A randomized trial of *Bacteroides fragilis 839* on preventing chemotherapy-induced myelosuppression and gastrointestinal adverse effects in breast cancer patients

doi: 10.6133/apjcn.202401/PP.0003
Published online: January 2024

Running title: *Bacteroides fragilis 839* prevents myelosuppression

Ting Zeng MM[1†], Yu-hong Deng [1†], Chu-hui Lin MM[1], Xin-xin Chen PhD[2], Hai-xia Jia PhD[2], Xiao-wu Hu MM[2], Ting Xia MM[2], Yun Ling MM[2], Le-hong Zhang MM[2], Teng-fei Cao MM[2]

[1]Clinical Nutrition, The Second Affiliated Hospital of Guangzhou Medical University
[2]Breast Surgery, The Second Affiliated Hospital of Guangzhou Medical University
Both authors contributed equally to this manuscript

Authors' email addresses and contributions:
Conception and design: Le-hong Zhang, Yu-hong Deng, Teng-fei Cao, Ting Zeng, Chu-hui Lin
Patient enrollment: All the authors
Experimental, clinical, data collection: All the authors
Data analysis and interpretation: Ting Zeng, Teng-fei Cao, Le-hong Zhang, Yu-hong Deng
Preparation of draft manuscript, doing revisions or providing critique: All the authors
Overall scientific management: Le-hong Zhang, Yu-hong Deng, Ting Zeng, Chu-hui Lin, Teng-fei Cao.

Corresponding Author: Dr Le-hong Zhang, No.250 Changgang Dong Road, Guangzhou City, Guangdong Province, 510260, China. Tel: +86-159-9997-8156. Email: ctf1985@163.com; Dr Teng-fei Cao, No.250 Changgang Dong Road, Guangzhou City, Guangdong Province, 510260, China. Tel: +86-159-9997-8156. Email: ctf1985@163.com

ABSTRACT

Background and Objectives: To evaluate the potential benefits of Bacteroides fragilis 839(BF839), a next-generation probiotics, in reducing myelosuppression and gastrointestinal toxicity associated with chemotherapy in breast cancer patient. **Methods and Study Design:** 40 women with early breast cancer were randomly assigned to theBF839 (n=20) or placebo (n=20) during the administration of adjuvant chemotherapy (4 cycles of epirubicin 100mg/m^2 and cyclophosphamide 600mg/m^2). Myelosuppression and gastrointestinal adverse effects were monitored in both groups. **Results:** Throughout the four treatment cycles, the percentage of patients experiencing myelosuppression was 42.5% in the *BF839* group, significantly lower than the 66.25% observed in the control group (p=0.003). Two patients in the *BF839* group and three patients in the placebo group received recombinant human granulocyte colony-stimulating factor (rhG-CSF) due to leukopenia/neutropenia. When considering an ITT analysis, which included all patients regardless of rhG-CSF treatment, the *BF839* group exhibited less reduction from baseline in white blood cells (-0.31±1.19 vs -1.15±0.77, p=0.012) and neutrophils (0.06±1.00 vs -0.84±0.85, p=0.004) compared to the placebo group. The difference became even more significant when excluding the patients who received rhG-CSF injections. Throughout the four treatment cycles, compared to the placebo group, the *BF839* group had significantly lower rates of 3-4 grade nausea (35.00% vs 71.25%, p=0.000), vomiting (20.00% vs 45.00%, p=0.001), and diarrhea (15.00% vs 30.00%, p=0.023). **Conclusions:** These findings suggest that *BF839* has the potential to effectively mitigate myelosuppression and gastrointestinal toxicity associated with chemotherapy in breast cancer patients.

Key Words: Bacteroides fragilis, prevention, chemotherapy, myelosuppression

INTRODUCTION

Breast cancer is the most common cancer in women worldwide.[1] Cyclophosphamide and epirubicin are commonly used chemotherapy drugs after surgical resection of breast cancer. However, chemotherapy often leads to myelosuppression, with a high incidence rate of over 60%, which can cause treatment delays and severe infections.[2,3] To address chemotherapy-induced neutropenia, recombinant human granulocyte colony-stimulating factor (rhG-CSF) is commonly used but can have side effects like bone/musculoskeletal pain and increased risk of hyperleukocytosis.[4-8] Chemotherapy also frequently causes gastrointestinal toxicity, resulting in symptoms like nausea, vomiting, and diarrhea, impacting treatment schedules and patient

outcomes. Therefore, it is crucial to explore alternative strategies to effectively prevent and manage chemotherapy-induced side effects in breast cancer patients.

Recent evidence suggests that the gut microbiome plays a role in cancer development and affects the effectiveness and side effects of anti-tumor therapies.[9,10] *Bacteroides fragilis* (BF) is a promising next-generation probiotic.[11] with immunogenicity and anti-cancer properties.[12] *BF839*, a non-toxic strain derived from infant feces and available in China for two decades, has shown benefits in preventing intestinal and respiratory diseases and promoting children's growth.[13-15] Our recent findings demonstrated its efficacy in treating psoriatic disease.[16] Given the strong immunogenicity properties of this strain and the paramount importance of long-term safety considerations, we conducted a clinical trial aimed at investigating whether *BF839* can alleviate chemotherapy-induced myelosuppression and gastrointestinal adverse effects in breast cancer patients, while ensuring its safety.

MATERIALS AND METHODS

Study design

The study was a single-center, randomized, double-blinded, and placebo-controlled clinical trial. A total of 40 breast cancer patients were recruited from the Breast Surgery Department of the Second Affiliated Hospital of Guangzhou Medical University. Once enrolled, the patients were assigned to receive either *BF839* or a placebo prior to their first cycle of chemotherapy, which consists of epirubicin and cyclophosphamide. The allocation was determined through randomization. Blood samples were collected for monitoring purposes before the start of chemotherapy and on day 7 and day 14 during each treatment cycle. (Figure 1)

Ethics statement

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Guangzhou Medical University with the approval number of 2019-hs-36, and was registered at the China Clinical Trial Registry (http://www.chictr.org.cn/) with the identification number of ChiCTR2100054876. Before starting the trial, all patients provided written informed consent.

Patients

The patients recruited for this study were selected from the Breast Surgery Department of the Second Affiliated Hospital of Guangzhou Medical University, spanning from December 1st,

2019, to October 31st, 2021. In order to be eligible for participation, patients needed to fulfill the following inclusion criteria: (1) Diagnosis of early-stage breast cancer and undergoing their initial chemotherapy following surgery. (2) Normal peripheral blood count, liver and kidney function, and electrocardiogram results prior to chemotherapy. (3) Age between 18 and 75 years. (4) Expected survival time of more than 3 months. (5) No history of allergy to probiotic preparations. The exclusion criteria were as follows: (1) Patients undergoing concurrent radiotherapy. (2) Individuals who had taken other probiotics or participated in other clinical trials within the past 3 months. (3) Presence of bone marrow or blood-related diseases. (4) Serious heart, lung, liver, or kidney diseases. (5) Concurrent tumors in other organs.

Randomization

Prior to the initiation of the first chemotherapy cycle, eligible patients were randomly allocated into two groups at a 1:1 ratio: the *BF839* group (n=20) and the placebo group (n=20).

Treatment

All patients received four cycles of epirubicin (100mg/m^2) plus cyclophosphamide (600mg/m^2). The BF839 group was administered four cycles of *BF839* (2 packs per day, each containing 10^6 CFU, 21 days per cycle), while the control group received placebo for the same duration, starting from the first day of chemotherapy. The ingredient of placebo was maltodextrin, which was also the main auxiliary ingredient of *BF839*. They had similar odor and taste, with identical packaging. All patients included in the study received a single dose of tropisetron 5mg intravenous (IV) and metoclopramide 10mg intramuscular before the start of chemotherapy. Besides *BF839* and Placebo, none of the patients consumed any special foods, including immune-boosting health foods.

Efficacy assessment

Main observation indicator

Frequency of myelosuppression: The frequency of myelosuppression was assessed to determine the severity of this condition. The grading was based on the "Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0)" developed by the National Cancer Institute of the United States.[17] It included monitoring white blood cell (WBC) counts, absolute neutrophil counts, platelet counts and hemoglobin level. We determined whether bone marrow suppression had occurred based on the results of peripheral blood routine tests,

including WBC, neutrophils, hemoglobin, and platelets, and then we listed their respective gradings. Myelosuppression grade was determined by the highest grade of each respective gradings. (Supplementary Table 1)

Blood cells counts changes：We examined the changes in peripheral WBC, neutrophils, red blood cells, and platelets changes from the baseline during the treatment cycles. This analysis covered each chemotherapy cycle intervals (on days 5-7, 11-14, and 21).

Secondary observation indicator

Frequency of gastrointestinal symptoms: the frequency of nausea, vomiting, and diarrhea at grade 3/4 are used as evaluation indicators. The grading was also based on the CTCAE v3.0. Grade 3/4 nausea was defined as inadequate oral caloric or fluid intake: IV fluids, tube feedings, or total parenteral nutrition (TPN) indicated ≥24 hours; or Life-threatening consequences. Grade 3/4 diarrhea was defined as increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hours; hospitalization; or Life-threatening consequences. Grade 3/4 vomiting was defined as ≥6 episodes in 24 hours; IV fluids, or TPN indicated ≥24 hours; hospitalization; or Life-threatening consequences. (Supplementary Table 1)

Sample size evaluation:

By utilizing the PASS 15.0 software for sample size calculation, we conducted a pilot experiment which revealed a difference of greater than 1.5×10^9/L in WBC counts between the two groups. With a significance level (α) of 0.05 and a test power (1-β) of 80%, the calculation determined that each group would require 17 cases. Considering a dropout rate of 15%, we decided to enroll 40 cases.

Statistical analysis

We calculated the change number for each cycle and obtained the total number by summing up across total cycles. Both Intention-to-treat (ITT) and Per-protocol (PP) analyses were performed using SPSS version 22.0. Continuous variables were presented as mean ± standard deviation (x ± SD) and compared using the t-test. Count data were expressed as the number of cases (%) and using the chi-square test or Fisher's exact test. Statistical significance was defined as $p < 0.05$.

Adverse events

Any unexpected adverse events were recorded.

RESULTS

Patient characteristics

A total of 40 patients participated in the trial. In the *BF839* group, 17 patients completed the study, with 1 due to diarrhea, 1 missing more than 20% of the medication, 1 loss of follow-up during the first cycle. In the placebo group, 19 patients completed the study, with 1 loss of follow-up during the first cycle. Baseline characteristics of the patients were no significantly difference between two groups. (Table 1)

BF839 significantly reduced frequency of myelosuppression

In ITT analysis， throughout total cycles, the incidence of all grade and I-II grade myelosuppression were significantly lower in the *BF839* group than in the placebo group (42.5% vs 66.25%, p=0.003); (35.00% vs 55.00%, p=0.0110). Especially in the fourth cycle, the reduction was the most significant. Similar results were seen in the PP analysis. (Table 2)

BF839 significantly reduced the degree of WBC and neutrophil counts reduction from baseline

Two patients in the *BF839* group received rh G-CSF injection, with WBC counts of 1.74×10^9/L and 1.72×10^9/L, and neutrophil counts of 0.11×10^9/L and 0.21×10^9/L, respectively. In the placebo group, three patients received rh G-CSF injection, with WBC counts of 1.68×10^9/L, 1.62×10^9/L, and 1.27×10^9/L, and neutrophil counts of 0.19×10^9/L， 0.46×10^9/L， 0.21×10^9/L, respectively. In an ITT analysis that included these five patients, the *BF839* group showed a significantly fewer decrease from baseline in WBC counts (-0.31±1.19 vs -1.15±0.77, p=0.012) and neutrophil counts (0.06±1.00 vs -0.84±0.85, p=0.004) after chemotherapy compared to the placebo group (Table 3) in total cycles. A similar trend was observed in the PP analysis (Table 3). To account for the effect of rh G-CSF injection on WBC and neutrophil counts, we excluded the aforementioned five patients and conducted ITT and PP analyses again, revealing an even more significant difference between the two groups (Table 4). We analyzed the continuous changes of WBC and neutrophils at each timepoint, *BF839* group also showed a fewer decrease in WBC and neutrophils (Figures 2 and 3). The

changes in red blood cells and platelets did not differ between the two groups; details are provided in Supplementary Tables 2 and 3.

BF839 significantly reduced frequency of gastrointestinal symptoms

In the ITT analysis, the proportion of patients experiencing grade 3-4 nausea, vomiting, and diarrhea in the *BF839* group was significantly lower than that in the placebo group, with a reduction of about half in total cycles (nausea: 35.00% vs 71.25%, p=0.000; vomiting: 20.00% vs 45.00%, p=0.001; diarrhea: 15.00% vs 30.00%, p=0.023). A similar trend was observed in the PP analysis (Table 5).

Safety assessment

One case in the *BF839* group dropped out due to diarrhea. Since diarrhea is also a common side effect of chemotherapy, it cannot be determined whether this diarrhea was related to the experiment. No other adverse effects were reported.

DISCUSSION

The study found that *BF839* reduces bone marrow suppression and gastrointestinal side effects in breast cancer patients undergoing chemotherapy. It decreases leukocyte and neutrophil reduction, lowers the incidence of nausea, vomiting, and diarrhea, while not affecting red blood cells and platelets.

Animal studies have demonstrated that specific strains of *Lactobacillus* bacteria, such as *Lactobacillus paracasei CRL431* or *Lactobacillus reuteri CRL1506*, can enhance the production of immature myeloid stem cells in the bone marrow, facilitating faster recovery of myeloid cells and neutrophils following cyclophosphamide treatment.[9,18] The precise mechanism by which gut bacteria influence peripheral blood cells remains unclear. Previous research has shown that patients who underwent chemotherapy-induced immune system destruction and subsequently received a fecal microbiota transplant during bone marrow transplantation experienced increased peripheral WBC counts, likely attributed to the successful restoration of a diverse microbial community with its associated metabolic functions.[19] Additionally, systemic recognition of microbiota-derived products through Toll-like receptors has been identified as crucial for maintaining an adequate pool of bone marrow myeloid cells, suggesting a reliance on microbiota-derived signals for infection vigilance.[20] Notably, commensal bacterium *Bacteroides fragilis* can regulate the balance of Th1/Th2 lymphocytes cell and rectify immune developmental defects in germ-free mice, thus restoring

normal levels of CD4+ T lymphocytes.[21,22] We speculate that *BF839* may stimulate the immune system through signals from the microbial community, aiding in the recovery of bone marrow function following chemotherapy-induced damage. However, our understanding of the mechanism is limited due to the intricate interplay between the host and microbial components.

Chemotherapy-induced gastrointestinal mucositis is characterized by villous atrophy and loss of intestinal epithelial cells.[23] This condition often leads to life-threatening systemic infections, as well as nausea, vomiting, and diarrhea in patients. Probiotics have shown potential in alleviating chemotherapy-related gastrointestinal side effects, including diarrhea, nausea, vomiting, constipation, and bloating, with no reported safety concerns.[24] Our study found that *BF839* significantly reduces nearly half of gastrointestinal side effects associated with the epirubicin and cyclophosphamide chemotherapy regimen. This further supports the use of probiotics to improve chemotherapy-related gastrointestinal issues. The mechanism of chemotherapy-induced mucositis is complex, with symbiotic gut microbiota potentially influencing mucositis through pathways involving inflammatory processes and oxidative stress, intestinal permeability, mucus layer composition, resistance to harmful stimuli and epithelial repair mechanisms, and immune effector molecule activation and release.[25] Polysaccharide A from Bacteroides fragilis, a recognized commensal symbiosis factor,[26] promotes the development of Foxp3+ regulatory T-cells in the intestine, enhancing interleukin 10 production,[22] thus effectively preventing intestinal inflammation.[27] Our recent study indicates that *BF839* can restore decreased expression of epithelial tight junction proteins, such as zonula occludens 1 and occludin, reducing intestinal permeability (under review). Future research may uncover additional mechanisms.

A recent review of 21 studies involving 2,600 patients using probiotics during chemotherapy and/or radiotherapy found no confirmed adverse reactions.[24] This small-sample study with *BF839* also showed no cases of bacteremia or severe adverse reactions. However, caution should be exercised as patients undergoing treatment may be more susceptible to probiotic-related adverse effects compared to healthy individuals. Larger studies are needed to confirm safety.

Despite promising findings, our study had limitations. Firstly, unlike some studies that extensively monitor blood cell count,[4,28,29] we only assessed it three times after chemotherapy on days 7, 14, and 21, which may result in insufficient data. However, our study design captured the period when patients typically experience the lowest white cell/neutrophil count after 7-14 days of chemotherapy, and reduced blood sampling improved patient compliance.

Secondly, we did not analyze fecal microbiota before and after the intervention, which could be assessed over a longer duration in future studies to explore its relationship with efficacy and uncover its mechanism. It is also essential to acknowledge the limitation imposed by the relatively small sample size, which may hinder the generalizability of our findings. Additionally, the exclusive inclusion of early-stage breast cancer patients who underwent a uniform chemotherapy regimen (EC) should be taken into account as another factor affecting the external validity of the results.

Our study suggests that *BF839* has the potential to effectively mitigate myelosuppression and gastrointestinal toxicity associated with chemotherapy of epirubicin and cyclophosphamide in breast cancer patients. This study adds to the increasing evidence supporting the use of probiotics as adjunct therapy in cancer patients to enhance treatment tolerability and improve patient outcomes. However, further research and clinical trials are needed to validate these initial findings and assess the effectiveness of *BF839* with other chemotherapy drugs and in different types of cancer.

ACKNOWLEDGEMENTS

We would like to thank Guangzhou Totem Life Medical Research Company Ltd. In this study, *BF839* and placebo were provided by them for free. We thank Mr Sun Changchun from Totem Company for providing product information. Guangzhou Totem Life Medical Research Company Ltd. was not involved in the study design, collection, analysis, interpretation of data, and the writing of this article or the decision to submit it for publication. We thank the patients who consented to this study.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors declare that they had no conflict of interest.

REFERENCES

1. 1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-49. doi: 10.3322/caac.21660.
2. Jones S, Holmes FA, O'Shaughnessy J, Blum JL, Vukelja SJ, McIntyre KJ et al. Docetaxel With Cyclophosphamide Is Associated With an Overall Survival Benefit Compared With Doxorubicin and

Cyclophosphamide: 7-Year Follow-Up of US Oncology Research Trial 9735. J Clin Oncol. 2009;27:1177-83. doi: 10.1200/JCO.2008.18.4028.

3. Zhang Peiying, Wu Dongfang. Combined chemotherapy with epirubicin, cyclophosphamide, and vincristine causes severe cardiac and blood toxicity reactions leading to death. Adverse Drug Reactions Journal, 2019;06:459-60. doi:10.3760/cma.j.issn.1008-5734.2019.06.017.
4. Holmes FA, O'Shaughnessy JA, Vukelja S, Jones SE, Shogan J, Savin M et al. Blinded, randomized, multicenter study to evaluate single administration pegfilgrastim once per cycle versus daily filgrastim as an adjunct to chemotherapy in patients with high-risk stage II or stage III/IV breast cancer. J Clin Oncol. 2002;20:727-31. doi: 10.1200/JCO.2002.20.3.727.
5. Dale DC, Crawford J, Klippel Z, Reiner M, Osslund T, Fan E et al. A systematic literature review of the efficacy, effectiveness, and safety of filgrastim. Support Care Cancer. 2018;26:7-20. doi: 10.1007/s00520-017-3854-x.
6. Chavda R, Herrington JD. Pegfilgrastim-induced hyperleukocytosis leading to hospitalization of a patient with breast cancer. Proc (Bayl Univ Med Cent). 2019;32:261-2. doi: 10.1080/08998280.2018.1547873.
7. Snyder RL, Stringham DJ. Pegfilgrastim-induced hyperleukocytosis. Ann Pharmacother. 2007;41:1524-30. doi: 10.1345/aph.1K093.
8. Gong J, Wu B, Guo T, Zhou S, He B, Peng X. Hyperleukocytosis: A report of five cases and review of the literature. Oncol Lett. 2014;8:1825-7. doi: 10.3892/ol.2014.2326.
9. Gori S, Inno A, Belluomini L, Bocus P, Bisoffi Z, Russo A et al. Gut microbiota and cancer: How gut microbiota modulates activity, efficacy and toxicity of antitumoral therapy. Crit Rev Oncol Hematol. 2019;143:139-47. doi: 10.1016/j.critrevonc.2019.09.003.
10. Bender MJ, McPherson AC, Phelps CM, Pandey SP, Laughlin CR, Shapira JH et al. Dietary tryptophan metabolite released by intratumoral Lactobacillus reuteri facilitates immune checkpoint inhibitor treatment. Cell. 2023;186:1846-62. doi: 10.1016/j.cell.2023.03.011.
11. Chang CJ, Lin TL, Tsai YL, Wu TR, Lai WF, Lu CC et.al. Next generation probiotics in disease amelioration. J Food Drug Anal. 2019;27:615-622. doi: 10.1016/j.jfda.2018.12.011.

12 Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350:1079-84. doi: 10.1126/science.aad1329.

13. Zhang Jijie, Yu Lixian, Xu Linzhi, Wang Wenyu, Zhang Hongjing, Pan Zhonghua et al. Isolation and identification of a Non-toxic Bacteroides fragilis. Ningxia Medical Journal, 1991;13: 216-218. (in Chinese).

14 Feng ShuZhen, Zhang HePing.The research progress of Bacteroides fragilis. 2015;42:1366-1371. doi: 10.13344/j.microbiol.china.140778. (in Chinese)

15 Zhang Jijie, Zhang Hongmei, Zhang Yi, Zhang Honggui, Bi Li, Zhang Xiuchen et al. Study on clinical application of Bacteroides fragilis (BF839). Chinese Journal of Biologicals, 1995;8: 63-65. (in Chinese). doi:10.13200/j.cjb.1995.02.63.zhangjj.006.

16 Lin C, Zeng T, Deng Y, Yang W, Xiong J. Treatment of psoriasis vulgaris using Bacteroides fragilis BF839: a single-arm, open preliminary clinical study. Sheng Wu Gong Cheng Xue Bao. 2021 Nov 25;37:3828-35. doi:10.13345/j.cjb.210198.

17 Common Terminology Criteria for Adverse Events v3.0. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf

18. Gramajo Lopez A, Gutiérrez F, Saavedra L, Hebert EM, Alvarez S, Salva S. Improvement of Myelopoiesis in Cyclophosphamide-Immunosuppressed Mice by Oral Administration of Viable or Non-Viable Lactobacillus Strains. Front Immunol. 2021;12:647049. doi: 10.3389/fimmu.2021.647049.

19 Schluter J, Peled JU, Taylor BP, Markey KA, Smith M, Taur Y et al. The gut microbiota is associated with immune cell dynamics in humans. Nature. 2020;588:303-7. doi: 10.1038/s41586-020-2971-8.

20 Balmer ML, Schürch CM, Saito Y, Geuking MB, Li H, Cuenca M et al. Microbiota-derived compounds drive steady-state granulopoiesis via MyD88/TICAM signaling. J Immunol. 2014;193:5273-83. doi: 10.4049/jimmunol.1400762.

21. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122:107-18. doi: 10.1016/j.cell.2005.05.007.

22. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107:12204-9. doi: 10.1073/pnas.0909122107.

23 Keefe DM, Brealey J, Goland GJ, Cummins AG. Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. Gut. 2000;47:632-7. doi: 10.1136/gut.47.5.632.

24 Garczyk A, Kaliciak I, Drogowski K, Horwat P, Kopeć S, Staręga Z et al. Influence of Probiotics in Prevention and Treatment of Patients Who Undergo Chemotherapy or/and Radiotherapy and Suffer from Mucositis, Diarrhoea, Constipation, Nausea and Vomiting. J Clin Med. 2022;11:3412. doi: 10.3390/jcm11123412.

25 Van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog. 2010;6:e1000879. doi: 10.1371/journal.ppat.1000879.

26 Erturk-Hasdemir D, Kasper DL. Finding a needle in a haystack: Bacteroides fragilis polysaccharide A as the archetypical symbiosis factor. Ann N Y Acad Sci. 2018;1417:116-129. doi: 10.1111/nyas.13660..

27 Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453:620-5. doi: 10.1038/nature07008.

28 Xu F, Zhang Y, Miao Z, Zeng X, Wu B, Cai L et al. Efficacy and safety of mecapegfilgrastim for prophylaxis of chemotherapy-induced neutropenia in patients with breast cancer: a randomized, multicenter, active-controlled phase III trial. Ann Transl Med. 2019;7:482. doi: 10.21037/atm.2019.07.95.

29 Green MD, Koelbl H, Baselga J, Galid A, Guillem V, Gascon P et al. A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol. 2003;14:29-35. doi: 10.1093/annonc/mdg019.

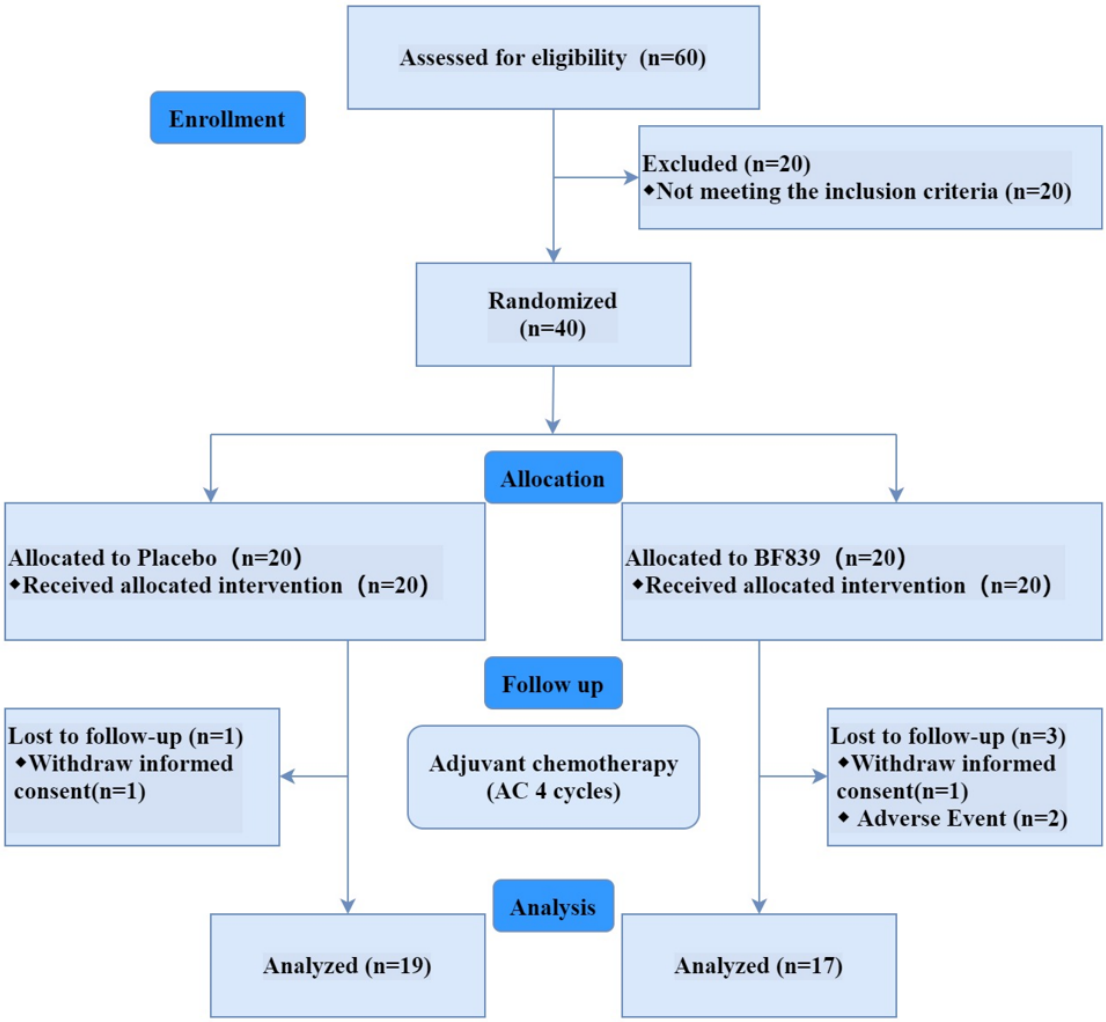
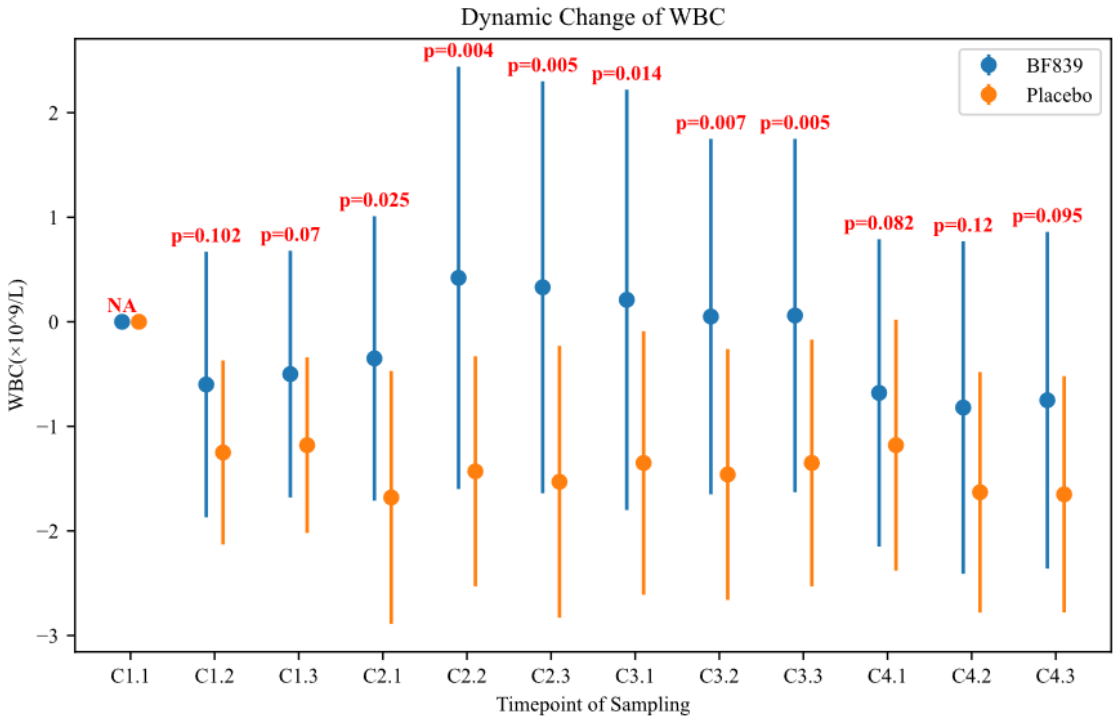
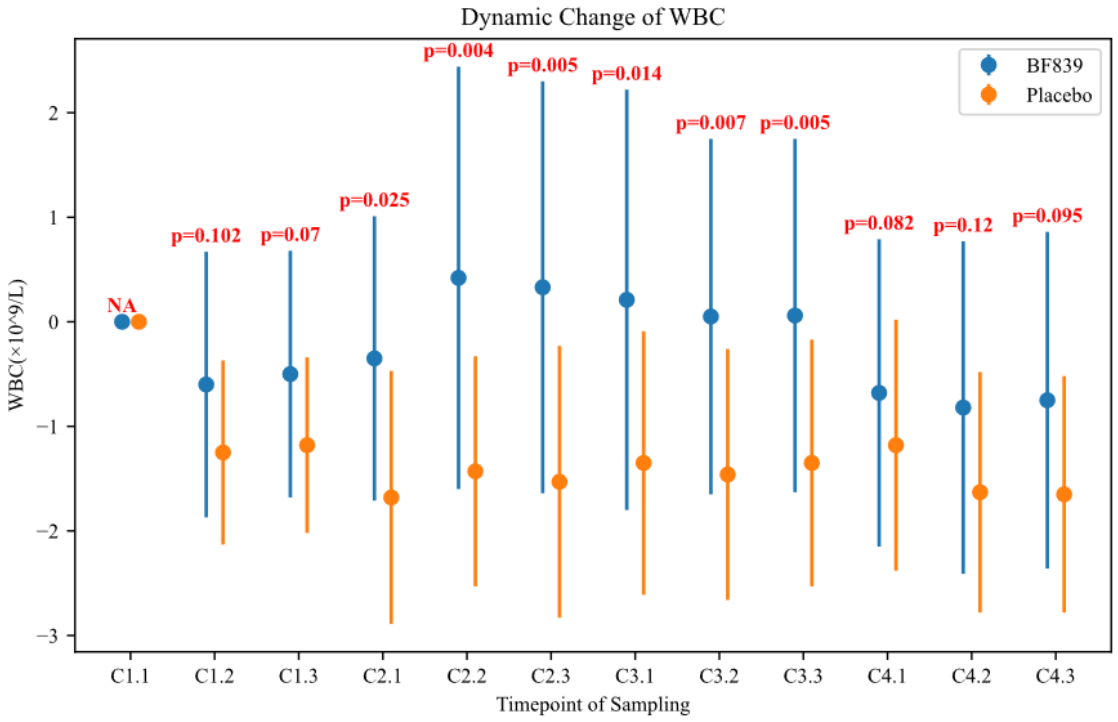


Figure 1. The design of the trial. *BF839*, *Bacteroides fragilis 839*; AC, epirubicin + cyclophosphamide



Timepoint of Sampling	WBC change in BF839 Group (n=15)	WBC change in Placebo Group (n=16)	p-value
C1.1	0.00±0.00	0.00±0.00	NA
C1.2	-0.60±1.27	-1.25±0.88	0.102
C1.3	-0.50±1.18	-1.18±0.84	0.07
C2.1	-0.35±1.36	-1.68±1.21	0.025
C2.2	0.42±2.02	-1.43±1.10	0.004
C2.3	0.33±1.97	-1.53±1.30	0.005
C3.1	0.21±2.01	-1.35±1.26	0.014
C3.2	0.05±1.70	-1.46±1.20	0.007
C3.3	0.06±1.69	-1.35±1.18	0.005
C4.1	-0.68±1.47	-1.18±1.20	0.082
C4.2	-0.82±1.59	-1.63±1.15	0.12
C4.3	-0.75±1.61	-1.65±1.13	0.095

Figure 2. Dynamic change of WBC between the *BF839* group and placebo group (excluding 5 patients who received rh G-CSF). WBC, white blood cells. In each cycle we did blood test at 3 points:1. before chemotherapy (day 21 of previous cycle); 2. 5-7 days after chemotherapy; 3. 10-14 after days chemotherapy. Timepoint C1.3 mean the blood sampling in 1st chemotherapy cycle at 10-14 after days chemotherapy



Timepoint of Sampling	WBC change in BF839 Group (n=15)	WBC change in Placebo Group (n=16)	p-value
C1.1	0.00±0.00	0.00±0.00	NA
C1.2	-0.60±1.27	-1.25±0.88	0.102
C1.3	-0.50±1.18	-1.18±0.84	0.07
C2.1	-0.35±1.36	-1.68±1.21	0.025
C2.2	0.42±2.02	-1.43±1.10	0.004
C2.3	0.33±1.97	-1.53±1.30	0.005
C3.1	0.21±2.01	-1.35±1.26	0.014
C3.2	0.05±1.70	-1.46±1.20	0.007
C3.3	0.06±1.69	-1.35±1.18	0.005
C4.1	-0.68±1.47	-1.18±1.20	0.082
C4.2	-0.82±1.59	-1.63±1.15	0.12
C4.3	-0.75±1.61	-1.65±1.13	0.095

Figure 3. Dynamic change of Neutrophil between the *BF839* group and placebo group (excluding 5 patients who received rh G-CSF). In each cycle we did blood test at 3 points:1. before chemotherapy (day 21 of previous cycle); 2. 5-7 days after chemotherapy; 3. 10-14 after days chemotherapy. Timepoint C1.3 mean the blood sampling in 1st chemotherapy cycle at 10-14 after days chemotherapy

Table 1. Baseline characteristics of patients

Characteristics	BF839 group (n=17)	Placebo group (n=19)	*p*
Age (year)	52.06±10.04	53.37±9.60	0.692
BMI (kg/m^2)	24.33±3.07	25.41±3.03	0.295
Type of the tumors n (%)			
Invasive ductal carcinoma	17 (100%)	19 (100%)	0.528
Stage n (%)			
II	6 (35.3%)	8 (42.1%)	0.742
III	11 (64.7%)	11 (57.9%)	
WBC ($10^9/L$)	5.48±1.28	5.56±0.80	0.84
Neutrophil ($10^9/L$)	3.13±0.87	3.47±0.80	0.27
RBC ($10^{12}/L$)	4.35±0.35	4.37±0.47	0.88
PLT ($10^9/L$)	286.67±112.24	267.63±60.58	0.62

WBC, white blood cells; RBC, red blood cells; PLT, platelets.

Table 2. Comparison of frequency of myelosuppression between the *BF839* and placebo group

Chemotherapy cycle/ Bone marrow suppression	ITT analysis			PP analysis		
	BF839 group (n=20)	Placebo group (n=20)	*p*	*BF839* group (n=17)	Placebo group (n=19)	*p*
Cycle 1						
All Grade	45.00% (9/20)	60.00% (12/20)	0.342	52.94% (9/17)	63.16% (12/19)	0.736
Grade 1-2	30.00% (6/20)	40.00% (8/20)	0.507	35.29% (6/17)	42.10% (8/19)	0.742
Grade 3-4	15.00% (3/20)	20.00% (4/20)	0.677	17.65% (3/17)	21.05% (4/19)	0.566
Cycle 2						
All Grade	45.00% (9/20)	75.00% (15/20)	0.053	52.94% (9/17)	78.95% (15/19)	0.158
Grade 1-2	45.00% (9/20)	65.00% (13/20)	0.204	52.94% (9/17)	68.42% (13/19)	0.495
Grade 3-4	0	10.00% (2/20)	0.147	0	10.52% (2/19)	0.271
Cycle 3						
All Grade	35.00% (7/20)	50.00% (10/20)	0.337	41.18% (7/17)	52.63% (10/19)	0.525
Grade 1-2	30.00% (6/20)	45.00% (9/20)	0.327	35.29% (6/17)	47.37% (9/19)	0.516
Grade 3-4	5.0% (1/20)	5.0% (1/20)	1.000	5.88% (1/17)	5.26% (1/19)	0.729
Cycle 4						
All Grade	45.00% (9/20)	80.00% (16/20)	0.022*	52.94% (9/17)	84.21% (16/19)	0.042*
Grade 1-2	35.00% (7/20)	70.00% (14/20)	0.027*	41.18% (7/17)	73.68% (14/19)	0.090
Grade 3-4	10.00% (2/20)	10.00% (2/20)	1.000	11.76% (2/17)	10.53% (2/19)	0.655
Total Cycles						
All Grade	42.5% (34/80)	66.25% (53/80)	0.003**	50.00% (34/68)	69.74% (53/76)	0.018*
Grade 1-2	35.00% (28/80)	55.00% (44/80)	0.011*	41.18% (28/68)	64.71% (44/76)	0.045*
Grade 3-4	7.5% (6/80)	11.25% (9/80)	0.416	8.82% (6/68)	11.84% (9/76)	0.906

* $p<0.05$，**$p<0.01$.

Table 3. Comparison of frequency of myelosuppression between the *BF839* and placebo group

	ITT analysis			PP analysis		
Average value compared with baseline	*BF839* group (n=20)	Placebo group (n=20)	*p*	*BF839* group (n=17)	Placebo group (n=19)	*p*
WBC (× 10^9/L)						
Total cycles	-0.31±1.19	-1.15±0.77	0.012*	-0.36±1.29	-1.21±0.74	0.020*
No. of cycle						
Cycle 1	-0.47±1.24	-1.10±0.88	0.060	-0.55±1.24	-1.16±0.86	0.095
Cycle 2	0.20±1.75	-0.98±1.48	0.027*	0.23±1.90	-1.03±1.50	0.033*
Cycle 3	-0.27±1.81	-1.36±1.17	0.050	-0.32±1.93	-1.27±1.04	0.071
Cycle 4	-0.70±1.81	-1.36±1.17	0.177	-0.83±1.94	-1.44±1.15	0.255
Neutrophils (× 10^9/L)						
Total cycles	0.06±1.00	-0.84±0.85	0.004*	0.069±1.09	-0.88±0.85	0.006**
No. of cycle						
Cycle 1	-0.28±0.98	-0.86±0.84	0.050	-0.32±1.05	-0.90±0.84	0.075
Cycle 2	0.50±1.79	-0.68±1.39	0.026*	0.58±1.93	-0.72±1.42	0.027*
Cycle 3	0.20±1.70	-0.91±1.12	0.019*	0.24±1.85	-0.95±1.13	0.024*
Cycle 4	-0.22±1.40	-0.87±1.17	0.122	-0.26±1.52	-0.92±1.19	0.158

* p<0.05，**p<0.01.

Table 4. Comparison of WBC and neutrophils changes from baseline between the BF839 and placebo group (excluding 5 patients who received rh G-CSF)

	ITT analysis			PP analysis		
Average value compared with baseline	*BF839* group (n=18)	Placebo group (n=17)	*p*	*BF839* group (n=15)	Placebo group (n=16)	*p*
WBC (×10^9/L)						
Total cycles	-0.31±1.19	-1.15±0.77	0.012*	-0.36±1.29	-1.21±0.74	0.020*
No. of cycle						
Cycle 1	-0.45±1.11	-1.15±0.93	0.052	-0.54±1.2	-1.22±0.91	0.080
Cycle 2	0.36±1.69	-1.25±1.09	0.002**	+0.44±1.85	-1.33±1.07	0.004**
Cycle 3	0.05±1.47	-1.33±1.08	0.004**	+0.06±1.62	-1.41±1.06	0.006**
Cycle 4	-0.69±1.58	-1.45±1.12	0.116	-0.83±1.71	-1.54±1.12	0.180
Neutrophils (×10^9/L)						
Total cycles	0.11±1.01	-0.28±0.98	0.001**	0.14±1.11	-1.02±0.71	0.002**
No. of cycle						
Cycle 1	-0.28±0.98	-0.96±0.84	0.034	-0.34±1.07	-1.02±0.83	0.054
Cycle 2	0.64±1.79	-0.98±0.95	0.002**	0.76±1.95	-1.03±0.94	0.004**
Cycle 3	0.48±1.50	-0.97±1.08	0.003**	0.58±1.63	-1.03±1.09	0.003**
Cycle 4	-0.18±1.23	-0.97±1.14	0.061	-0.23±1.35	-1.03±1.15	0.080

* p<0.05，**p<0.01.

Table 5. Comparison of grade 3/4 gastrointestinal side effects between the BF839 and placebo group

	ITT analysis			PP analysis		
Chemotherapy cycle	*BF839* group (n=20)	Placebo group (n=20)	*p*	*BF839* group (n=17)	Placebo group (n=19)	*p*
Nausea (Grade 3-4)						
Cycle 1	25.00% (5/20)	60.00% (12/20)	0.025*	29.41% (5/17)	63.16% (12/19)	0.043*
Cycle 2	35.00% (7/20)	70.00% (14/20)	0.027*	41.18% (7/17)	73.68% (14/19)	0.048*
Cycle 3	35.00% (7/20)	75.00% (15/20)	0.011*	41.18% (7/17)	78.95% (15/19)	0.020*
Cycle 4	45.00% (9/20)	80.00% (16/20)	0.022*	52.94% (9/17)	84.21% (16/19)	0.042*
Total cycles	35.00% (28/80)	71.25% (57/80)	0.000***	41.17% (28/68)	75% (57/76)	0.000***
Vomiting (Grade 3-4)						
Cycle 1	25.00% (5/20)	35.00% (7/20)	0.490	29.41% (5/17)	36.84% (7/19)	0.732
Cycle 2	20.00% (4/20)	45.00% (9/20)	0.091	23.53% (4/17)	47.37% (9/19)	0.177
Cycle 3	15.00% (3/20)	50.00% (10/20)	0.018*	17.56% (3/17)	52.63% (10/19)	0.041*
Cycle 4	20.00% (4/20)	50.00% (10/20)	0.047	23.53% (4/17)	52.63% (10/19)	0.097
Total cycles	20.00% (16/80)	45.00% (36/80)	0.001**	24.53% (16/68)	47.37% (36/76)	0.003**
Diarrhea (Grade 3-4)						
Cycle 1	10.00% (2/20)	30.00% (6/19)	0.114	11.76% (2/17)	31.58% (6/19)	0.236
Cycle 2	15.00% (3/20)	25.00% (5/20)	0.429	17.65% (3/17)	26.32% (5/19)	0.695
Cycle 3	15.00% (3/20)	30.00% (6/19)	0.256	17.65% (3/17)	31.58% (6/19)	0.451
Cycle 4	20.00% (4/20)	35.00% (7/20)	0.288	23.53% (4/17)	36.84% (7/19)	0.481
Total cycles	15.00% (12/80)	30.00% (24/80)	0.023*	17.64% (12/68)	31.58% (24/76)	0.054

* p<0.05，**p<0.01，***p<0.001.

Supplementary Table

Supplementary Table 1. Grading of myelosuppression and gastrointestinal symptoms

Items	Grade 1	Grade 2	Grade 3	Grade 4
Myelosuppression				
WBC	＜LLN-3.0×10^9/L	＜3.0-2.0×10^9/L	＜2.0-1.0×10^9/L	＜1.0×10^9/L
Neutrophils	＜LLN-1.5×10^9/L	＜1.5-1.0×10^9/L	＜1.0-0.5×10^9/L	＜0.5×10^9/L
Platelets	＜LLN-75×10^9/L	＜75-50×10^9/L	＜50-25×10^9/L	＜25×10^9/L
Hemoglobin	＜LLN-100g/L	＜100-80g/L	＜80-65g/L	＜65g/L
Gastrointestinal Symptoms				
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences
Diarrhea	Increase of <4 stools per day over baseline;	Increase of 4 – 6 stools per day over baseline; IV fluids indicated <24hrs;	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalization;	Life-threatening consequences
Vomiting	1 episode in 24 hrs	2 – 5 episodes in 24 hrs; IV fluids indicated <24 hrs	≥6 episodes in 24 hrs; IV fluids, or TPN indicated ≥24 hrs	Life-threatening consequences

WBC: white blood cells; LLN: Lower Level of Normal; hrs: hours; IV: intravenous; TPN: total parenteral nutrition
[†]Modified from CTCAE (Common Terminology Criteria for Adverse Events) v3.0
.

Supplementary Table 2. Comparison of RBC changes between the two groups

RBC	Changes compared with baseline	*BF839* group (n=17)	Placebo group (n=19)	*p*
Mean value	All cycles	-0.19±0.30	-0.13±0.32	0.589
(× 10^{12}/L)	Cycle 1	-0.19±0.35	-0.13±0.26	0.560
	Cycle 2	-0.15±0.30	-0.15±0.39	0.989
	Cycle 3	-0.20±0.33	-0.13±0.42	0.596
	Cycle 4	-0.26±0.40	-0.13±0.39	0.320
Minimum value	All cycles	-0.34±0.34	-0.24±0.36	0.400
(× 10^{12}/L)	Cycle 1	-0.38±0.55	-0.27±0.27	0.445
	Cycle 2	-0.30±0.35	-0.29±0.48	0.957
	Cycle 3	-0.28±0.35	-0.20±0.43	0.546
	Cycle 4	-0.42±0.44	-0.21±0.40	0.158

RBC: red blood cells
.

Supplementary Table 3. Comparison of PLT changes between the two groups

RBC	Changes compared with baseline	*BF839* group (n=17)	Placebo group (n=19)	*p*
Mean value ($\times 10^{12}$/L)	All cycles	12.18±56.19	-8.80±45.36	0.224
	Cycle 1	9.94±55.40	-16.34±62.61	0.193
	Cycle 2	6.51±65.33	1.03±45.13	0.769
	Cycle 3	24.61±62.26	4.39±53.69	0.303
	Cycle 4	22.28±79.38	-8.35±60.28	0.198
Minimum value ($\times 10^{12}$/L)	All cycles	-22.57±50.86	-34.83±47.15	0.458
	Cycle 1	-39.55±68.23	-67.16±102.91	0.355
	Cycle 2	-32.00±68.00	-36.58±51.78	0.820
	Cycle 3	-12.95±57.94	-12.58±53.66	0.984
	Cycle 4	-5.67±87.78	-23.00±62.84	0.499

PLT: blood platelet