Neoadjuvant *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) and chemotherapy versus placebo plus chemotherapy in patients with HER2-negative breast cancer: a randomized, controlled, double-blind trial

Yue Gong[1,2,3#], Hua Zuo[1,2,3,4#], Ying Zhou[1,2,3], Ke-Da Yu[1,2,3], Guang-Yu Liu[1,2,3], Gen-Hong Di[1,2,3], Jiong Wu[1,2,3], Zhe-Bin Liu[1,2,3], Zhi-Ming Shao[1,2,3]

[1]Department of Breast Surgery, Fudan University Shanghai Cancer Center, Shanghai, China; [2]Key Laboratory of Breast Cancer in Shanghai, Shanghai, China; [3]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China; [4]Department of Breast Surgery, Renhe Hospital, Shanghai, China

Contributions: (I) Conception and design: ZB Liu, ZM Shao; (II) Administrative support: Y Gong, H Zuo, Y Zhou, ZB Liu, ZM Shao; (III) Provision of study materials or patients: Y Gong, H Zuo, Y Zhou, KD Yu, GY Liu, GH Di, ZB Liu, ZM Shao; (IV) Collection and assembly of data: Y Gong, H Zuo, Y Zhou, ZB Liu, ZM Shao; (V) Data analysis and interpretation: Y Gong, H Zuo, ZB Liu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Prof. Zhi-Ming Shao; Prof. Zhe-Bin Liu. Department of Breast Surgery, Fudan University Shanghai Cancer Center, 270 Dong-An Road, Shanghai 200032, China. Email: zhi_ming_shao@163.com; zhebinliu@shca.org.cn.

Background: According to preclinical experiments, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) exerts antiproliferative effects against breast cancer cells. It has been approved by the State Food and Drug Administration in China for complementary cancer treatment, and its safety has been confirmed in previous clinical trials. The present randomized, controlled, double-blind clinical trial was conducted to investigate the efficacy and safety of neoadjuvant PA-MSHA and placebo with chemotherapy in patients with human epidermal growth factor receptor 2 (HER2)-negative breast cancer.

Methods: Eligible patients aged 18 years or older with previously untreated HER2-negative stage II–III breast cancer were enrolled and randomly assigned at a 1:1 ratio to receive neoadjuvant chemotherapy with PA-MSHA or a placebo. The Response Evaluation Criteria in Solid Tumors (RECIST) was used to assess clinical response every 2 cycles. The primary endpoint was the objective response rate (ORR) based on the clinical response following neoadjuvant chemotherapy.

Results: A total of 75 patients were randomly assigned to either the PA-MSHA group (37 patients) or the control group (38 patients). The ORR was found to be significantly higher in the PA-MSHA group compared with the control group [86.5% versus 60.5%; rate difference 26.0; 95% confidence interval (CI): 5.9–43.5%; P=0.011]. The pathological complete response (pCR) and survival outcomes did not differ significantly between the 2 groups. Patients with immune-related adverse events (irAEs) appeared to benefit from the PA-MSHA treatment, with greater disease-free, relapse-free, and overall survival. The application of PA-MSHA to neoadjuvant chemotherapy did not increase the incidence of severe adverse events. Moreover, the addition of PA-MSHA increased serum interferon-γ levels and the percentage of peripheral blood T cells, $CD8^+/CD4^+$ T cells, $CD8^+CD28^+$ T cells, and natural killer (NK) cells, and decreased serum interleukin 4 levels.

Conclusions: The addition of PA-MSHA to neoadjuvant chemotherapy is an effective alternative regimen for HER2-negative breast cancer. Patients with irAEs caused by PA-MSHA may obtain more benefits from this treatment.

Trial Registration: Chinese Clinical Trial Registry ChiCTR-TRC-10000794.

Keywords: HER2-negative breast cancer; clinical trial; *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA); neoadjuvant chemotherapy; efficacy

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Introduction

Neoadjuvant therapy has become the standard treatment for patients with locally advanced breast cancer due to its clinical advantages, including tumor downstaging and the customization of adjuvant systemic therapy (1). Patients with different molecular subtypes of breast cancer are recommended to receive selective and appropriate neoadjuvant therapy regimens to improve the clinical and pathological responses (2). However, some patients risk disease progression during neoadjuvant treatment. Patients with hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer are less sensitive to neoadjuvant chemotherapy, with a low objective response rate (ORR) of approximately 60–70% and a pathological complete response (pCR) rate ranging from 5% to 10% (2-4). Even for patients with triple-negative breast cancer (TNBC) who have shown a relatively high ORR and pCR rate during neoadjuvant treatment, the long-term prognosis is not ideal (5). Thus, novel neoadjuvant treatment strategies are urgently needed to improve the overall response.

Highlight box

Key findings

- The addition of PA-MSHA to neoadjuvant chemotherapy for HER2-negative breast cancer could improve the tumor clinical response, modulate the immune response, and be well tolerated by patients. Patients with immune-related adverse events could benefit more from the PA-MSHA treatment.

What is known and what is new?

- The antitumor effect of PA-MSHA has been established in preclinical studies and clinical trials for patients with HER2-negative metastatic breast cancer.
- This clinical trial revealed the efficacy and safety of neoadjuvant PA-MSHA in combination with weekly paclitaxel and carboplatin treatments in patients with HER2-negative breast cancer.

What are the implications, and what should change now?

- The addition of PA-MSHA to neoadjuvant chemotherapy could be considered an effective alternative regimen for HER2-negative breast cancer.

Engineered bacteria have been used to treat cancer for decades (6). *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) is a genetically established, engineered, heat-killed *Pseudomonas aeruginosa* strain characterized by the expression of mannose-sensitive hemagglutination type I fimbriae on its surface (7). The State Food and Drug Administration in China approved PA-MSHA for complementary cancer treatment in 1998. Recent studies have shown that PA-MSHA exhibits antitumor efficacy in numerous cancer types, including breast cancer, lung cancer, hepatocellular carcinoma, bladder cancer, and gastric cancer (7-11). The antitumor effect of PA-MSHA is attributed to both its direct tumoricidal activity and immune response (12,13). Our previous study indicated that PA-MSHA exerts antiproliferative effects against HER2-negative breast cancer cells by inducing apoptosis mediated by modulating caspase family proteins and by affecting the cell cycle regulation machinery (7). We also found that PA-MSHA can suppress mammary tumorigenesis and decrease lung metastasis *in vivo* by inhibiting the expression of several oncogenes, including vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and cathepsin-D, and by promoting the expression of the tumor-suppressor gene, E-cadherin, in breast cancer cells (12). Moreover, PA-MSHA was found to induce the maturation of dendritic cells in a Toll-like receptor 4 (TLR4)-dependent manner and to promote the activation, expansion, and interferon (IFN)-γ secretion of TLR4-mediated T cells in a mouse model of lung cancer (13). In addition, PA-MSHA can increase the proportion of mature macrophages and induce M1 macrophage polarization in bladder cancer (14,15). Based on these preclinical findings, several clinical trials have been conducted to prove that a regular dose of PA-MSHA according to the manufactory's instructions could improve the therapeutic effects of chemotherapy in patients with malignancies without adding severe toxicities (16-18). Recently, a phase II clinical trial was conducted

and demonstrated that PA-MSHA in combination with capecitabine has a good safety profile in patients with HER2-negative metastatic breast cancer and possesses superior clinical benefit in patients with moderate immune-related adverse events (irAEs), highlighting the potential benefits of using PA-MSHA in the clinical treatment for breast cancer (16).

However, the role of PA-MSHA in the neoadjuvant treatment of breast cancer remains unclear and needs to be clarified. Considering the differences between the preferred treatment regimens for patients with early and metastatic breast cancer, we chose weekly paclitaxel and carboplatin (PCb) treatment as the combination regimen for the neoadjuvant chemotherapy of breast cancer (19). We conducted the present clinical trial to investigate the efficacy and safety of neoadjuvant PA-MSHA in combination with weekly PCb treatments in patients with HER2-negative breast cancer. We present the following article in accordance with the CONSORT reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4093/rc).

Methods

Study design and participants

This prospective, randomized, controlled, two-arm parallel, double-blind, phase II clinical trial was conducted at the Fudan University Shanghai Cancer Center in China. The eligibility criteria were as follows: (I) females aged 18 to 70 years with newly diagnosed, operable or locally advanced stage IIA to stage IIIC breast cancer according to the American Joint Committee on Cancer staging system; (II) a measurable and evaluable primary breast tumor that was pathologically confirmed as invasive ductal carcinoma; (III) no evidence of metastasis; (IV) histologically confirmed HER2-negative status defined by a score of 0 or 1 in the immunohistochemical analysis or the absence of HER2 amplification by fluorescence *in situ* hybridization with an immunohistochemistry score of 2 (20); (V) no prior treatment with surgery, chemotherapy, endocrine therapy, or radiotherapy for invasive breast cancer; (VI) a Karnofsky score greater than or equal to 70 and an Eastern Cooperative Oncology Group performance status of 0 to 2; (VII) normal renal, hepatic, and cardiac function; and (VIII) adequate hematologic function and normal blood counts.

The key exclusion criteria were as follows: (I) the presence of distant metastasis; (II) current pregnancy or lactation; (III) other invasive malignant diseases in addition to breast cancer (except for excised basal cell skin carcinoma and cervical carcinoma in situ) within the past 5 years; (IV) participation in another clinical trial; (V) any other physical or psychological condition that affected the patient's health or compliance; and (VI) known hypersensitivity to the treatment agents used in the study. The full inclusion and exclusion criteria can be found in Table S1.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the ethics committee of Fudan University Shanghai Cancer Center (ethics approval number: 100181-4). Informed consent was obtained from all individual participants.

Randomization and masking

The eligible patients were randomly assigned at a 1:1 ratio to receive either PCb combined with PA-MSHA (PA-MSHA group) or PCb combined with a placebo (control group). Randomization was performed using computer-generated permuted blocks with no stratification factors. A specific statistician who did not participate in the clinical trial was responsible for generating the randomization sequence and distributing the numbers to the experimental drugs. The clinical investigators randomly assigned the drugs based on enrollment order and recorded the randomization sequence. The investigators, study site personnel, and patients were masked to the treatment assignment. The treatment assignment was sealed and reserved by the principal investigator. Participants and clinical investigators could only notice the individual random number. Masking was achieved by ensuring that the placebo was not distinguishable from PA-MSHA.

Procedures

PA-MSHA or the matching placebo was dispensed to patients via subcutaneous injection to the upper arm at a dose of 1 mL every other day (0.5 mL on the first day) from the first day of neoadjuvant chemotherapy until 3 days before surgery. Both groups received PCb; this consisted of paclitaxel 80 mg/m^2 and carboplatin (area under the time curve =2) on days 1, 8, and 15 every 28 days for 4 cycles. If patients had a fever of more than 38.5 ℃ and were accompanied by neutropenia, the prophylactic use of antibiotics was required for at least 24 hours; the chemotherapy regimens were kept unchanged with no

dose reduction, and granulocyte colony-stimulating factor (G-CSF) could be supplemented in subsequent cycles.

The clinical response was routinely assessed based on physical examination, ultrasonography, and magnetic resonance imaging after the second and fourth cycles according to the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) (21). The imaging assessments were performed independently by 2 experienced radiologists in a blinded manner.

The patients underwent definitive breast surgery after the completion of neoadjuvant chemotherapy. Axillary lymph node dissection was required unless a preoperative sentinel lymph node biopsy was negative. Subsequent radiotherapy and postoperative adjuvant chemotherapy and endocrine therapy were administered at the discretion of the oncologist, and patients were followed up for recurrence and survival.

Pathological assessment

The baseline biomarkers, including tumor type, estrogen receptor (ER) status, progesterone receptor (PgR) status, and HER2 status, were determined using the baseline core needle biopsy from the primary site. The expression levels of ER and PgR were visually scored based on the percentage of nuclear staining in invasive tumor cells. Tumors were regarded as hormone receptor-positive if ER or PgR was present in ≥10% of the tumor cells. TNBC was defined as a tumor with hormone receptor–negative and HER2-negative status. The pathological responses of breast and axillary lymph nodes were assessed by local pathologists. Pathological reports were reviewed by 1 independent experienced certified pathologist from whom the treatment assignments were masked.

Outcomes

The primary study end point was ORR, defined as the proportion of patients who achieved a complete response (CR) with the clinical disappearance of the tumor in the breast or a partial response (PR) with at least a 30% decrease from the baseline of the sum of the diameters according to the RECIST guidelines (version 1.1) (21). The secondary end points included pCR; disease-free survival (DFS), relapse-free survival (RFS), distant disease-free survival (D-DFS), overall survival (OS); safety; and immunological indexes. pCR was defined as the absence of residual invasive breast cancer with or without ductal carcinoma *in situ* in the breast and lymph nodes (ypT0/TisN0). DFS was defined as the time from randomization to the first occurrence of any event, including noninvasive and invasive breast cancer recurrences (local, regional, or distant), contralateral breast cancer, second primary cancer, or death from any cause. RFS was defined as the time from randomization to the date of diagnosis of invasive breast cancer recurrence or death. D-DFS was defined as the time from randomization to the date of diagnosis of distant recurrence or death. OS was defined as the time from randomization to the date of death from any cause (22).

Adverse events were recorded at the time of every patient visit and were assessed and graded in patients who had received at least 1 cycle of neoadjuvant chemotherapy according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3.0). Adverse event data were collected throughout 28 days after the last dose of the study drug. All adverse events suggestive of immune-mediated events (e.g., skin induration at the injection site, rash, and fever) were defined as irAEs. A prespecified subgroup analysis for irAEs was performed to investigate the association between irAEs and treatment efficacy.

Immunological index testing

For all participants, blood samples were collected at baseline and alongside the second and fourth cycles. Peripheral blood mononuclear cells were separated and obtained to determine the percentage of T cells, $CD8^+$ T cells, $CD4^+$ T cells, $CD8^+CD28^+$ T cells, $CD4^+CD25^+CD127^{low/-}$ T cells, natural killer (NK) cells, and B cells using flow cytometry analysis. The sera were also extracted from the blood samples, and the levels of IFN-γ and interleukin (IL)-4 were detected by enzyme-linked immunosorbent assay according to the manufacturer's protocol.

Statistical analysis

Based on a review of previous data and our preliminary study, an ORR of 55% was expected for neoadjuvant chemotherapy among patients with HER2-negative breast cancer, and the estimated ORR for the PA-MSHA group was 85% (23,24). A sample size of 34 patients per group was calculated to achieve a power of 80% and to detect a 30% difference between the study group and the control group at

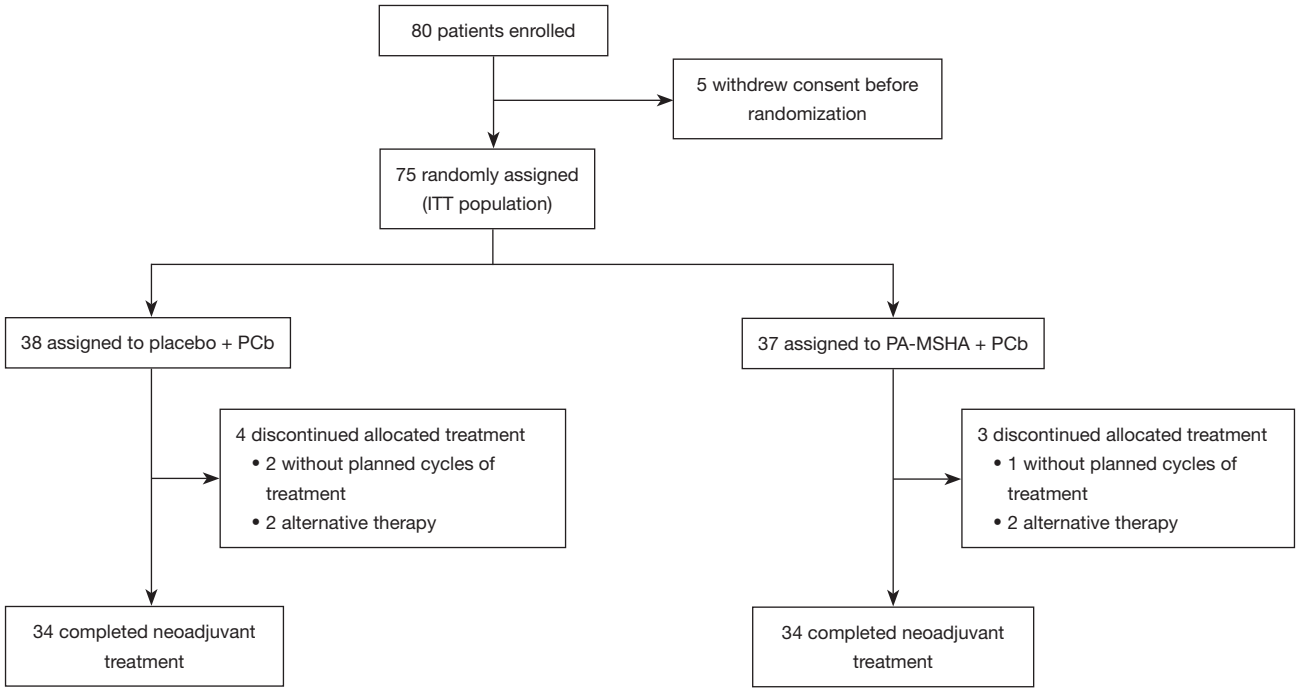

Figure 1 CONSORT diagram of the trial. ITT, intention to treat; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

a 5% significance level (two-sided). Considering a dropout rate of 10%, we ultimately set the estimated enrollment size to 80, with 40 patients per group.

All analyses were performed based on an intention-to-treat (ITT) principle in all randomly assigned patients with follow-up. Assessments up to discontinuation were used to determine the ORRs of the patients that discontinued treatment. For continuous and categorical factors, the Mann-Whitney test and the chi-squared test (or Fisher exact test when necessary) were applied to evaluate the difference between the two groups, respectively. Survival outcomes were estimated using the Kaplan-Meier method and compared using log-rank tests. Subgroups were analyzed according to age, menopausal status, clinical T classification, N classification, TNM stage, ER status, PgR status, and molecular subtype. Two-sided 95% confidence intervals (CIs) for the difference in the ORR between two subgroups were estimated by the Wilson score method (25). A two-sided P value <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software (version 22.0; IBM Corporation, Armonk, NY, USA).

Results

Patient characteristics

Between July 18, 2010, and April 11, 2014, a total of 80 patients were enrolled. Of these, 5 patients withdrew consent before randomization. Thus, 75 patients were randomly assigned to the 2 treatment groups, including 38 patients in the control group and 37 patients in the PA-MSHA group. Among them, 2 patients in the control group and 1 patient in the PA-MSHA group did not receive the planned cycles of treatment, while 2 patients in each group were switched to other alternative therapies according to their attending physicians. No patient discontinued the study due to severe adverse events.

In total, 34 patients in the control group and 34 patients in the PA-MSHA group received all scheduled courses of neoadjuvant treatment (*Figure 1*). The baseline demographic and clinicopathological characteristics were well balanced between the groups (*Table 1*). The median age of the patients was 50 years (interquartile range, 43–55 years) at the time of enrollment. Most patients had stage III disease (64.0%) and luminal subtype breast cancer (77.3%).

Table 1 Patient demographics and tumor clinicopathological characteristics by treatment group

Characteristics	All patients (n=75)	Placebo + PCb (n=38)	PA-MSHA + PCb (n=37)
Age, years			
Median [IQR]	50 [43–55]	50 [45–55]	49 [42–55]
≤50, n (%)	41 (54.7)	21 (55.3)	20 (54.1)
>50, n (%)	34 (45.3)	17 (44.7)	17 (45.9)
Menopausal status, n (%)			
Premenopausal	36 (48.0)	18 (47.4)	18 (48.6)
Postmenopausal	39 (52.0)	20 (52.6)	19 (51.4)
Clinical T classification, n (%)			
cT1–3	39 (52.0)	20 (52.6)	19 (51.3)
cT4	36 (48.0)	18 (47.4)	18 (48.7)
Clinical N classification, n (%)			
cN0	8 (10.7)	4 (10.5)	4 (10.8)
cN1	38 (50.7)	19 (50.0)	19 (51.4)
cN2–3	29 (38.7)	15 (39.5)	14 (37.8)
Clinical stage, n (%)			
IIA–IIB	27 (36.0)	12 (31.6)	15 (40.5)
IIIA–IIIC	48 (64.0)	26 (68.4)	22 (59.5)
ER status, n (%)			
Positive	56 (74.7)	28 (73.7)	28 (75.7)
Negative	19 (25.3)	10 (26.3)	9 (24.3)
PgR status, n (%)			
Positive	54 (72.0)	27 (71.1)	27 (73.0)
Negative	21 (28.0)	11 (28.9)	10 (27.0)
Molecular subtype, n (%)			
Luminal	58 (77.3)	29 (76.3)	29 (78.4)
Triple-negative	17 (22.7)	9 (23.7)	8 (21.6)

IQR, interquartile range; ER, estrogen receptor; PgR, progesterone receptor; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

Primary end point: clinical response

Of the 37 patients in the PA-MSHA group, 4 (10.8%) patients experienced CR and 28 (75.7%) patients achieved PR. In contrast, 1 (2.6%) patient experienced CR and 22 57.9%) patients achieved PR in the control group (*Table 2*). Overall, the ORR of the PA-MSHA group (32 of 37 patients, 86.5%) was significantly higher than that of the control group (23 of 38 patients, 60.5%), with a rate difference of 26.0% (95% CI: 5.9–43.5%; P=0.011; Figure S1). The post hoc exploratory subgroup analysis for the ORR is shown in Figure S1. An ORR benefit was consistently observed across the subgroups relative to the baseline characteristics.

Pathological response and survival

The pCR status was unknown for 1 patient in the PA-

Table 2 Tumor clinical response and pathological response upon completion of neoadjuvant treatment in the ITT population

Response	Placebo + PCb (n=38)	PA-MSHA + PCb (n=37)	P value
Clinical response, n (%)			0.011
CR	1 (2.6)	4 (10.8)	
PR	22 (57.9)	28 (75.7)	
SD	12 (31.6)	5 (13.5)	
PD	3 (7.9)	0 (0.0)	
CR + PR	23 (60.5)	32 (86.5)	
Pathological response, n (%)			0.516
Non-pCR	32 (84.2)	30 (81.1)	
pCR	4 (10.5)	6 (16.2)	
NA	2 (5.3)	1 (2.7)	

ITT, intention to treat; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; pCR, pathological complete response; NA, not accessed; PCb, paclitaxel and carboplatin; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin.

MSHA group and 2 patients in the control group who did not undergo surgery. These patients were counted as non-pCR during the analysis. Overall, pCR was observed in 6 of 37 patients (16.2%) in the PA-MSHA group versus 4 of 38 patients (10.5%) in the control group (P=0.516; *Table 2*).

Figure 2 shows the Kaplan-Meier curves for DFS, RFS, OS, and D-DFS according to the treatment group. After a median follow-up of 95 months (interquartile range, 53–113 months), a total of 38 events (contralateral breast cancer, second primary cancer, distant or local relapse, and death) occurred, with 19 events (51.4%) in the PA-MSHA group and 19 (50.0%) events in the control group. Treatment with PA-MSHA showed no significant difference in terms of DFS [hazard ratio (HR) 0.99; 95% CI: 0.53–1.88; P=0.982], RFS (HR 0.96; 95% CI: 0.48–1.93; P=0.913), OS (HR 0.87; 95% CI: 0.39–1.98; P=0.747), or D-DFS (HR 0.78; 95% CI: 0.38–1.63; P=0.509) compared with the control.

Safety outcomes

The main treatment-related adverse events are listed in *Table 3*. Overall, 24 (64.9%) patients in the PA-MSHA group and 27 (71.1%) patients in the control group experienced 1 or more grade 3 or 4 adverse events, and the rates of grade 3 or 4 adverse events did not significantly differ between the 2 treatment groups. As expected, hematologic toxicity was predominant with both treatments, and PA-MSHA did not increase the hematologic toxicity of chemotherapy. The most common grade 3 or 4 hematologic toxicities were neutropenia (59.5% in the PA-MSHA group versus 57.9% in the control group) and leukopenia (40.5% in the PA-MSHA group versus 39.5% in the control group). Peripheral neuropathy was the most common grade 3 or 4 nonhematological adverse event (5.4% in the PA-MSHA group versus 7.9% in the control group). The majority of adverse events related to PA-MSHA, including skin induration at the injection site, rash, and fever, were mild or moderate and manageable. Any-grade irAEs were more common in the PA-MSHA group (64.9% in the PA-MSHA group versus 28.9% in the control group), with the most notable differences observed in the rates of skin induration at the injection site (56.8% in the PA-MSHA group versus 13.2% in the control group). Both treatments were generally well tolerated, and no episodes of treatment-related death or life-threatening event were recorded. Only 1 patient in each group experienced temporary grade 4 neutropenia, and there were no grade 4 nonhematological adverse events.

Preplanned subgroup analysis by irAEs

Subgroup analysis was performed based on the irAEs, which indicated that only patients with irAEs were found to demonstrate a significantly better clinical response to the PA-MSHA treatment (95.8% in the PA-MSHA group

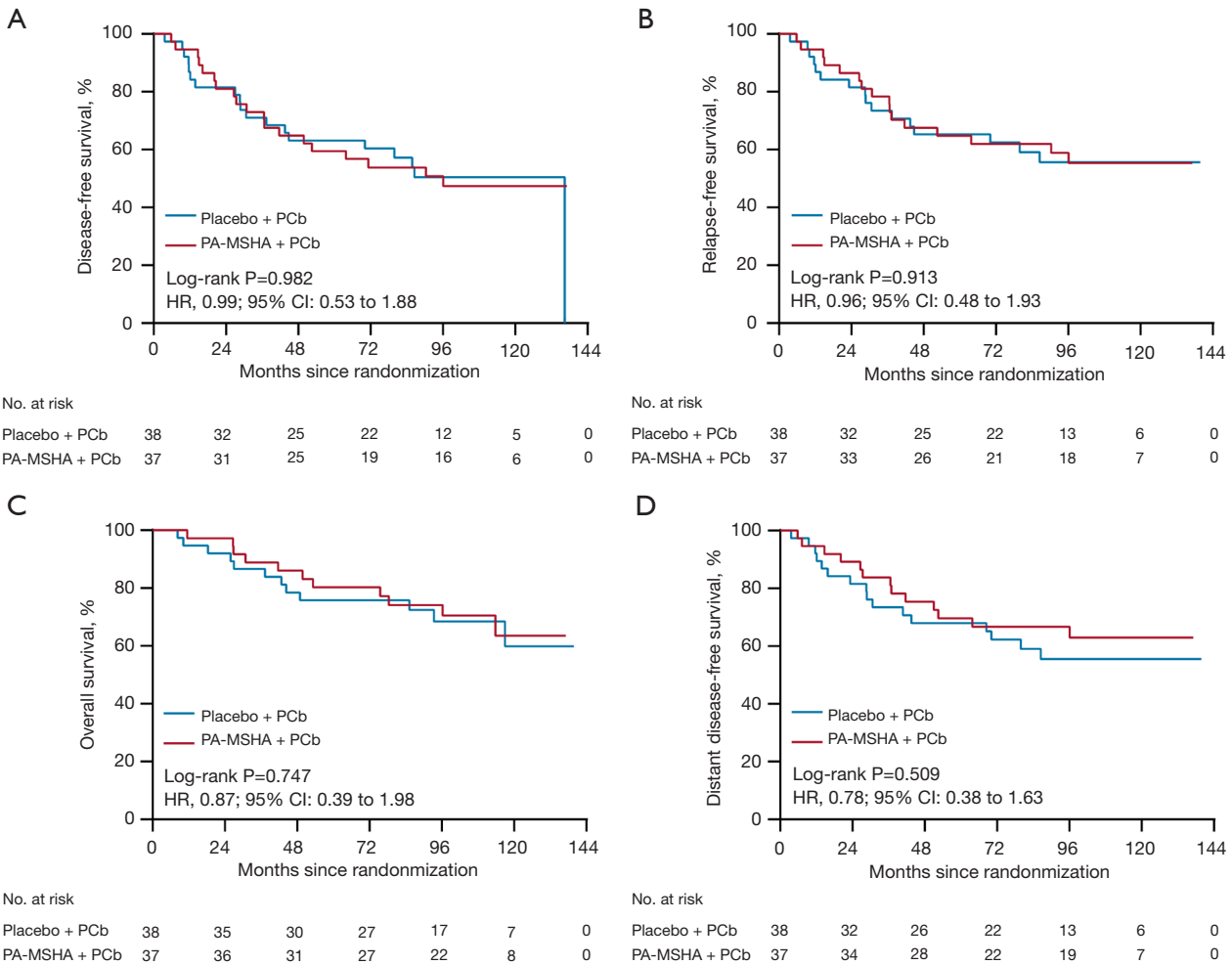

Figure 2 Kaplan-Meier plots of disease-free survival (A), relapse-free survival (B), overall survival (C), and distant disease-free survival (D) between the 2 treatment groups. HR, hazard ratio; CI, confidence interval; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

versus 63.6% in the control group; P=0.026), with a P value for interaction of 0.014 (Table S2). The ORR was also significantly improved in patients with irAEs compared to those without irAEs in the PA-MSHA group (95.8% versus 69.2%; P=0.042). Moreover, patients with irAEs displayed significantly better DFS (HR 0.20; 95% CI: 0.08–0.52; P<0.001), RFS (HR 0.38; 95% CI: 0.14–1.01; P=0.044), and OS (HR 0.23; 95% CI: 0.07–0.80; P=0.012) than did those without irAEs in the PA-MSHA group, with P values of 0.088, 0.197 and 0.217 for interaction, respectively (*Figure 3*). The D-DFS difference between patients with and without irAEs was not significant in the PA-MSHA group (HR 0.36; 95% CI: 0.12–1.09; P=0.061).

Immunological index changes by treatment group

Peripheral blood mononuclear cells were available in 68 patients (90.7% of the study participants; 34 in the PA-MSHA group and 34 in the control group). There was no significant difference in peripheral blood mononuclear cells at baseline between the 2 treatment groups (*Figure 4* and Figure S2). Following neoadjuvant therapy, we observed a significantly higher percentage of T cells (P=0.004) and a lower $CD4^+$ to $CD8^+$ T cell ratio (P=0.020) in the PA-MSHA group. Moreover, the absolute change in the percentage of T cells (P=0.019), $CD4^+/CD8^+$ T cells (P=0.004), $CD8^+CD28^+$ T cells (P=0.022), and NK cells (P=0.039) between posttreatment and baseline were more

Table 3 Summary of adverse events occurring during the neoadjuvant phase

Adverse event	Placebo + PCb (n=38)		PA-MSHA + PCb (n=37)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Hematologic toxic effects				
Neutropenia	16 (42.1)	22 (57.9)	15 (40.5)	22 (59.5)
Leukopenia	23 (60.5)	15 (39.5)	22 (59.5)	15 (40.5)
Anemia	29 (76.3)	4 (10.5)	35 (94.6)	0 (0)
Thrombocytopenia	10 (26.3)	2 (5.3)	9 (24.3)	1 (2.7)
Nonhematologic toxic effects				
Alopecia	30 (78.9)	0 (0)	32 (86.5)	0 (0)
Nausea	24 (63.2)	1 (2.6)	22 (59.5)	0 (0)
Diarrhea	19 (50.0)	0 (0)	18 (48.6)	0 (0)
Constipation	12 (31.6)	0 (0)	12 (32.4)	0 (0)
Vomiting	11 (28.9)	1 (2.6)	11 (29.7)	1 (2.7)
Skin induration at the injection site	5 (13.2)	0 (0)	21 (56.8)	0 (0)
Fever	5 (13.2)	0 (0)	10 (27.0)	0 (0)
Rash	2 (5.3)	1 (2.6)	3 (8.1)	2 (5.4)
Peripheral neuropathy	18 (47.4)	3 (7.9)	16 (43.2)	2 (5.4)
Fatigue	11 (28.9)	1 (2.6)	10 (27.0)	1 (2.7)
Increased ALT and/or AST	11 (28.9)	0 (0)	8 (21.6)	0 (0)
Headache	7 (18.4)	0 (0)	5 (13.5)	0 (0)
Arthralgia/myalgia	6 (15.8)	0 (0)	5 (13.5)	0 (0)
Stomatitis	3 (7.9)	1 (2.6)	1 (2.7)	1 (2.7)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

significant in the PA-MSHA group, while the absolute change in the percentage of $CD4^+CD25^+CD127^{low/-}$ T cells and B cells showed no difference between the 2 treatment groups.

We further assessed the levels of serum IFN-γ and IL-4 in 57 patients (76.0% of the study participants; 28 in the PA-MSHA group and 29 in the control group). The levels of serum IFN-γ and IL-4 at baseline showed no significant difference between the 2 treatment groups (*Figure 5*). The levels of serum IFN-γ were elevated after the neoadjuvant therapy, while the levels of serum IL-4 were reduced. Higher levels of serum IFN-γ (P<0.001) and IFN-γ/IL-4 ratio (P<0.001), as well as lower levels of serum IL-4 (P=0.022), were observed in the PA-MSHA group following treatment. We also noticed that the absolute change in the levels of serum IFN-γ (P<0.001) and IL-4 (P=0.029) as well as the IFN-γ to IL-4 ratio (P<0.001) during the neoadjuvant therapy were more significant in the PA-MSHA group.

Discussion

To our knowledge, the current study is the first randomized controlled trial to investigate the efficacy and safety of PA-MSHA in combination with weekly PCb as neoadjuvant treatment for patients with HER2-negative breast cancer. The results indicated that the addition of PA-MSHA to neoadjuvant chemotherapy could improve the tumor overall response, modulate the immune response, and be well tolerated by patients.

The weekly PCb regimen has been proven to be a

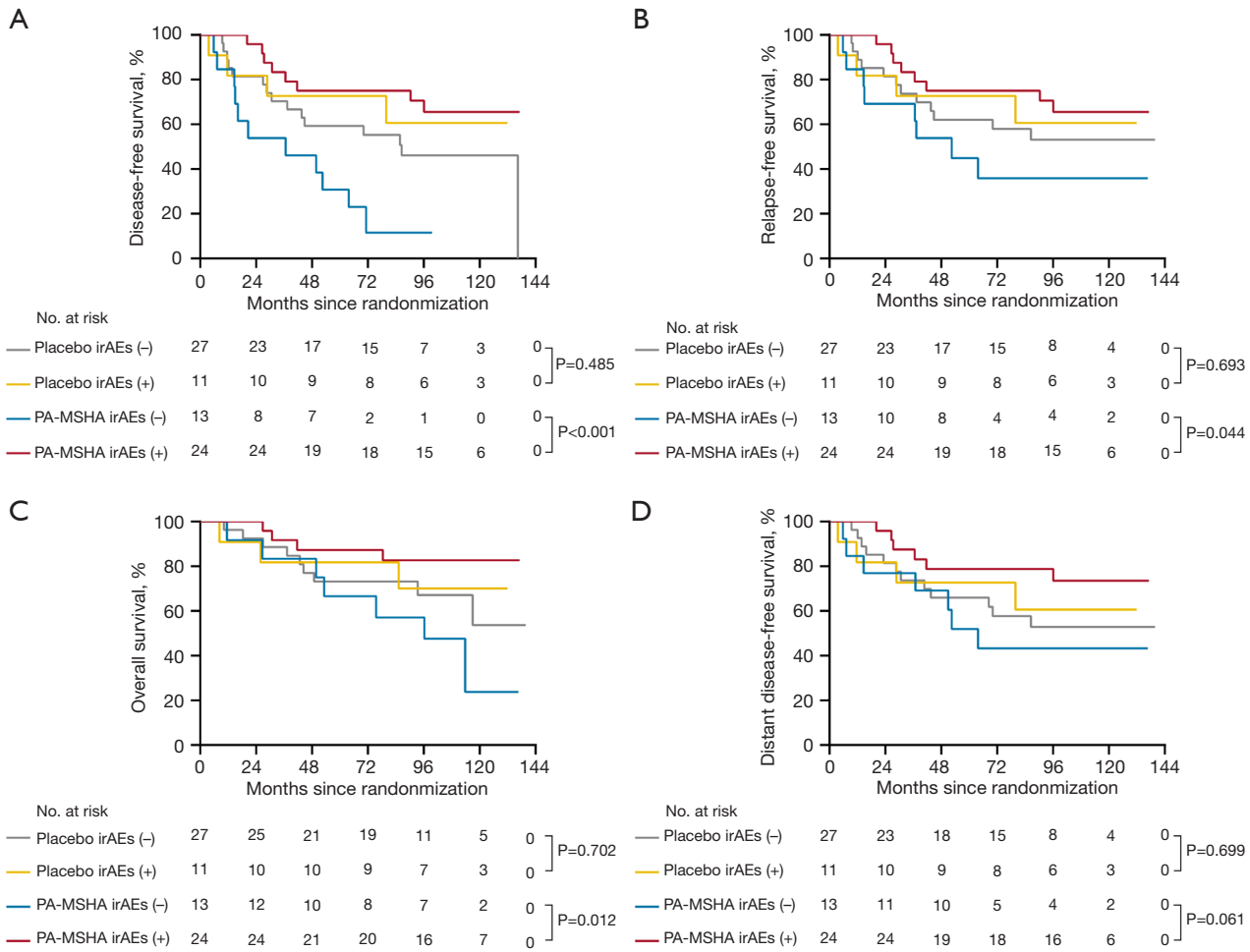

Figure 3 Kaplan-Meier plots of disease-free survival (A), relapse-free survival (B), overall survival (C), and distant disease-free survival (D) according to irAEs. irAEs, immune-related adverse events; PA-MSHA, *Pseudomonas aeruginosa*-mannose-sensitive hemagglutinin.

reasonable nonanthracycline-containing option for the neoadjuvant treatment of breast cancer, with active efficacy and acceptable safety profiles (19). The addition of PA-MSHA to this regimen could significantly increase the clinical response rate in patients with HER2-negative breast cancer. Although patients should receive 1 injection of PA-MSHA or placebo every other day as described in previous studies, no patients included in the study complained about the inconvenience of injection (16-18). We speculated that its subcutaneous injection, which is similar to insulin injection, contributed considerably to this. However, further studies should also be designed to investigate whether the interval of injection could influence the efficacy of PA-MSHA and whether the interval of injection could be extended to enhance convenience. Moreover, we noticed that the clinical response rate in the control group was lower than the ORR of approximately 70–80% reported in previous studies (19,24,26). This difference might be attributable in part to differences in the study population. The current study included a high proportion of patients with hormone receptor-positive breast cancer (77.3% in total and 76.3% in the control group), which is less sensitive to neoadjuvant chemotherapy (27). Subgroup analysis also demonstrated that the ORR of patients with TNBC was much higher than that of patients with luminal breast cancer in the control group.

No significant difference was noted with respect to the rate of pCR. The pCR rate of 10.5% observed in the control group appeared to be lower than that reported in previous trials with this regimen (19,26,28). We attribute this difference to the enrollment of patients with HER2-positive breast cancer and a higher proportion of patients

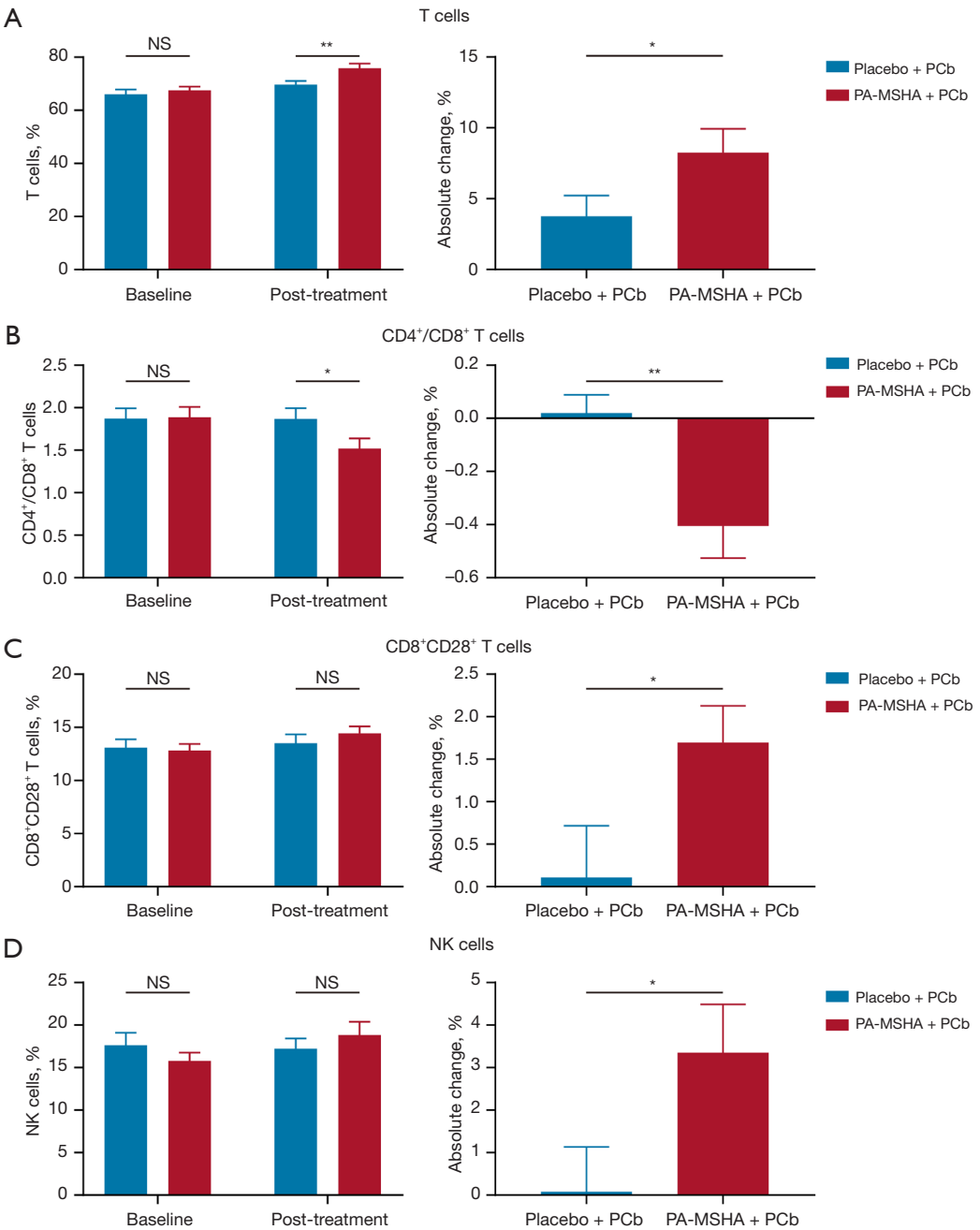

Figure 4 Comparison of the percentage of peripheral blood T cells (A), ratio of $CD4^+/CD8^+$ T cells (B), $CD8^+CD28^+$ T cells (C), and NK cells (D) at baseline and posttreatment, as well as the absolute change during the neoadjuvant treatment between the 2 treatment groups. *, P<0.05; **, P<0.01. NS, not significant; PCb, paclitaxel and carboplatin; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; NK, natural killer.

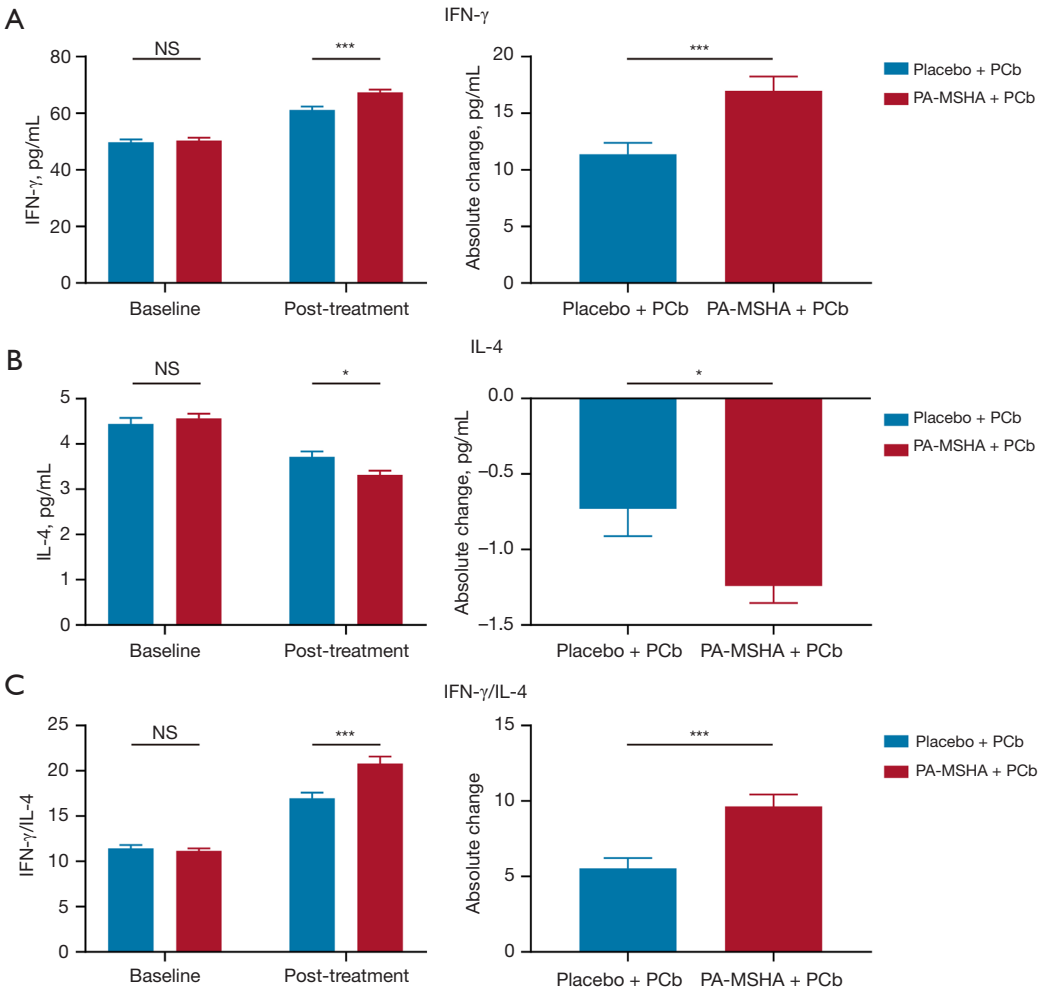

Figure 5 Comparison of serum levels of IFN-γ (A), IL-4 (B) and the ratio of IFN-γ/IL-4 (C) at baseline and posttreatment, as well as the absolute change during the neoadjuvant treatment between the 2 treatment groups. *, P<0.05; ***, P<0.001. IFN, interferon; NS, not significant; IL, interleukin; NS, not significant; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

with TNBC in those studies, which could have increased the pCR rate. Indeed, the rates of pCR in patients with TNBC treated with PCb in this trial were consistent with data presented in previous trials. Moreover, several previous meta-analyses of randomized studies that tested neoadjuvant treatments for breast cancer indicated that pCR is not a valid surrogate end point for DFS and OS, especially in patients with ER-positive, HER2-negative breast cancer, which constituted the majority of enrolled patients in this study (29,30). Thus, we selected the ORR as the primary study end point in the current study.

The indication for neoadjuvant chemotherapy in ER-positive, HER2-negative breast cancer remains controversial, as the rates of ORR and pCR are significantly lower in this subtype of breast cancer than in TNBC or HER2-positive disease (31). Previous evidence has highlighted the efficacy of neoadjuvant chemotherapy in increasing the breast-conserving surgery rate in ER-positive, HER2-negative breast cancers (32). Thus, patients with ER-positive, HER2-negative breast cancer requiring downsizing of the primary tumor to undergo breast-conserving surgery may be considered for neoadjuvant chemotherapy. Considering this, the ORR may also be a more reliable end point as opposed to the total pCR. Our study revealed that the ORR increased from 55.2% in the control group to 86.2% in the PA-MSHA group in the

luminal breast cancer subgroup, indicating the potential application of PA-MSHA in neoadjuvant chemotherapy for ER-positive, HER2-negative breast cancer.

A previous study that investigated PA-MSHA plus capecitabine treatment for HER2-negative metastatic breast cancer reported that the combination regimen had a good safety profile (16). Consistent with this previous study, the addition of PA-MSHA to PCb regimen in our study did not increase the toxicity and was also well tolerated. Only irAEs such as skin indurations, fever, and rash occurred more frequently in the PA-MSHA group than in the control group. This phenomenon could be attributed to the immune response caused by PA-MSHA (13,14). Interestingly, the efficacy in patients that experienced irAEs was significantly improved in the PA-MSHA group, and the ORR was much higher in these patients (95.8%). It might be expected that an improved prognosis would be achieved with a favorable response to neoadjuvant therapy. Although the survival outcomes did not significantly differ between the 2 treatment groups in the current study, we observed that DFS, RFS, and OS were significantly prolonged in patients with irAEs in the PA-MSHA group. We speculate that patients who had obvious immune reactions may have a better response to PA-MSHA, suggesting that early recognition and proper management of irAEs might be required to maximize the therapeutic effect of PA-MSHA in patients with breast cancer. The development of irAEs caused by immune-checkpoint inhibitors has been found to be associated with survival benefits in melanoma, non-small cell lung cancer, and other cancer types (33). However, the precise mechanisms remain unknown. Thus, consistent with immune-checkpoint inhibitors, the mechanisms underlying the association between irAEs and the outcomes of PA-MSHA treatment require further investigation.

To confirm the function of PA-MSHA in regulating the immune response of patients with breast cancer, we collected blood samples for the detection of peripheral blood mononuclear cells and cytokines. PA-MSHA has been shown to trigger naive immune responses by activating NK cells, monocytes, dendritic cells, and antigen-presenting cells (34). Previous preclinical studies have also reported that the numbers of tumor-infiltrating and peripheral blood $CD8^+$ and $CD4^+$ T cells were increased after PA-MSHA treatment *in vivo* (13,17). PA-MSHA can induce dendritic cell maturation to enable T-cell priming for the activation, expansion, and proliferation of T cells via TLR4. Additionally, the antitumor effect of PA-MSHA persists when T cells are deficient, suggesting that PA-MSHA may also be involved in the modulation of other immune cells, such as NK cells or B cells, due to the high expression of TLR4 in NK cells and B cells. Consistent with previous studies, our trial found that PA-MSHA treatment could increase the percentage of T cells, $CD8^+/CD4^+$ T cells, $CD8^+CD28^+$ T cells, and NK cells in the peripheral blood of patients. However, the influence of PA-MSHA on tumor-infiltrating lymphocytes, which have been found to be associated with improved pathological response to neoadjuvant chemotherapy and improved DFS and OS in TNBC, should be further explored. Moreover, PA-MSHA has been found to increase the secretion of antitumor cytokines, including IFN-γ and IL-2, and decrease the secretion of protumor cytokines, such as IL-4 and IL-10 (13,15). Additionally, we observed similar effects of PA-MSHA on the serum cytokine levels during the neoadjuvant treatment.

Although our study provides intriguing data that support the addition of PA-MSHA in the neoadjuvant treatment of HER2-negative breast cancer, it has some limitations. First, the sample size was relatively small, and the optimal subgroup population for neoadjuvant PA-MSHA plus chemotherapy was not identified in the current study. Moreover, this study only included Chinese patients. Thus, our findings need to be confirmed by larger randomized clinical trials and cohorts with other ethnicities. Second, the trial was first designed and launched in 2010. However, several novel regimens have been proposed and recommended as the optimal choices for the neoadjuvant treatment of breast cancer over the past 10 years. It still needs to be confirmed whether PA-MSHA could increase the efficacy of these regimens. Third, the trial included both patients with hormone receptor-positive, HER2-negative breast cancer and patients with TNBC; however, the response to neoadjuvant therapy differs for patients with distinct molecular subtypes. The subgroup analysis was also underpowered because of the relatively small sample size; thus, the efficacy of PA-MSHA for different molecular subtypes of breast cancer remains to be explored. Fourth, the measure of outcome to neoadjuvant chemotherapy needs to be examined more extensively with evaluation indexes other than ORR.

In conclusion, this randomized clinical trial demonstrated that the addition of PA-MSHA to neoadjuvant chemotherapy is an alternative regimen for treating HER2-negative breast cancer, with meaningful improvement in tumor clinical response and modest toxicity. The benefits of neoadjuvant PA-MSHA plus chemotherapy for tumor clinical response

would facilitate subsequent surgery for patients with locally advanced disease. Moreover, the PA-MSHA-induced irAEs may have a favorable therapeutic effect on the outcomes of treatment.

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Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4093/rc

Trial Protocol: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4093/tp

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4093/dss

C*onflicts of Interest*: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4093/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the ethics committee of the Fudan University Shanghai Cancer Center (ethics approval number: 100181-4). Informed consent was obtained from all individual participants.

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Supplementary

Table S1 Inclusion and exclusion criteria of the study

Inclusion criteria
(I) Females aged 18–70 years old
(II) Patient has operable or locally advanced breast cancer with stage IIA to stage IIIC based on the AJCC staging system. The primary breast tumor could be measured and evaluated and has been pathologically confirmed as invasive ductal carcinoma. No clinical or imaging evidence of metastasis based on abdominal ultrasound, chest CT and whole-body bone scan
(III) The estrogen receptor, progesterone receptor, and HER2 status of primary breast tumor were confirmed by histopathology, of which HER2-negative status was defined as a score of 0 or 1 by IHC analysis or the absence of HER2 amplification by FISH with an immunohistochemistry score of 2
(IV) No prior treatment with surgery, chemotherapy, endocrine therapy, or radiotherapy for invasive breast cancer
(V) A Karnofsky score ≥70 and an ECOG performance status of 0 to 2
(VI) Normal renal, hepatic, and cardiac function
(VII) Adequate hematologic function and normal blood counts: leukocyte count ≥4×10^9/L; hemoglobin ≥90 g/L; platelet ≥100×10^9/L
(VIII) Participants voluntarily joined the study and signed the informed consent before any trial related activities were conducted
Exclusion criteria
(I) Presence of distant metastasis
(II) Pregnant or lactating women, or women of childbearing age who cannot practice effective contraceptives
(III) Other invasive malignant diseases in addition to breast cancer (except for excised basal cell skin carcinoma and cervical carcinoma in situ) within the past 5 years
(IV) Patients participating in other similar clinical trials within the last 2 months
(V) Severe or uncontrolled systemic diseases or infections
(VI) Evidence of sensory or motor disease
(VII) Known hypersensitivity to the treatment agents used in the study
(VIII) Patients unable to understand the purpose of the study or unable to agree to the requirement of the study

AJCC, American Joint Committee on Cancer; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemical.

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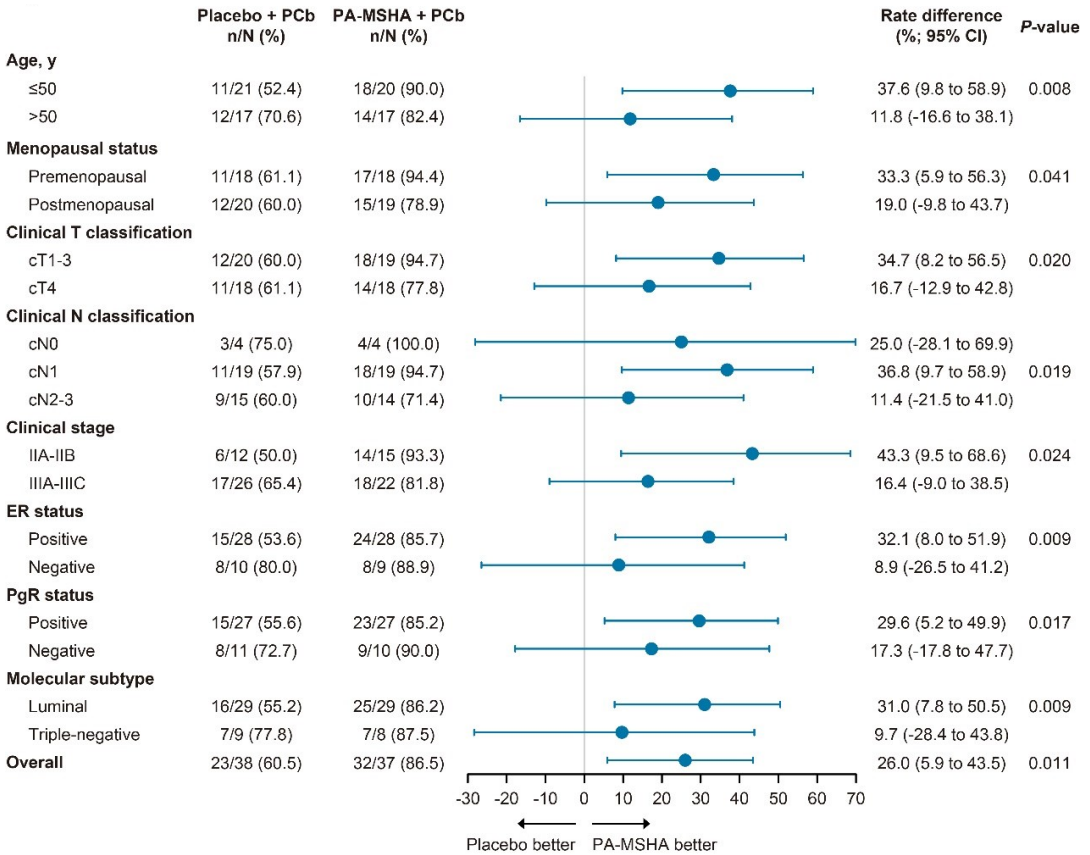

Figure S1 Subgroup analysis of differences in the percentages of patients with objective response rates. CI, confidence interval; ER, estrogen receptor; ITT, intention-to-treat; PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin; PgR, progesterone receptor.

Table S2 Tumor clinical response in the subgroups stratified by irAEs

Subgroup	Placebo + PCb (n=38), n (%)	PA-MSHA + PCb (n=37), n (%)	P value	P for interaction
Without irAEs (n=40)	16 (59.3)	9 (69.2)	0.730	0.014
With irAEs (n=35)	7 (63.6)	23 (95.8)	0.026	

irAEs, immune-related adverse events; PA-MSHA, Pseudomonas aeruginosa mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin.

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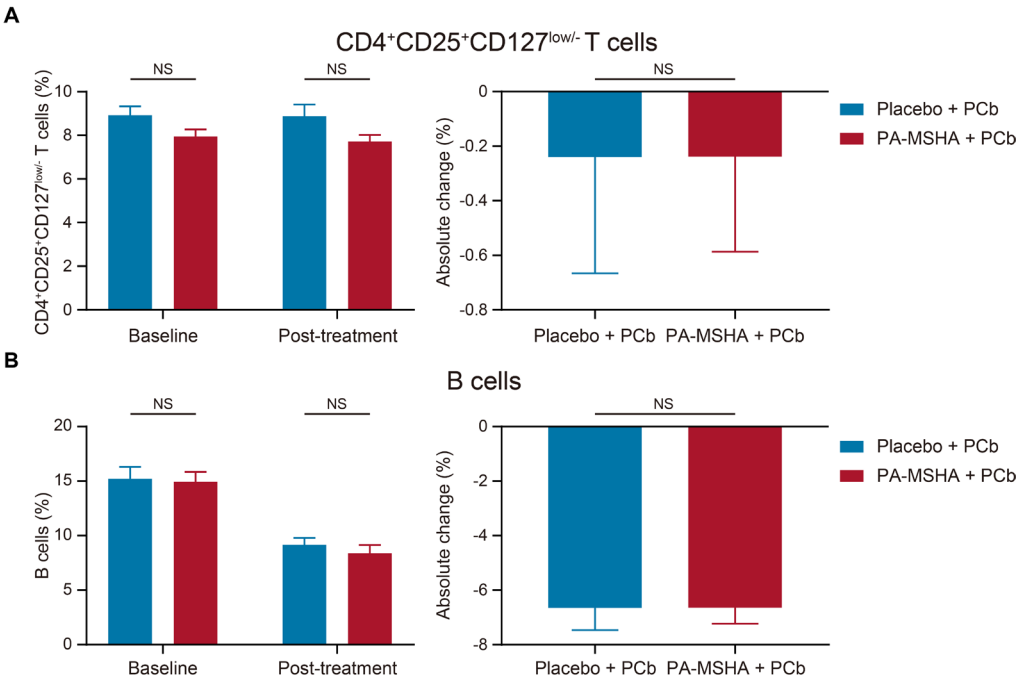

Figure S2 Comparison of the percentage of peripheral blood $CD4^+$ $CD25^+$ $CD127^{low/-}$ T cells (A) and B cells (B) at baseline and posttreatment, as well as the absolute change during the neoadjuvant treatment between the 2 treatment groups. PA-MSHA, *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin; PCb, paclitaxel and carboplatin; NS, not significant.

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