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ORIGINAL ARTICLE

Safety and efficacy of PD-1 blockade-activated multiple antigen-specific cellular therapy alone or in combination with apatinib in patients with advanced solid tumors: a pooled analysis of two prospective trials

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Abstract

Background The lethal effects of multiple antigen-specific cellular therapy (MASCT) may be enhanced by blocking PD-1 in vitro and vascular endothelial growth factor receptor 2 inhibitor (apatinib). We analyzed the pooled data from our phase I/II trials to determine the toxicity and efficacy of PD-1 blockade (SHR-1210)-activated MASCT (aMASCT) alone or in combination with apatinib in advanced solid tumors.

Methods Patients with advanced solid tumors received aMASCT alone ($n=32$) or aMASCT plus apatinib (500 mg q.d., $n=38$) after standard treatment. The safety profile was the primary end point. The secondary end points were antitumor response, progression-free survival (PFS), and overall survival (OS). The circulating T cells were quantified before and after aMASCT infusion.

Results Treatment-related adverse events (AEs) occurred in 18/32 (56.3%) and 25/38 (65.8%) patients in the aMASCT and aMASCT plus apatinib groups, respectively. No serious AEs were reported, and apatinib did not increase immunotherapy-related toxicity. The objective response rate (34.2% and 18.8%) and PFS (median 6.0 and 4.5 months, $P=0.002$) were improved in the aMASCT plus apatinib group compared with the aMASCT group; however, the OS was not improved (median 10.0 and 8.2 months, $P=0.098$). Multivariate analyses indicated that two or more cycles of aMASCT treatment was an independent and favorable prognostic factor of PFS and OS. The circulating T cells increased and Tregs decreased in both groups after one cycle of aMASCT treatment.

Conclusions Treatment with aMASCT plus apatinib was safe and effective for the management of advanced solid tumors.

Keywords Safety · PD-1 blockade · Apatinib · DC-CIK · Anti-angiogenic drugs

Lijun Liang, Yixuan Wen and Rong Hu contributed equally to this article.

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Abbreviations

ACT	Adoptive cellular therapy
AEs	Adverse events
CI	Confidence intervals
CR	Complete response
CTL	Cytotoxic T lymphocytes
DCs	Dendritic cells
DCR	Disease control rate
PD	Disease progression
ELISPOT	Enzyme-linked immunospot assay
HFSR	Hand–foot syndrome
HR	Hazard ratios
MASCT	Multiple antigen-specific cellular therapy
MDSC	Myeloid-derived suppression cells
ORR	Objective response rate

OS	Overall survival
PR	Partial response
aMASCT	PD-1 blockade (SHR-1210)-activated MASCT
PBMCs	Peripheral blood mononuclear cells
PD-1	Programmed cell death-1
PD-L1	Programmed cell death ligand-1
PFS	Progression-free survival
Tregs	Regulatory T cells
RECIST	Response Evaluation Criteria in Solid Tumors version 1.1
SD	Stable disease
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2

Introduction

Immune checkpoint inhibitors and cell-based cancer immunotherapies have a broad therapeutic application across a range of advanced-stage malignancies via modulation of immune response [2]. Programmed cell death 1 (PD-1)/PD-L1 blockade therapy only brings clinical benefits to less than 20% cancer patients, suggesting that the patients using these drugs just depend on their insufficient pre-existing endogenous antitumor-specific T cells [3]. Indeed, the immune system is commonly destroyed in most patients with advanced cancer, especially those exposed to multiple rounds of high-dose chemotherapy [4]. For these patients, the immune system may be rescued by cell-based immunotherapies.

Cell-based cancer immunotherapies, including dendritic cells (DCs)-based therapeutic cancer vaccines and adoptive cellular therapy (ACT), have attracted much attention as potential clinical treatments, particularly for patients with late-stage disease [5]. Multiple antigen-stimulating cell therapy (MASCT) is the first therapeutic intervention combining DCs vaccines and ACT in a single treatment modality to elicit both active and passive immune response [6]. MASCT has been used to treat patients with hepatocellular carcinoma after curative treatment; however, it appears to be unsatisfactory [7]. An emerging body of evidence suggests that negative costimulatory receptors are also expressed on adoptive cells, and inhibition of these molecules in vitro resulted in reinvigorated T cell-mediated cytotoxicity against tumor cells [8–10]. Therefore, it is plausible that PD-1 inhibitor-activated MASCT (aMASCT) therapy may enhance the objective clinical response, even in immunocompromised patients.

The vascular endothelial growth factor (VEGF)/VEGF receptor 2 (VEGFR2) pathway is critical to angiogenesis associated with tumor growth [11]. Apatinib is a novel, small molecule, selective VEGFR2 tyrosine kinase inhibitor and has been approved in China as a subsequent treatment for metastatic gastric cancer [12, 13]. Furthermore, apatinib showed promising clinical efficacy against a variety of other advanced solid tumors, as well as a tolerable toxicity profile as a single agent [14, 15]. In addition to the known anti-angiogenic properties of apatinib, the inhibition of VEGFR2 also has immunomodulatory effects mediated via decreased regulatory T cells (Tregs) and myeloid-derived suppression cells (MDSC), and enhanced DCs maturation and effector T cells infiltration [16–22]. The efficacy of aMASCT may be enhanced further by the addition of apatinib to counteract VEGFR2-mediated immunosuppression.

Based on the accumulation of the relevant evidence, we designed two proof-of-principle phase I/II clinical trials to investigate the safety and clinical efficacy of aMASCT (NCT02858232) or concurrent aMASCT and apatinib (NCT02844881) in advanced solid tumors after curative treatment. However, because of slow accrual, both trials were terminated early. As the entry criteria for the two trials were similar, we pooled the data for the analysis of toxicity, clinical efficacy, and immune responses.

Patients and methods

Patients

Between July 26, 2016, and May 1, 2018, patients aged between 18 and 80 years were enrolled in the study (Fig. S1a) if they met the following criteria: a documented diagnosis of stage IV or recurrent metastatic solid cancer refractory or intolerant to standard therapy; a life expectancy greater than 3 months; cessation of other antitumor therapies for at least 1 month before enrollment; a baseline Eastern Cooperative Oncology Group performance status of 0 or 1; acceptable hematologic function; and tumor lesions that could be measured by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST). Patients were excluded if they had previously received immunotherapy. The exclusion criteria were as follows: uncontrolled blood pressure with medication (> 140/90 mmHg); untreated metastases of the central nervous system; serious infection or autoimmune disease; bleeding tendency; hepatopathy, nephropathy, cardiopathy, respiratory disease, or uncontrollable diabetes.

Study design and treatment

The physical examination of each patient was conducted by professional oncologists before treatment. Patients who met the eligibility criteria were classified into two groups: the aMASCT group, in which patients received PD-1 blockade (SHR-1210)-activated MASCT alone, and the aMASCT + apatinib group, in which patients received

concurrent aMASCT infusion and oral apatinib, both in combination with the best supportive care. A treatment cycle was defined as 28 days (4 weeks). Eligible participants were exposed to repeated cycles of therapeutic regimen until one of the following events occurred: disease progression (PD); death; unmanageable toxic effects; consent withdrawn from the study; or discontinuation owing to the physician's decision. The initial dose of oral apatinib was 500 mg in a tablet form once daily for 28 consecutive days. If the adverse events (AEs) were uncontrollable and intolerable, the dose could be decreased to 250 mg. Dose re-escalation was not permitted. All the enrolled patients were treated with a subcutaneous injection of $1–10\times10^7$ mature DCs (m DCs) loaded with multiple antigens on Day 8 and an intravenous infusion with PD-1 blockade-activated autologous cytotoxic T lymphocytes (CTL) induced by mDCs on Day 27 ($1–10\times10^9$ per infusion cycle, Fig. S1b). No crossover between treatment groups was permitted.

Endpoints and assessments

The primary end point was safety and the secondary end points included overall survival (OS, the duration from the time of enrollment to death), progression-free survival (PFS, the duration from time of enrollment to PD), objective response rate [ORR; including rate of complete response (CR) plus partial response (PR)], and disease control rate [DCR; including CR, PR, and stable disease (SD)]. Toxicity assessments were conducted throughout the study and until at least 28 days after the final cycle. AEs were classified and graded using the National Cancer Institute Common Toxicity Criteria (version 4.0). In each patient, tumors were evaluated at baseline, after three cycles, and every 12 weeks thereafter until PD occurred or until the patients were lost to follow-up; assessments were based on computed tomography and/or magnetic resonance imaging.

Generation and assessment of aMASCT

MASCT was prepared as previously described [6]. As indicated in Fig. S1b, patients' peripheral blood mononuclear cells (PBMCs) were obtained by density gradient centrifugation using Lymphoprep (Nycome-dPharma, Oslo, Norway) and incubated at 37 °C in a saturated 5% CO_2 incubator for 1.5 h. Subsequently, adherent monocytes were cultured in AIM-V (Gibco, Carlsbad, CA) with GM-CSF (1000 U/mL) and IL-4 (500 U/mL) for differentiation into immature DCs (imDCs). The imDCs were pulsed with a peptide pool of multiple tumor antigens (1 μg/mL/peptide) and cultured with a maturation cocktail (IL-6, 1000 U/mL; TNF-α, 1000 U/mL; IL-1β, 10,000 U/mL; PEG2, 1 μg/mL; Poly I:C, 10 μg/mL) to differentiate into antigen-presenting mDCs. To prepare the activated CTL for infusion, the frozen non-adherent PBMCs were co-cultured with antigen-loaded mDCs for approximately 4 weeks in the presence of IL-2 (1000 U/mL; R&D Systems Inc, Minneapolis, MN). The anti-CD3 antibody (50 ng/mL; eBioscience, Inc., San Diego, CA) was added 3 days after the co-culture. The autologous T cells of MASCT were then incubated with 1.5 mg SHR-1210 (a fully human IgG4 monoclonal antibody against PD-1) [23] ex vivo for 40 min in a 37 °C thermostat, referred to as aMASCT, and finally transferred to patients.

All the tumor types received identical tumor-associated basic antigens overexpressed in most types of cancers (Supplementary Table 1) and each tumor histology received different species-specific antigens which were highly expressed only in specific tumors (Supplementary Table 2).

Flow cytometry analysis

The percentage of imDCs and mDCs immunophenotypes, the subset composition and activated markers of CTL before infusion, the PD-1 expression of CTL before PD-1 blockade was activated, and the circulating Tregs from patients were assessed using flow cytometry as previously described [6]. Antibodies for surface markers and intracellular protein staining were obtained from BD Biosciences including anti-CD80, -CD83, -CD86, -HLA-DR, -CD3, -CD4, -CD8, -CD25, -CD127, -PD-1, -IFNγ, and -granzyme B. All the flow cytometry assays were determined using a FACSCalibur Flow Cytometer (BD Pharmingen), and the data were analyzed by the FlowJo software (Tree Star Inc.).

Enzyme-linked immunospot assay (ELISPOT)

ELISPOT was used to detect the levels of peptide reactivity in the CTL. About 2×10^5 cells were incubated with the basic antigens peptide pool or irrelevant peptides using a 96-well ELISPOT plate (U-CyTech Biosciences, Netherlands) overnight at 37 °C in a saturated 5% CO_2. The assay was conducted and analyzed according to the manufacturer's instructions.

Statistical methods

Statistical analyses were performed using SPSS 22.0 or GraphPad Prism 7.0 software. A two-sample *t* test was used to compare the quantitative variables between the two groups. Where appropriate, the Pearson χ^2 test or Fisher exact test was employed to analyze the categorical variables. PFS and OS were estimated using Kaplan–Meier curves and compared using a stratified log-rank test. A Cox proportional hazards regression analysis of PFS and OS was performed to quantify the prognostic significance of risk factors. The results were reported as hazard ratios (HR) and their 95% confidence intervals (CI); HR > 1 indicates an increased

risk relative to the reference category. A CI that does not include a value of 1 is statistically significant at the 5% level. $P < 0.05$ was considered statistically significant in all the analyses.

Results

Patient characteristics

Based on the inclusion and exclusion criteria, 75 patients were screened in total and divided into two groups: aMASCT group ($n = 35$) and aMASCT plus apatinib group ($n = 40$). Four patients (three in the aMASCT group and one in the aMASCT plus apatinib group) did not continue treatment or undergo follow-up, and one patient in the aMASCT plus apatinib group died before receiving treatment. The detailed clinical characteristics of these patients are presented in Supplementary Table 3. In total, 32 patients in the aMASCT group and 38 patients in the aMASCT plus apatinib group underwent subsequent clinical assessments. The participant flow through this study is outlined in Fig. S1.

The baseline characteristics of the patients are summarized in Table 1. The median number of cycles of aMASCT therapy was 2 (range 1–10) in the aMASCT group and 2 (range 1–9) in the aMASCT plus apatinib group.

Table 1 Demographics and baseline characteristics of patients

Patient characteristic	aMASCT No. (%)	aMASCT + apatinib No. (%)
Age (range), years		
Median	59.5	58
Range	33–82	27–72
Sex		
Male	21 (65.625)	22 (57.9)
Female	11 (34.375)	16 (42.1)
ECOG PS		
0	22 (68.75)	25 (65.8)
1	10 (31.2)	13 (34.2)
Treatment cycle		
Median	2	2
Range	1–10	1–9
Tumor histology		
CRC	4 (12.5)	2 (5.3)
NSCLC	8 (25)	7 (18.4)
Sarcoma	3 (9.375)	14 (36.8)
Ovarian cancer	3 (9.375)	1 (2.6)
HCC	4 (12.5)	2 (5.3)
Fallopian tube carcinoma	0	1 (2.6)
Melanoma	1 (3.125)	6 (15.8)
Cervical cancer	3 (9.375)	1 (2.6)
Gastroesophageal tumor	1 (3.125)	2 (5.3)
RCC	5 (15.625)	2 (5.3)
Prior antitumor treatment		
Radiotherapy	12 (31.6)	10 (31.25)
Chemotherapy treatment	18 (47.4)	17 (53.125)
Surgery	22 (57.9)	21 (65.625)

ECOG Eastern Cooperative Oncology Group, *PS* performance status, *NSCLC* non-small cell lung cancer, *CRC* colorectal cancer, *HCC* hepatocellular carcinoma, RCC renal cell carcinoma, *aMASCT* PD-1 blockade (SHR-1210)-activated multiple antigen-specific cellular therapy

Immunophenotypes and characteristics of mDCs or CTL

Before treatment, the immunophenotypes and characteristics of mDCs or CTL were evaluated. Almost all mDCs expressed the molecular markers of maturation ($CD80^+$, $CD83^+$, $CD86^+$, HLA-DR^+); however, no imDCs expressed these markers (Fig. S2a). Moreover, compared with PBMC, a considerable percentage of CTLs expressed granzyme B and IFN-γ (Fig. S2b), indicating that mDCs and CTL were activated before infusion. Furthermore, nearly all of the CTL were $CD3^+$ T cells including $CD3^+$ $CD8^+$ T cells, $CD3^+$ $CD4^+$ T cells, and a few $CD4^+CD25^+CD127^-$ Tregs (Fig. S2c).

Notably, nearly 17% CTL expressed PD-1 before adding PD-1 blockade during the generation procedure (Fig. S2d). ELISPOT revealed a higher number of IFN-γ positive spots in the PD-1 blockade-activated CTL upon stimulation with antigenic peptides pool compared with non-activated CTL (Fig. S2e), which demonstrated that PD-1 blockade could enhance the specific antigen response of CTL.

Treatment toxicity

Treatment-related AEs of all grades occurred in 18/32 patients (56.3%) in the aMASCT group and 25/38 patients (65.8%) in the aMASCT plus apatinib group (Table 2). The most frequently observed immune-related AEs, as determined by the investigator, was transient fever (12/32, 37.5% in the aMASCT group and 13/38, 34.2% in the aMASCT plus apatinib group); however, almost all fevers were minor and less than 38 °C and spontaneously resolved within 12 h. Other AEs caused by immunotherapy were rarely observed, and no immune-related serious AEs were observed in any of the patients among the two treatment groups. The anti-angiogenic drug-related AEs that occurred in the aMASCT plus apatinib group included hand–foot syndrome (HFSR 12/38, 31.6%), hypertension (10/38, 26.3%), and proteinuria (9/38, 23.7%); most of these AEs were grade 1 or 2, and none was grade 5. Other common treatment-related toxicities included elevated levels of transaminase (5/32, 15.6% in the aMASCT group vs. 11/38, 28.9% in the aMASCT

Table 2 Summary of AEs in patients in response to therapy

AEs	No. of patients (%)	
	aMASCT ($n=32$)	aMASCT + apatinib ($n=38$)
Overall incidence	18 (56.3)	25 (65.8)
Immune-related AEs		
Fever	12 (37.5)	13 (34.2)
Flu-like symptoms	3 (9.4)	2 (5.3)
Chills	3 (9.4)	4 (10.5)
Anti-angiogenic drugs-related AEs		
HFSR	0 (0)	12 (31.6)
Hypertension	1 (3.1)	10 (26.3)
Proteinuria	0 (0)	9 (23.7)
AEs related to any treatment		
Elevated transaminase	5 (15.6)	11 (28.9)
Neutropenia	7 (21.9)	10 (26.3)
Leukopenia	4 (12.5)	6 (15.8)
Thrombocytopenia	5 (15.6)	6 (15.8)
Anemia	1 (3.1)	5 (13.2)
Nausea	3 (9.4)	5 (13.2)
Fatigue	3 (9.4)	2 (5.3)

AEs adverse events, *HFSR* hand–foot syndrome, *aMASCT* PD-1 blockade (SHR-1210)-activated multiple antigen-specific cellular therapy

plus apatinib group) and neutropenia (7/32, 21.9% in the aMASCT group vs. 10/38, 26.3% in the aMASCT plus apatinib group).

Overall, none of the patients who received aMASCT failed to complete a full cycle of therapy, and no patients were excluded from either group because of AEs. However, eight patients experienced apatinib dose reduction or interruption attributable to the following reasons: proteinuria (4/8, 50.0%), HFSR (2/8, 25.0%), and hypertension (2/8, 25.0%). Finally, among the patients in the aMASCT plus apatinib group, the median duration of treatment with apatinib was 5.1 months (range 0.6–22.2).

Efficiency

Three (7.9%) patients in the aMASCT plus apatinib group manifested CR, and their images before and after treatment are presented in Fig. S3. The tumor response of the remaining 35 patients is shown in Fig. 1a. PR, SD, and PD were detected in 10 (26.3%), 14 (36.8%), and 11 (28.9%) patients, respectively, with an ORR of 34.2% and a DCR of 71.1% (Supplementary Table 4). None of the 32 patients enrolled in the aMASCT group exhibited CR, whereas 6 (18.8%) showed PR, 14 (43.8%) displayed SD, and 12 (37.5%) manifested PD (Supplementary Table 4). The ORR and DCR were 18.8% and 62.5%, respectively (Supplementary Table 4).

As of May 1, 2018, the median PFS was 6.0 months (95% CI 4.0–8.0) in the aMASCT plus apatinib group and 4.5 months (95% CI 2.1–6.9) in the aMASCT group ($P<0.05$; Fig. 1b). The median OS of the aMASCT plus apatinib group was slightly longer than in the aMASCT group; however, the difference between the two groups was not significant (10.0 months, 95% CI 3.8–16.3 in the aMASCT plus apatinib group vs. 8.2 months, 95% CI 5.5–10.9; $P=0.098$; Fig. 1c).

Predictors associated with clinical outcomes

Because not all patients were responsive to the treatment in this study, it was important to consider the factors that might improve the antitumor effect. We performed a stratified analysis among all the patients and found that the patients who received two or more cycles of aMASCT had a longer median PFS and median OS than patients who received one cycle of aMASCT therapy (median PFS 7.2 months vs. 4.0 months, $P<0.05$; median OS 13.1 months vs. 8.0 months, $P<0.05$; Fig. 2a, b). Moreover, among the patients who received aMASCT plus apatinib, the PFS and the OS were much longer in patients who received two or more cycles of aMASCT than in patients who received only one cycle of aMASCT therapy (median PFS 8.0 vs. 3.7 months, $P<0.05$; median OS 16.0 vs. 8.0 months, $P<0.05$; Fig. 2c, d).

The effects of aMASCT treatment on the prognosis of patients with advanced solid tumors were evaluated in multivariate Cox proportional hazards regression analyses. As shown in Table 3, receipt of two or more cycles of aMASCT treatment showed a significant improvement in PFS and OS (HR 0.473, 95% CI 0.252–0.886, $P=0.019$ and HR 0.311, 95% CI 0.146–0.663, $P=0.002$). In addition, the number of metastases > 1 was an independent prognostic factor of PFS and OS (HR 3.510, 95% CI 1.716–7.181, $P=0.001$ and HR 2.367, 95% CI 1.074–5.219, $P=0.033$).

Analysis of the circulating T cells

As shown in Fig. 3, no significant differences in $CD4^+CD25^-$ T cells and $CD4^+CD25^+CD127^-$ Tregs among PBMCs were observed between the two groups at the beginning of the first treatment ($P>0.05$). However, after one cycle of aMASCT treatment, the frequency of $CD4^+CD25^-$ T cells was significantly increased and that of Tregs was decreased. Moreover, the percentage of $CD4^+CD25^-$ T cells from patients in the aMASCT plus apatinib group was significantly higher than that of the aMASCT group after aMASCT treatment ($P<0.05$). Conversely, Tregs were remarkably lower in the combination group than in the aMASCT group. Collectively,

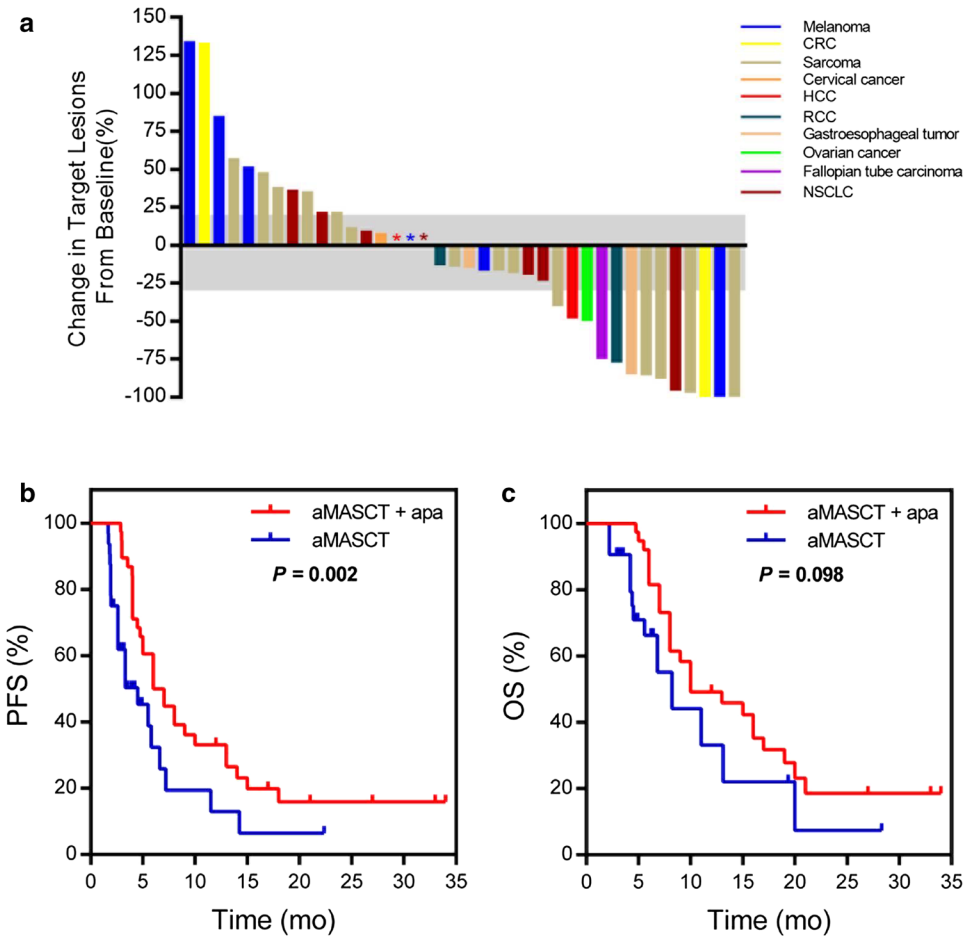

Fig. 1 Antitumor activity of aMASCT alone or combined with apatinib treatment in advanced solid cancers. **a** Waterfall plot of the best percentage change from baseline in measurable tumor lesions of patients treated with aMASCT plus apatinib; **b–c** PFS, and OS were estimated using the Kaplan–Meier curves in patients with advanced solid tumors. *NSCLC* non-small cell lung cancer, *CRC* colorectal cancer, *HCC* hepatocellular carcinoma, *RCC* renal cell carcinoma, *apa* apatinib

these results indicated that the addition of apatinib induced further reversal of immunosuppression in patients undergoing aMASCT treatment.

Discussion

Poor outcomes for advanced carcinoma remain a challenge, especially for patients who failed to respond to conventional surgery, radiation therapy, chemotherapy, and targeted therapy. Currently, a series of clinical trials have shown that the antitumor response is induced by immunotherapies, such as DCs vaccines, ACT, and PD-1/PD-L1 blockade therapy, in patients with various types of advanced cancer. However, these immunotherapeutic strategies still present great challenges. aMASCT is the first immunotherapy combining these three in a single treatment [6]. In aMASCT, tumor cells are annihilated via synergistic mechanisms including activation of multiple tumor antigen-loaded DCs and amplification of tumor-specific T cells in the autologous T cell repertoire and blockade of PD-1 immunosuppression ex vivo prior to infusion to restore the immune responsiveness of CTL, suggesting that aMASCT is a highly promising anti-cancer strategy [6, 10]. Unfortunately, many other immunosuppressive factors in the tumor microenvironment, such as Tregs and MDSCs, can shut down the immune surveillance mechanism via dysfunction of tumor-specific effector T cells, as well as the loss of antigen expression in tumor cells. Moreover, the durable antitumor response of CTL requires increased infiltration of T cells into the tumor parenchyma. Based on the favorable immunomodulatory effects of anti-angiogenic molecules [16–22], we first designed this prospective study and demonstrated the safety of aMASCT combined with apatinib with satisfactory antitumor efficacy in patients with advanced solid tumors.

In this study, patients who received aMASCT exhibited a promising clinical response, with 7/32 patients achieving objective tumor regression (Supplementary Table 4). Consistent with these results, recent preclinical and clinical data also revealed that the specific inhibition of the PD-1 checkpoint significantly increased the antitumor efficacy of adoptive T cell immunotherapy performed with DC-CIK [10] or chimeric antigen receptor T cells [24]. Moreover, in patients who received aMASCT plus apatinib, a significant improvement in PFS (Fig. 1b) and a stronger change in the circulating T cells and Tregs level (Fig. 3) were observed. Patients

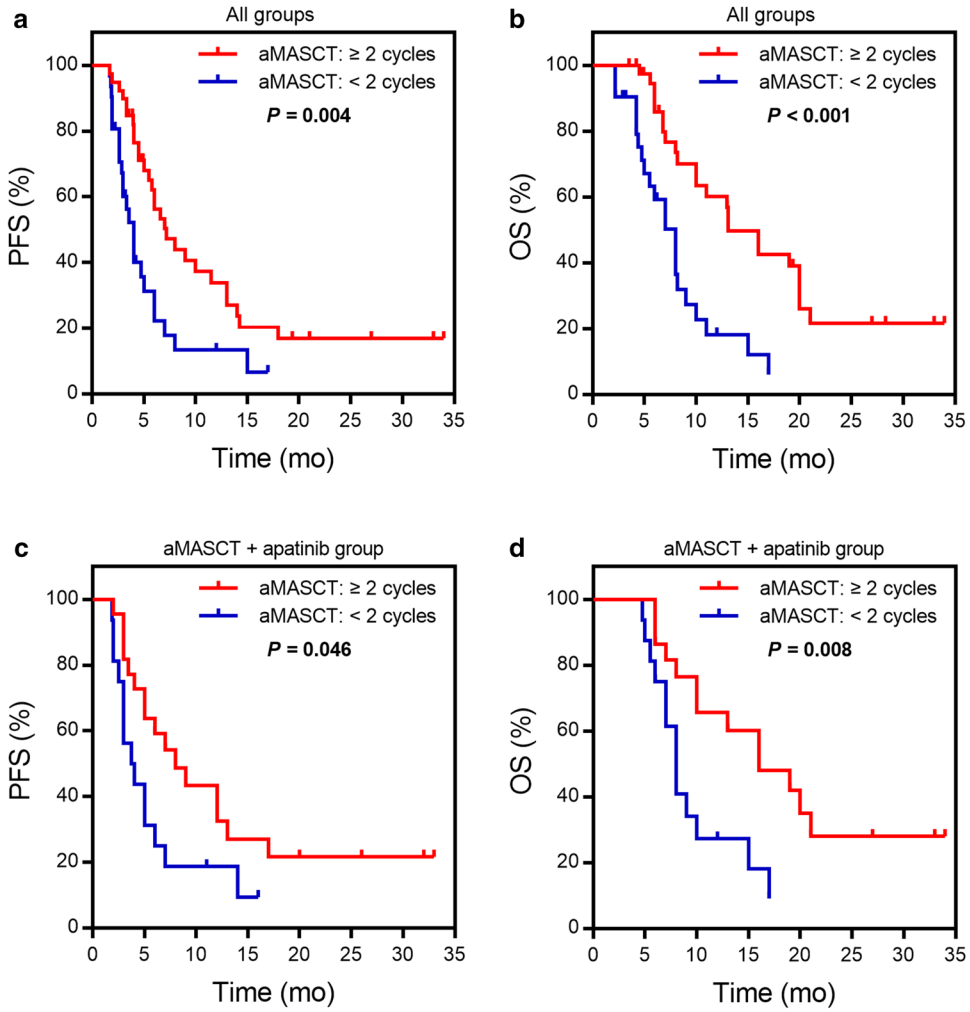

Fig. 2 PFS and OS analyses of patients who received aMASCT therapy were divided into one cycle and two or more cycles for each treatment group. PFS (**a**) and OS (**b**) of patients in all groups, and PFS (**c**) and OS (**d**) of patients in the aMASCT plus apatinib group were divided into one cycle and two or more cycles

Table 3 Multivariable analysis of 70 patients' demographic and clinical characteristics and survival

Variables	PFS		OS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (> 60 vs. ≤ 60)	0.864 (0.474–1.575)	0.632	1.208 (0.639–2.282)	0.561
Sex (male vs. female)	1.108 (0.599–2.051)	0.743	0.693 (0.334–1.441)	0.326
ECOG PS (1 vs. 0)	1.928 (0.977–3.807)	0.058	1.050 (0.427–2.581)	0.915
No. of metastases (> 1 vs. 1)	3.510 (1.716–7.181)	0.001*	2.367 (1.074–5.219)	0.033*
Treatment (aMASCT vs. aMASCT + apatinib)	2.982 (1.582–5.620)	0.001*	2.080 (1.024–4.225)	0.043*
Treatment cycle (≥ 2 vs. < 2)	0.473 (0.252–0.886)	0.019*	0.311 (0.146–0.663)	0.002*

ECOG Eastern Cooperative Oncology Group, *PS* performance status, *PFS* progression-free survival, *OS* overall survival, *CI* confidence interval, *aMASCT* PD-1 blockade (SHR-1210)-activated multiple antigen-specific cellular therapy

*$P < 0.05$

who received aMASCT plus apatinib tended to exhibit longer OS, although the difference was not statistically significant (Fig. 1c). The Phase III IMpower150 study showed that the combination chemotherapy using bevacizumab, atezolizumab, and chemotherapy as the first-line treatment for non-squamous metastatic NSCLC resulted in a significant improvement in both PFS and OS, regardless of PD-L1 expression and EGFR or ALK genetic alteration status [22]. Notably, in our study, the tumor histology differed between the groups. There were 14 sarcoma patients in the apatinib

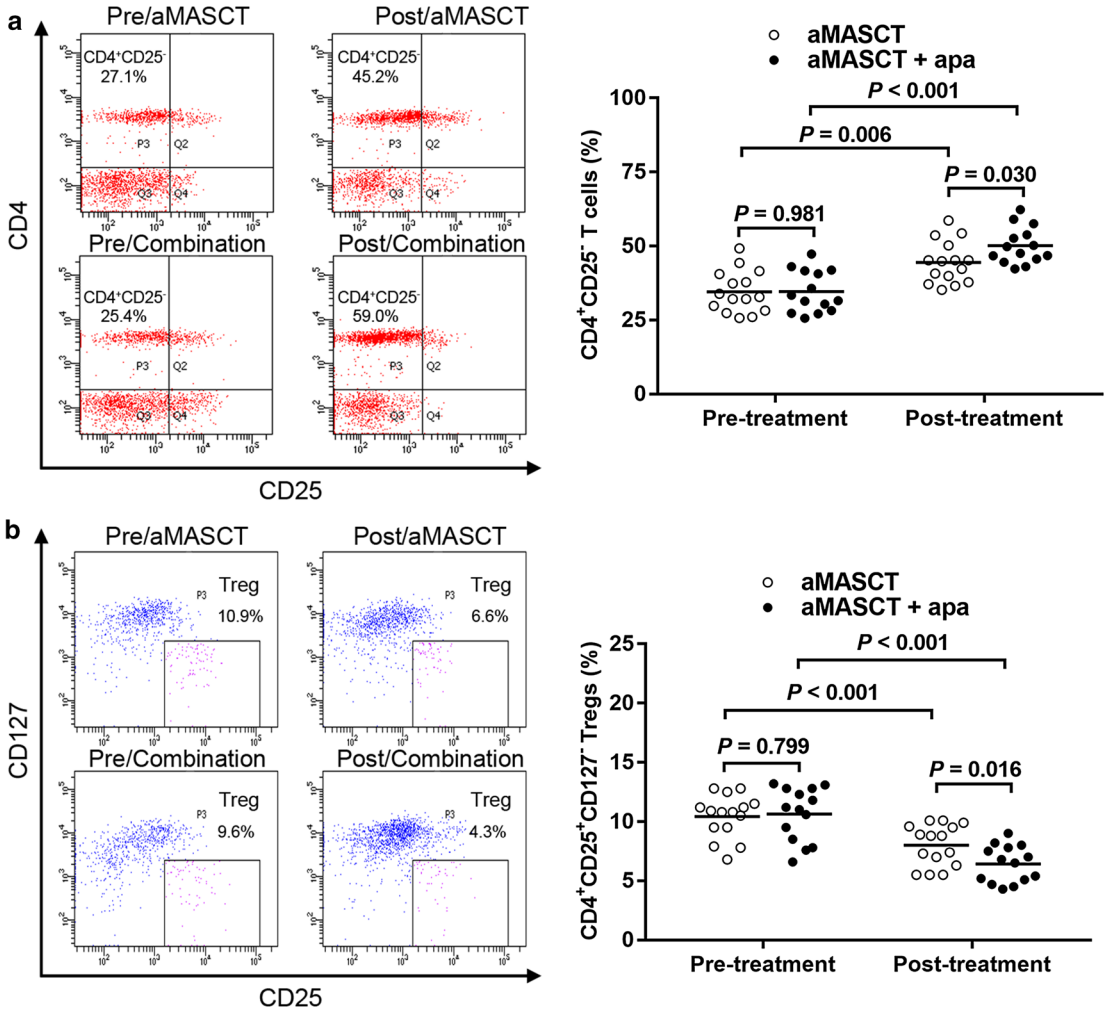

Fig. 3 Trends in circulating T cells before and after treatment. The frequency of $CD4^+CD25^-$ T cells (**a**) and $CD4^+CD25^+CD127^-$ Tregs (**b**) in patients' PBMCs was measured at the beginning of the first treatment and measured after one cycle of aMASCT infusion by using flow cytometry. *apa* apatinib

plus aMASCT group, and 1 reached CR, 4 reached PR, and the final ORR was 35.6% (5/14). In an exploratory trial of patients with 34 osteosarcoma patients receiving apatinib monotherapy, the ORR was 20.5% (1 patient reached CR and 6 patients reached PR) [25], which was mildly lower than that in our study. The OS was not significantly prolonged by the addition of apatinib to aMASCT in this study, which may be attributed to the higher number of sarcomas (metastatic melanoma was most often fatal until checkpoint blockade developed) and melanomas in the combined group with relatively poor prognosis, while the aMASCT group exhibited a higher incidence of renal cancers with better prognosis [26].

Previous studies suggested that patients with hepatocellular carcinoma who received multiple courses (≥ 5) of MASCT treatment after curative treatment may receive greater benefits from cell-based immunotherapy [7]. The cycles of treatment prescribed in different studies are not always consistent, patients enrolled in our study were all with advanced cancer who had failed multi-line therapy, and nearly half of the patients received less than two cycles of aMASCT therapy. Therefore, the cutoff point of the cycle count in this study was determined to be two cycles. In this study, we presented the result that two or more cycles of aMASCT treatment was significantly associated with improved PFS and OS in both patients from the aMASCT plus apatinib group or two groups (Fig. 2). After adjustment for competing risk factors, treatment with two or more cycles of aMASCT was an independent and robust predictor of PFS and OS (Table 3). Jiang et al. [27] and colleagues also reported that DCR and OS were significantly improved in patients with advanced pancreatic cancer that received two or more cycles of immunotherapy than patients that received only one cycle. These data indicated that a cumulative effect of immunotherapy was necessary to prolong patient survival.

Although previous clinical trials showed that PD-1 blockade therapies were associated with immune-related AEs, including serious AEs [28], these toxicities appear to be less frequent and no immune-related serious AEs were observed in patients who received aMASCT in this study. Most of the events observed in this study, including fever, chills, and others, were similar to those reported in previous studies of MASCT [6, 7]. The safety and tolerance of patients to aMASCT may result from the dose of SHR-1210 (2 μg per million cells) added in vitro prior to infusion in this study, which was much lower than that used in other studies (60–400 mg per cycle of infusion) [23], with limited toxicity. A fine balance between maintaining efficacy and reducing toxicity is necessary in apatinib treatment. The initial dose of apatinib selected in this study was 500 mg, rather than 850 mg, and the anti-angiogenic drug-related AEs were adequately controlled. No new safety signals were identified in the aMASCT plus apatinib group. A Phase IV trial also demonstrated that the administration of apatinib at 500 mg/day was clinically more beneficial and safer than that at 850 mg/day [29].

This study was a pooled analysis of two prospective trials, which were terminated prematurely because of slow enrollment. Although the entry criteria were similar, it was undoubtedly difficult for physicians and patients to be unbiased during the enrollment of the two treatment groups. A multi-center randomized trial with larger numbers of patients would provide more accurate and reliable evidence for the efficacy and safety of aMASCT plus apatinib.

Conclusions

In summary, we provide the first data showing that SHR-1210-activated MASCT cells have a promising safety profile and favorable clinical response in patients with advanced solid tumors. Concurrent apatinib and aMASCT could result in further improvements in the clinical response and PFS without increasing the toxicity profile. We also demonstrated that a cumulative effect of aMASCT immunotherapy was necessary to prolong patient survival. Thus, the combination of aMASCT and apatinib may be an effective and safe treatment option for advanced cancers.

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Author contributions XJ, LL, YW, and KH participated in the conception and design of this study. LW, YX, YQ, TC, JF, and XJ contributed to the recruitment of patients and acquisition and analysis and review of the data. LL, YW, RH, and XG conducted the in vitro experiments, analyzed the data, and wrote the manuscript. XJ and KH supervised the drafting of the manuscript. All authors critically reviewed each draft and approved the version to be published.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and ethical standards The two single-center, open-label, phase I/II studies were conducted using a protocol approved by the medical ethics committee of the Affiliated Lianyungang Hospital of Xuzhou Medical University (2016016 and 2016018). All methods and procedures associated with this study were conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and the Chinese law. These trials were registered with the Clinical Trials Registry (NCT02844881, NCT02858232).

Informed consent Informed consent for protocol therapy, the analysis of blood samples for research purposes, and the use of generated data for publication was obtained from all patients enrolled in this study.

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