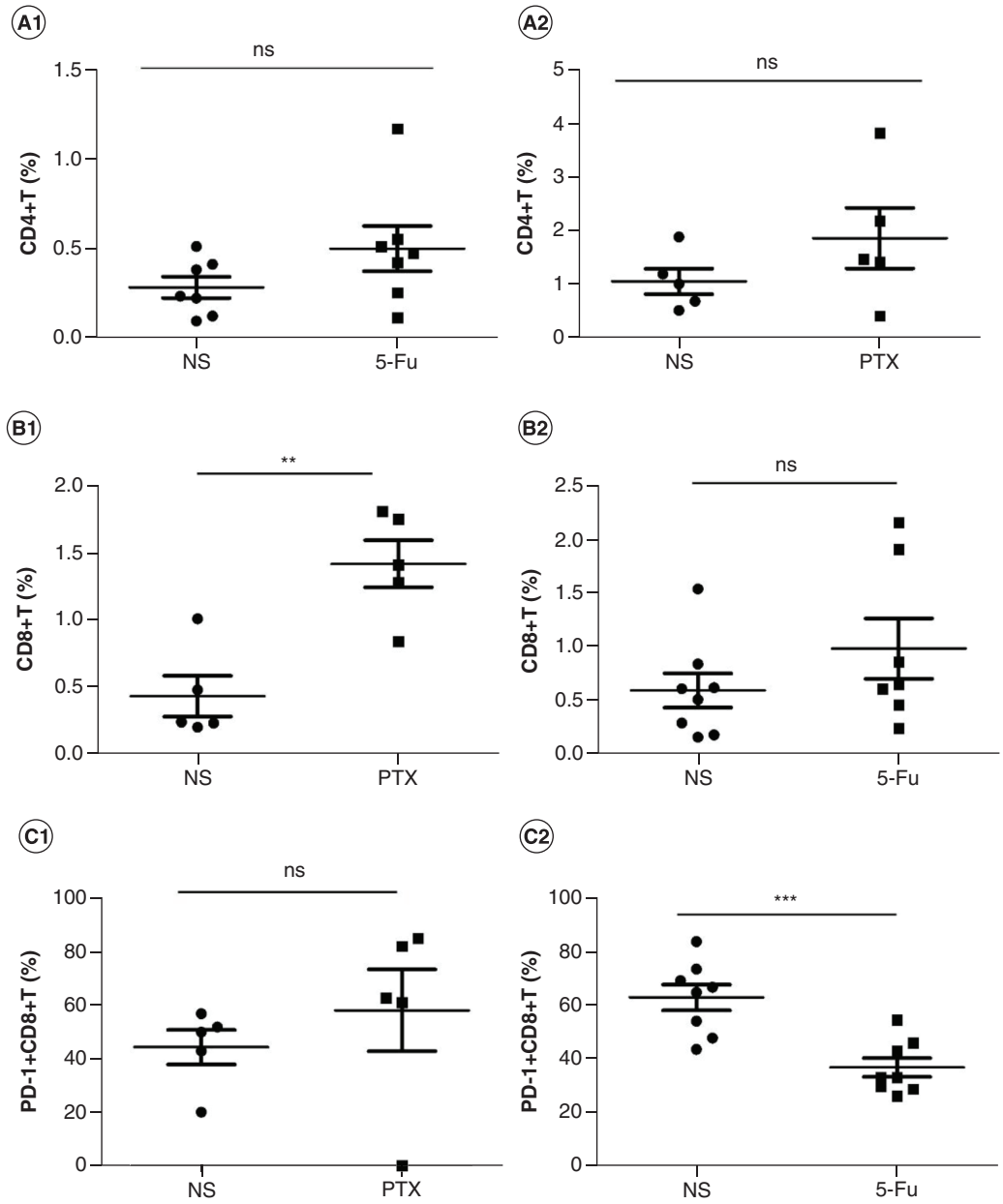

Figure 4. Effects of low-dose chemotherapy on T-lymphocyte subsets in the tumor microenvironment of gastric cancer xenografts. (A-1) Effect of low-dose PTX on the percentage of $CD4^+$ T cells in the TME of GC xenografts, n = 5. **(A-2)** Effect of low-dose 5-Fu on the percentage of $CD4^+$ T cells in the TME of GC xenografts, $n \geq 5$. **(B-1)** Effect of low-dose paclitaxel on $CD8^+$ T cells in the TME of GC xenografts, n = 5. **(B-2)** Effect of low-dose 5-fluorouracil on $CD8^+$ T cells in the TME of GC xenografts, $n \geq 5$. **(C-1)** Effect of low-dose paclitaxel on PD-1^+ $CD8^+$ T cells in the TME of GC xenografts, n = 5. **(C-2)** Effect of low-dose 5-fluorouracil on PD-1^+ $CD8^+$ T cells in the TME of GC xenografts, $n \geq 5$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
5-Fu: 5-fluorouracil; GC: Gastric cancer; PTX: Paclitaxel; TME: Tumor microenvironment.

Effects of low-dose chemotherapy on T-lymphocyte subsets in the TME of GC xenografts

Low-dose paclitaxel had no effect on the percentage of $CD4^+$ T cells in the TME ($1.85 \pm 0.56\%$ vs $1.05 \pm 0.23\%$; $p = 0.23$; Figure 4A-1) but increased the percentage of $CD8^+$ T cells in the TME ($1.42 \pm 0.17\%$ vs $0.43 \pm 0.15\%$; $p < 0.01$; Figure 4B-1). Low-dose paclitaxel had no effects on the number of PD-1^+ $CD8^+$ T cells in the TME of GC xenografts ($44.34 \pm 6.486\%$ vs $58.16 \pm 15.34\%$; $p > 0.05$; Figure 4C-1). Low-dose 5-fluorouracil had no effects on the percentages of $CD4^+$ T cells ($0.28 \pm 0.06\%$ vs $0.49 \pm 0.13\%$; $p = 0.15$; Figure 4A-2) and $CD8^+$ T

cells in the TME (0.98 ± 0.28% vs 0.59 ± 0.16%; p = 0.23; Figure 4B-2). Low-dose 5-fluorouracil reduced the percentage of PD-1$^+$ CD8$^+$ T cells in the TME (36.94 ± 4.50% vs 63.01 ± 4.778%; p < 0.001; Figure 4C-2).

Antitumor effects of low-dose chemotherapy combined with anti-PD-1 antibody in GC xenografts

Low-dose paclitaxel had no effect on the tumor growth of GC xenografts (1581.00 ± 381.70 vs 2003.00 ± 190.90; p > 0.05), while anti-PD-1 antibody suppressed the tumor growth (794.80 ± 214.90 vs 2003.00 ± 190.90; p < 0.01). Low-dose paclitaxel combined with anti-PD-1 antibody suppressed tumor growth (1155 ± 137.7 vs 2003.00 ± 190.90; p < 0.01), but the effects of combination therapy were comparable to those of anti-PD-1 antibody used alone (794.8 ± 214.9 vs 1155 ± 137.7; p > 0.05; Figure 5A & B). In studies of low-dose 5-fluorouracil combined with anti-PD-1 antibody, low-dose 5-fluorouracil and anti-PD-1 antibody alone suppressed tumor growth (1882 ± 152.3 vs 2500 ± 230.4, p < 0.05; 1371 ± 266.8 vs 2500 ± 230.4, p < 0.05). The combination of low-dose 5-fluorouracil with anti-PD-1 antibody suppressed tumor growth (1580 ± 159.0 vs 2500 ± 230.4; p < 0.05), but the efficacies were not superior tor either monotherapy used alone (Figure 5C–E).

Discussion

The US FDA has approved anti-PD-1 antibody (pembrolizumab) as the third-line compound for patients with GC with PD-L1$^+$ (≥1%) based on the clinical trial KEYNOTE-059. Besides, the FDA approved pembrolizumab as a treatment for patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair–deficient solid tumors that progressed following prior treatment. In ATTRACTION-2, a trial of nivolumab, another kind of anti-PD-1 antibody, showed a survival benefit in the third- or later-line treatment in an Asian patient population, which led to the approval of nivolumab as a treatment for GC in Japan [8], although its use still had many problems. First, whether the antitumor activity of the anti-PD-1/anti-PD-L1 antibody was superior to chemotherapy was yet to be proved. Some trials, such as JAVELIN Gastric 300, indicated that the anti-PD-1/anti-PD-L1 antibody therapy was not significantly superior to paclitaxel or irinotecan [9]. Second, how to improve the efficacy of anti-PD-1 antibody needed further investigation. A combination with chemotherapy might be a good choice. In KEYNOTE-059, the ORR was 60.0% (95% CI: 38.7–78.9) for combination therapy and 25.8% (95% CI: 11.9–44.6) for monotherapy [10]. Moreover, the trial ATTRACTION-4, which investigated the safety and efficacy of nivolumab in combination with S-1/capecitabine plus oxaliplatin in patients with previously untreated, unresectable and advanced or recurrent advanced gastric or gastroesophageal junction cancer, confirmed that nivolumab combined with chemotherapy led to manageable safety as well as clinically relevant antitumor activity [11]. Contrary to the findings of KEYNOTE-059 and ATTRACTION-4, the trial KEYNOTE-062 evaluated the antitumor activity of pembrolizumab, pembrolizumab plus chemotherapy or chemotherapy alone in patients with advanced gastric or gastroesophageal junction cancer. This trial found that anti-PD-1 antibody (pembrolizumab) alone or with chemotherapy was not superior to chemotherapy for the OS and progression-free survival end points tested [12].

As the results of the trials were diverse, finding a biomarker of anti-PD-1 antibody to maximize patient benefit, minimize the risk of toxicities and guide combination approaches was important. So far, no such biomarker of anti-PD-1 antibody in GC has been proved to work precisely. The major focus has been on the PD-L1 expression level in tumor tissues. The trials KEYNOTE-059 and KEYNOTE-061 suggested a higher PD-L1 expression level and a higher treatment effect of anti-PD-1 antibody (pembrolizumab). The trial ATTRACTION-2 failed to confirm the predictive value of PD-L1 expression [8]. Besides, the trials KEYNOTE-059 and KEYNOTE-062 also suggested that PD-L1 expression level might not be a good predictive factor for pembrolizumab combined with chemotherapy in GC [12,13]. TILs were also considered to be potential biomarkers of anti-PD-1 antibody therapy. Patients with cancer are classified into four types. A type I (PD-L1$^+$ TIL$^+$) tumor most likely responds to the anti-PD-1 antibody; however, a type III (PD-L1$^+$ TIL$^-$) tumor is prone to resist to anti-PD-1 antibody monotherapy [14]. Besides, based on TILs, the tumor immune microenvironment is classified into three subtypes: immune inflamed subtype, excluded infiltrate subtype and immune ignorance subtype [15]. Other biomarkers, such as tumor mutation burden (TMB) and microsatellite instability (MSI), are also being explored.

Besides the problems associated with anti-PD-1 antibody therapy, few patients with GC or other cancers benefit from this therapy. The present study explored the effect of low-dose chemotherapy on GC xenografts and the antitumor activity of low-dose chemotherapy combined with anti-PD-1 antibody to find a better combination therapy so as to improve its efficacy and reduce the side effects. In this study, GC xenograft models were established, which possessed functional immune responses; and these xenograft models could accurately reflect the effects

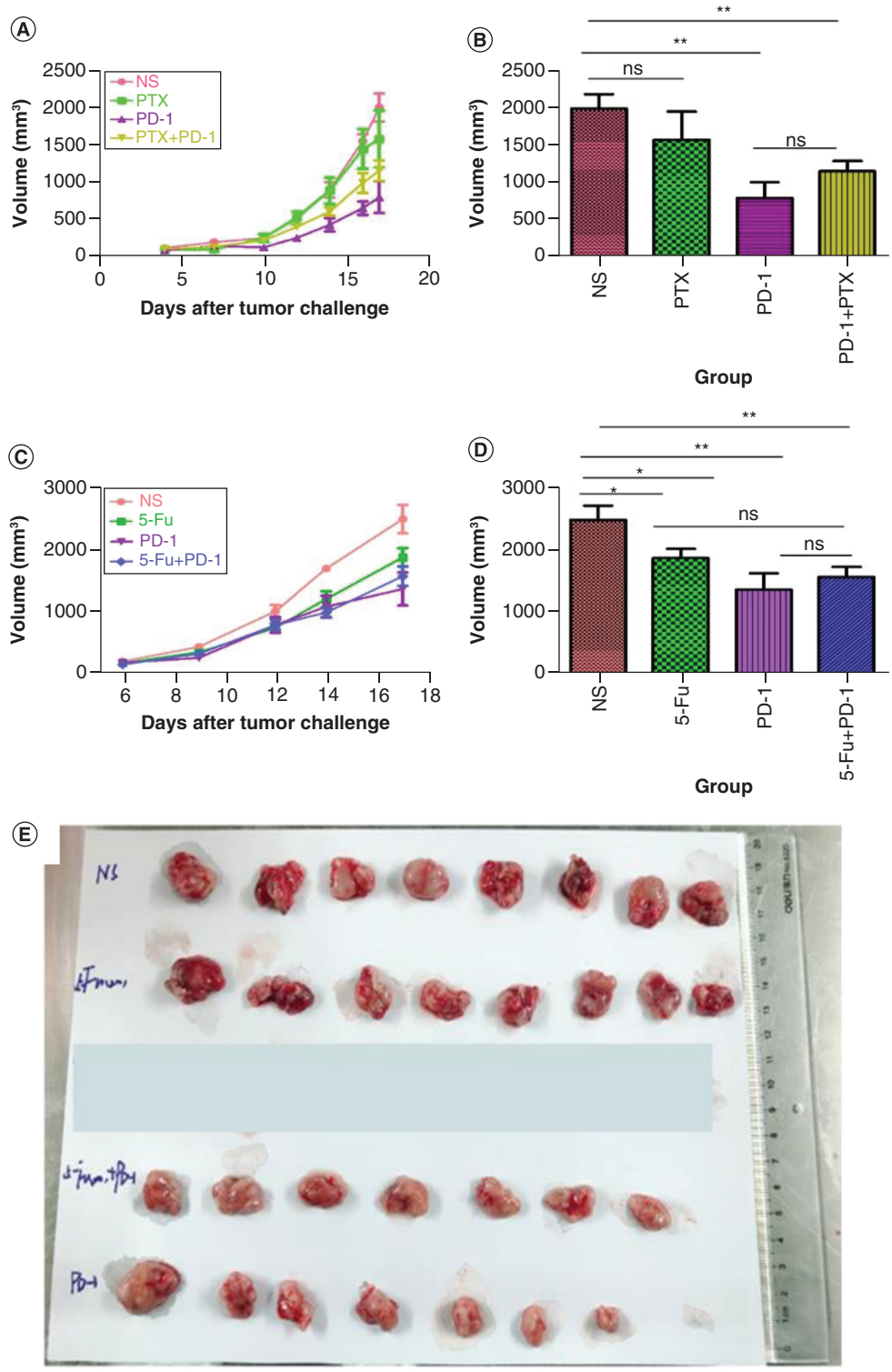

Figure 5. Tumor growth curve and volume under different treatments. (A) Tumor growth in different groups of low-dose paclitaxel combined with anti-PD-1 antibody. **(B)** Tumor volume in different groups of low-dose paclitaxel combined with anti-PD-1 antibody on the day the mice were euthanized. **(C)** Tumor growth in different groups of low-dose 5-fluorouracil combined with anti-PD-1 antibody. **(D)** Tumor volume in different groups of low-dose 5-fluorouracil combined with anti-PD-1 antibody on the day the mice were euthanized. **(E)** Tumor tissues in different groups of low-dose 5-fluorouracil combined with anti-PD-1 antibody on the day the mice were euthanized.
*$p < 0.05$; **$p < 0.01$.

of low-dose chemotherapy on the TME. This study offered valuable information for the discovery of the most reasonable combined therapy so as to improve the antitumor effect of anti-PD-1 antibody. Further, low-dose paclitaxel was found to increase the level of PD-L1 and the percentage of $CD8^+$ T cells in the tumor immune microenvironment. Low-dose 5-fluorouracil reduced the number of MDSCs and PD-1^+ $CD8^+$ T cells in the tumor immune microenvironment. A difference was found between the observed effects of paclitaxel and 5-fluorouracil. 5-Fluorouracil induced cell death through the inhibition of thymidylate synthase and through its misincorporation into newly synthesized DNA and RNA [16]. Increasing evidence has supported that the immune system played a role in the effectiveness of 5-fluorouracil treatment, including induction of heat shock protein 70 on tumor cells following treatment, leading to enhanced tumor uptake by dendritic cells, IL-12 secretion and enhanced antigen presentation. Moreover, induced expression of intercellular adhesion molecule 1 and Fas-ligand results in tumor cell elimination by T cells [17]. Reduction in the frequency of circulating and tumor-infiltrating MDSCs via induction of 5-fluorouracil-induced apoptotic cell death may itself promote changes in the expression of PD-L1. The antitumor actions of paclitaxel were attributed to their ability to suppress cellular division via microtubule stabilization. Studies found that paclitaxel regulated the production of various cytokines and stimulated/inhibited various lymphocytes to have inhibitory effects on tumor cells [18]. MDSCs were suppressive immune cells present in the TME that inhibited the activation of T cells. MDSCs produced nitric oxide and oxygen radicals, thus suppressing the tumor immune response [19–21]. Chemokine receptor 5 inhibitors that target MDSCs can promote antitumor effects in GC [22]. Therefore, reducing the number of MDSCs in tumor tissues represents a novel target for tumor therapy [23]. TILs, including $CD4^+$ T and $CD8^+$ T cells, play an important role in the tumor immune response. Previous studies identified a correlation between TILs and the prognosis of patients with GC [24]. $CD8^+$ T cells are key to tumor immunity and express a series of inhibitory molecules, including PD-1, T-cell immunoglobulin and mucin domain 3, lymphocyte-activation gene and cytotoxic T-lymphocyte-associated antigen 4. PD-1 expression on T cells affected the proliferative and apoptotic phenotypes of the cells [25–27]. A previous study reported that patients with head-and-neck cancer with a large number of PD-1^+ $CD8^+$ T cells had poorer disease-free survival. In colorectal cancer, the number of PD-1^+ $CD8^+$ T cells correlated with the prognosis in pancreatic cancer [28]. Therefore, this study confirmed that low-dose chemotherapy alters the suppressive TME in GC xenografts.

Low-dose chemotherapy combined with the anti-PD-1 antibody seemed to be a reasonable candidate. Thus, the antitumor effects of the aforementioned combination therapy were explored in this study. However, the combination therapy did not show a better antitumor effect compared with the monotherapy. The results suggested that the combination therapy with anti-PD-1 antibody was complicated. The time, order and frequency of drug delivery might affect the antitumor activity of combination therapy. The drug delivery frequency of low-dose chemotherapy was important. Studies found that more frequent administration of cyclophosphamide induced damage to NK cells, while less frequent Q9 day and Q12 day schedules resulted in tumor escape. Similar results were obtained for increasing the activation and functionality of $CD8^+$ T cells [29]. Thus, finding an appropriate timing and dosing schedule might dramatically improve the treatment outcome by both engaging and protecting antitumor immune responses. Finding such a combination for GC remains a challenge to be addressed in future research.

This study had some limitations. First, immunohistochemistry (IHC) was not performed to confirm the presence of a TME. IHC is a widely used diagnostic technique in tissue pathology that helps reveal the distribution of different types of immune cells in the TME and confirm the presence of a TME. However, this technique is associated with some limitations, including high interobserver variability and the capacity to label only one marker per tissue section. For the sake of time and expenditure, IHC was not performed. Also, a second cell line for the experiments would have been beneficial to learn the effect of low-dose chemotherapy on the TME, but no such second cell line was suitable for this study. In addition, the preliminary study showed that the mice with GC treated with regular doses of chemotherapy were too weak and died in less than 17 days after the tumor challenge; therefore, the effect of normal-dose chemotherapy on the GC TME was not investigated in this study. Some recent clinical trials observed improved outcomes with the combination of "normal"-dose chemotherapy and immunotherapy in patients with GC [10,11]. The results would have been different if regular doses of chemotherapy had been used, and it would have been interesting to look at the effect on MDSCs with varying doses of chemotherapy as well. Last but not least, the study was carried out within a short observation period; had it been conducted with a longer observation time, the results could have been different.

Conclusion

Low-dose chemotherapy altered the suppressive TME in GC xenografts but failed to improve responses to the anti-PD-1 antibody. Low-dose paclitaxel increased the level of PD-L1 expression in tumor tissues and regulated the suppressive TME by increasing the percentage of $CD8^+$ T cells. Low-dose 5-fluorouracil altered the suppressive TME by reducing the number of MDSCs and $PD\text{-}1^+$ $CD8^+$ T cells. The anti-PD-1 antibody inhibited tumor growth on GC xenografts. Low-dose chemotherapy combined with anti-PD-1 antibody did not have superior antitumor activity compared with monotherapy.

Summary points

- The anti-PD-1 antibody shows potential as an anticancer therapy for tumors such as gastric cancer (GC). However, the effect of PD-1 antibody monotherapy is still unsatisfactory.
- Chemotherapy combined with anti-PD-1 antibody treatment appears to improve the efficacy of anti-PD-1 antibody in some clinical trials. However, patients with GC benefiting from the combination therapy is controversial due to the inconsistent results of the trials.
- The biomarker for anti-PD-1 antibody therapy is unclear. The expression level of PD-L1 in tumor tissue, tumor mutation burden and microsatellite instability is also being explored.
- The present study explored the effects of low-dose chemotherapy on GC xenografts and its antitumor activity when combined with anti-PD-1 antibody to find a better combination therapy so as to improve its efficacy and reduce the side effects.
- Low-dose paclitaxel increased the PD-L1 expression level in tumor tissues. Low-dose paclitaxel regulated the suppressive immune microenvironment by increasing the percentage of $CD8^+$ T cells. Low-dose 5-fluorouracil altered the suppressive immune microenvironment by reducing the number of myeloid-derived suppressor cells and $PD\text{-}1^+$ $CD8^+$ T cells.
- Anti-PD-1 antibody inhibited tumor growth on GC xenografts. Low-dose chemotherapy combined with anti-PD-1 antibody did not have superior antitumor activity compared with monotherapy.
- The results suggest that the combination therapy with anti-PD-1 antibody was complicated and needed to be explored further to figure out the mechanism. The time, order and frequency of drug delivery might affect the antitumor activity of combination therapy.

Author contributions

F Lin designed and performed the experiments; acquired, analyzed and interpreted the data; and wrote the manuscript. H Chen participated in the experiments and interpretation of the data and wrote the manuscript. X Lin designed the experiments and revised the manuscript. T Jiang and J Zheng participated in the design of the experiments and interpretation of the data and revised the manuscript. Q Liu, B Yang and X Wang participated in revision of the manuscript.

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Financial & competing interests disclosure

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Ethical conduct of research

The authors state that all animal procedures were approved by the Animal Ethics Committee of Fujian Medical University (project approval number: FJMU IACUC 2018-090). They were in accordance with the ethical standards and guidelines for laboratory animals of Fujian Medical University.

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- **This paper discusses the origin, mechanisms of expansion and suppressive functions of myeloid-derived suppressor cells.**