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Clinical and biological effects of tumor-associated lymphocytes in the presence or absence of chemotherapy for malignant ascites in ovarian cancer patients

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Abstract. Tumor-associated lymphocytes (TALs) have been successfully isolated from ascites and solid tumors, however the clinical use of TALs in treating ovarian cancer (OC) has not yet been reported. The present study investigated the efficacy and toxicity of TALs in the presence or absence of chemotherapy in OC patients with malignant ascites (MA). A total of 32 patients were enrolled in this study. A total of 8 patients received treatment with TALs alone (TALs group), 11 patients received combined treatment of TALs and chemotherapy (combination group) and 13 patients received chemotherapy alone (chemotherapy group). The endpoints included Karnofsky performance status (KPS), ascites-associated symptoms (AAS), time to progression (TTP) and overall survival (OS). Compared with the TALs and chemotherapy group, the KPS and AAS in the combination group significantly improved following treatment. Patients in the TALs group (37.5%) and chemotherapy group (53.8%) achieved significantly fewer objective response rates of ascites compared with those in the combination group (90.9%). Furthermore, combination therapy significantly extended TTP (13 months) compared with TALs alone (1 month, P<0.001), and chemotherapy alone (6 months, P=0.027). Similar results were observed for OS between the combination group and the TALs group (25 vs. 7 months, P<0.001). The present study therefore demonstrates that combined therapy of TALs and chemotherapy is safe, feasible, and more effective than chemotherapy or TALs alone in controlling MA and improving the quality of life for OC patients.

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Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer death for women in the United States (1). Nearly two-thirds of OC patients will eventually suffered from malignant ascites (MA), which would negatively affect their quality of life and survival due to ascites-associated symptoms (AAS) (2). These symptoms include abdominal distention, abdominal pain, nausea, anorexia, vomiting, fatigue, dyspnea and weariness. Although there are several therapeutic strategies for MA, it is still prone to relapse. Once refractory ascites occurred, it is difficult to treat patients with OC (3). Therefore, it is urgent to find a novel and effective therapy for refractory MA in OC.

Adoptive cell transfer (ACT) is an emerging technique used to treat malignant carcinoma. During this process, T cells are firstly collected from one patient, expanded exponentially *in vitro*, and then re-infused to the patient to help the immune system targeting tumor cells. A seminal study by Rosenberg *et al* (4) demonstrated that tumor-infiltrating lymphocytes (TILs) extracted from freshly resected melanomas can be expanded *in vitro* and mediate specific lysis of autologous tumor cells. In their study, adoptive transfer of TILs led to objective regression of metastatic melanoma in 11 of 22 patients.

Additional studies showed that lymphodepletion prior to adoptive transfer of TILs led to 50-72% objective response rates (4-7). However, while tumor reactive lymphocytes which are isolated from ascites (8) or solid tumors (9-12) are an efficient and less toxic treatment method for OC, the reports on the intraperitoneal (IP) administration of tumor-associated lymphocytes (TALs) to treat MA arising from OC are rare. TALs are a population of antigen-specific cytotoxic cells which are easier obtained compared with TILs (13). Furthermore, previous studies (14-16) not only demonstrated that tumor reactive lymphocytes can be easierly purified from MA than from solid tissue of OC, but also showed that TALs which extracted from ascites had more significant NK activity against K562 cells than that from a series of disaggregated solid tumors. Therefore, we carefully analyzed the toxicity and efficacy of

TALs combined with or without chemotherapy in MA caused by OC.

Patients and methods

Patient cohort. Between January 2001 and December 2011, 32 patients with MA arising from OC were enrolled in this study at Nanfang Hospital of Southern Medical University (Guangzhou, China). All patients met the following inclusion criteria: Had measurable MA by ultrasound and computed tomography (maximal depth, ≥3.0 cm), and recovered from any toxic effects. Other requirements included: Age ≥18 years, Karnofsky performance status (KPS) score ≥40, life expectancy of 6 weeks or more, adequate bone marrow function, no serious damage to liver or kidneys, and no active infection. Patients that had intestinal dysfunction or uncorrectable obstruction, significant adhesions preventing free flow of fluid, prior intraperitoneal therapy with recombinant interleukin-2 (rIL-2), prior intraperitoneal chemotherapy unless free of extensive adhesions or significant medical/psychiatric disorders were excluded. All patients gave informed consent according to the Ethics Committee of Southern Medical University in China.

TALs preparation. MA from 19 of 32 patients, who received TALs immunotherapy, were collected under sterile conditions in centrifuge tubes containing preservative-free heparin (10 U/ml). Ascitic fluid samples were centrifuged at 600 g for 5 min, cell pellets containing tumor cells and lymphocytes were resuspended in Earle's balanced salt solution (EBSS), and passed through a 100 μm cell strainer to remove desquamation. Single cell suspensions were washed twice with RPMI 1640 and then cultured in RPMI 1640 supplemented with 10% human AB serum and 1,000 IU ml^{-1} rIL-2 (Double-aigrettes Pharmacy, Ltd., China). After overnight incubation, non-adhesive lymphocytes were passed through a 70 μm cell strainer to remove large aggregates of erythrocytes, tumor cells, macrophages, fibrocytes, and cell debris. If the total lymphocyte number exceeded $1x10^7$ cells, the remaining cell suspensions which resuspended in EBSS were layered onto a 100% Ficoll-Hypaque density cushion and centrifuged at 800 g for 30 min. Then, the lymphocytes cells were removed from the mid layer of Ficoll-Hypaque and EBSS. After washed twice with EBSS, the lymphocytes cells were activated and amplified by RPMI 1640 with 10% human AB serum and 1,000 IU ml^{-1} rIL-2. As previously described, the TALs can be easily distinguished from tumor cells by using morphology and size, if there were few tumor cells or macrophages adhered to the flask, we can collect the suspension TALs by gentle pipetting while leaving tumor cells and macrophages adherent (5,6). The procedure was repeated two to three times until TALs purity reached 100%. The complete medium and rIL-2 in media were supplemented according to the growth rate of TALs. When the total number of TALs exceeded $1x10^9$ after in 1- to 2-week culture, the phenotype of TALs were determined by flow cytometry. Then the TALs were administered to the peritoneal cavity with 250 ml sterile saline. If the total number was less than $1x10^7$, ficoll separation was postponed and the cells continued to be cultured with rIL-2.

FACS analysis. Flow cytometry was performed on cultured TALs immediately before the patient received their first IP injection. Briefly, single-cell TALs suspensions ($1x10^5$ cells) were labeled with CD8-PE/CD4-FITC/CD3-PE-Cy5 and their homeotype negative controls (BD Biosciences, San Jose, CA, USA), respectively.

Treatment design. Eight patients with MA caused by OC received IP injections of TALs in the TALs group, 13 patients received intravenous (IV) chemotherapy alone in the chemotherapy group, and 11 patients were treated with combined IP TALs and IV chemotherapy in the combination group. In chemotherapy group, 4 patients had a previously untreated OC and 9 patients had recurrent OC after surgery with or without chemotherapy. In the combination group, 5 patients had previously untreated OC and the remaining 6 patients had recurrent OC after chemotherapy. No patients were resistant to platinum. IV chemotherapy prior to IP TALs was administered as follows on day 1: Liposome-paclitaxel (135 mg/m^2) plus carboplatin (AUC 5) for at least two cycles. After recovery from toxicity due to the chemotherapy at least 5 days, the 19 patients in TALs and combination group were given IP injections of TALs.

Response assessment. Response assessments were done according to World Health Organization (WHO) and performed 3 weeks after 2 cycles of therapy or sooner in the event that there was evidence of clinical deterioration. Patients were considered to be in complete remission (CR) if the ascites disappeared or receded (100 ml; maximal depth, <2 cm) for 4 weeks. Partial remission (PR) involved a ≥50% decrease in ascites volume lasting for 4 weeks. Stable disease (SD) required a ≤25% increase or ≤50% decrease in ascites volume. Patients were considered to have progressive disease (PD) if the ascites volume increased by ≥25%. Combined CR and PR plus SD was defined as the MA controlled rate. The tumor response was assessed according to the criteria set forth by WHO (17). CR plus PR was defined as an objective response rate.

Follow-up evaluation. Patients were followed every month for 6 months, then every 2 months until December 2013. Complete medical histories, KPS scores (100-point), AAS (anorexia, insomnia, dyspnea, nausea, vomiting, abdominal pain, abdominal distention, fatigue, weariness), routine blood examinations, CA125 and albumin levels were conducted at enrollment (pre-treatment) and 3 weeks after the first cycle of therapy (post-treatment). A 5-point scale determined the severity of AAS (0, not at all; 1, very little; 2, somewhat; 3, moderately; 4, very much). Abdominal ultrasonography was repeated prior to each cycle of therapy. Survival data were obtained from the day of administration of TALs or chemotherapy until the death of patients or last contact when the patient is still alive. Time to progression (TTP) was defined as the time from treatment to PD or death. Overall survival (OS) was defined as the time from treatment to death.

Statistical analysis. Statistical analyses were performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). The Pearson Chi-square and one-way ANOVA compared the clinical parameters between patient cohorts. Scores and serum markers

before and after treatment were compared by a paired t-test. Survival rates between the combination group and chemotherapy or TALs group were compared using the Kaplan-Meier method and log-rank test P<0.05 was considered statistically significant and data were presented as the mean ± SD.

Results

The clinical characteristics of all 32 patients are illustrated in Table I. All patients had pathological staging according to the International Federation of Gynecologists and Obstetricians (FIGO). There were no statistical significances in clinical stage, histological subtype, histological grade or cytology of ascites between or within the three groups (P>0.05). The mean KPS score of 32 patients was 60.9±12.3 at enrollment, which was improved significantly to 73.4±15.6 after the first cycle of therapy (P<0.001). The mean score of AAS significantly decreased from 1.4±0.6 to 0.6±0.7 (P<0.001). Serum CA125 and albumin levels significantly changed from 797.2±998 to 407.2±631 (P=0.005) and 33.5±4.2 to 35.9±6.3 (P=0.04), respectively.

The TALs were successfully separated from ascites in patients who were received TALs therapy. With the time extension, the cellular morphology of TALs changed from initial round to round, branching and rods. The TALs will form sample colony growth when it at exuberant growth period (Fig. 1B). Before the first TALs therapy, the $CD3^+$, $CD4^+/CD3^+$ and $CD8^+/CD3^+$ T lymphocyte populations of TALs were also detected by flow cytometry (Fig. 1A).

In the TALs group, the mean ages of TALs ranged from 7 to 15 days (10.25±2.81), and the treatment doses of IP TALs ranged from $9x10^9$ to $3x10^{10}$ cells ($2.4±1.1 x10^{10}$). $CD3^+$, $CD4^+/CD3^+$ and $CD8^+/CD3^+$ T lymphocyte populations were 89.2±5.5, 37.9±11.5 and 45.5±13.4, respectively (Table II). However, there was no difference between the populations of $CD4^+/CD3^+$ and $CD8^+/CD3^+$ T lymphocytes. There were no significant changes in KPS and AAS scores or serum CA125 and albumin levels before or after treatment (Fig. 2A and Table III). According to the response criteria subscribed above, the MA controlled rate (CR+PR+SD) was 62.5% (5/8 patients), with a CR of 25% (2/8), a PR of 12.5% (1/8) and a SD of 25% (2/8). PD was observed in 3 patients (37.5%) (Table IV).

In the chemotherapy group, only symptoms of abdominal distension, anorexia, and fatigue significantly improved after carboplatin-based chemotherapy (all P<0.05) (Fig. 2B). As shown in Table IV, KPS scores and serum CA125 significantly changed after treatment from 63.1±14.4 to 74.6±16.1 (P=0.012) and 1294.9±1399.7 to 559.3±917.8 (P=0.021), respectively. Changes in serum albumin levels revealed no significant improvement. The MA controlled rate was 76.9%, with a CR of 46.2% (6/13), a PR of 7.6% (1/13), and a SD of 23.1% (3/13) (Table IV).

In the combined TALs and chemotherapy group, treatment doses of IP TALs (7 to 18 days old) ranged from $3x10^9$ to $5.4x10^{10}$ cells. The $CD3^+$, $CD4^+/CD3^+$ and $CD8^+/CD3^+$ T lymphocyte populations were 84.4±5.7, 34.2±11.7 and 45.5±14.3, respectively (Table II). All had no statistical significance between the TALs group and the combined therapy group. After the combined treatment, the mean score of overall AAS and six specific AAS (insomnia, weariness, fatigue, anorexia, abdominal pain, and abdominal distension) significantly improved (P<0.05) (Fig. 2C). Although serum CA125 levels decreased from 477.20±370.46 to 250.23±278.97 U/ml, there was no statistical significance (P=0.061). However, serum albumin levels significantly improved from 31.20±3.96 to 36.0±5.30 g/l (P=0.033). The MA controlled rate was 100%, with a CR of 54.5% (6/11), a PR of 36.4% (4/11), and a SD of 9.1% (1/11) (Tables III and IV).

Median TTP was significantly different in the combination group (13 months) compared to the TALs group (1 months, P<0.001), and the chemotherapy group (6 months, P=0.027). The median OS of patients in combination group (25 months) was significantly longer than the TALs group (7 months, P<0.001), but not the chemotherapy group (18 months, P=0.135). The objective response rate of MA was 90.9% in the combination group, which was higher than the TALs group (37.5%, P=0.009) and chemotherapy group (53.8%, P=0.047). However, tumor objective response was achieved in 7 of 11 patients (63.6%) in the combination group, 2 of 8 patients (25%) in the TALs group, and 4 of 13 patients (30.8%) in chemotherapy group. But the significance difference existed only in the combination group and TALs group (P=0.002) (Table IV).

There were no cases of treatment-related toxicities with IP TALs administration. Twelve patients receiving IV chemotherapy alone or combined with IP TALs experienced grades 1-3 bone marrow suppression as well as grades 1 and 3 vomiting. All side-effects were managed with routine medical treatments.

Discussion

The aim of this study was to assess the efficacy and toxicity of TALs in combination with or without chemotherapy in OC patients with MA. Our results indicated that combining therapy of TALs and chemotherapy is safe, feasible, and more effective than chemotherapy or TALs alone in controlling MA and improving quality of life in OC patients.

TALs is a unique subtype of TILs, and can be served as a suitable model for the study of TILs (18). Unlike the LAK and TILs, the effector TALs coexist with tumor target cells in a defined environment presented by MA. Previous study (19) showed that the non-specific cytotoxic potential of TALs against autologous tumor can be increased by incubation with IL-2. Melioli *et al* (20) reported that most TALs isolated from MA secondary to OC consist predominantly of T cells and almost lack of B and NK cells. A study by Ioannides *et al* (21) also demonstrated that $CD3^+CD4^+$ TALs isolated from OC ascites can be propagated in large numbers and for long time intervals as T-cell lines *in vitro*.

Previous clinical studies used adoptive cell therapy of young 'unselected' TILs to treat a variety of cancer, which are directly isolated from solid tumors without assessing the percentage of myeloid-derived suppressor cells (MDSCs) or macrophages (6,7,22-26). Other studies also demonstrated that the efficacy and tumor response rates of patients were similar in both 'selected' TILs group and 'unselected' TILs group (27,28). In addition, Allavena *et al* (29) reported that suppression by mature macrophages dose not play a major role in the determination of the low reactivity of the TALs

Table I. Clinical and treatment characteristics of patients.

Patient	Age (years)	Histology	Stage/ grade	Ascites cytology	Maximun depth of ascites (cm)		Previous chemotherapy
					Pre-	Post-	
A1	38	Serous	III/G2	-	9	10.5	Surgery, TC[a], PC, GP, endostar, radiotherapy
A2	56	Serous	IV/G2	+	12	<2	Surgery, DC, CC
A3	56	Serous	IV/G2	+	14.8	12.5	Surgery, PC, EP
A4	45	Serous	IV/G2	+	8	11.3	Surgery, TC[c]
A5	69	Serous	IV/G1	+	10	13	Surgery, TC[c]
A6	74	Transitional cell	III/G3	+	14	<2	Surgery, CC
A7	35	Mucus	IIb/G1	+	6.5	7.1	Surgery, PC
A8	55	Serous	III/G3	-	9.5	2.8	Surgery, TC[a], PC
B1	51	Endometroid	III/G2	+	15	<2	Surgery, TC[a]
B2	65	Serous	IV/G2	+	12.5	<2	None
B3	60	Serous	III/G3	+	15	<2	None
B4	42	Serous	IIIc/G2	+	11.3	4.3	Surgery, TC[b], CD
B5	44	Adenocarcinoma	III/G3	+	6	3	Surgery, DC, VIP
B6	71	Carcinosarcoma	IIIc/G3	+	14	2.1	Surgery, CP
B7	54	Adenocarcinoma	IV/G3	+	13	0	None
B8	50	Serous	IV/G3	-	12	7	None
B9	47	Serous	III/G2	+	10	5	PC
B10	69	Serous	IIIc/G3	+	11.3	3.2	Xeloda
B11	76	Serous	IIIc/G1	+	16	0	None
C1	47	Adenocarcinoma	IV/G3	+	8.2	0	None
C2	54	Adenocarcinoma	IV/G3	+	10	7	None
C3	57	Serous	III/G1	+	13	<2	Surgery, PC
C4	61	Serous	IIIc/G2	+	11	<2	Surgery, PC
C5	49	Serous	IV/G3	+	11	14.4	Surgery
C6	73	Serous	IIIc/G2	+	8.8	12.4	None
C7	50	Adenocarcinoma	IV/G3	+	10.5	8	Surgery, TC[a], GP
C8	56	Serous	IIIc/G2	+	10.1	10	Surgery, DC
C9	43	Serous	IIIc/G1	+	13.4	7	Surgery, CC
C10	57	Adenocarcinoma	IIIc/G3	-	10.7	14	Surgery, PC
C11	63	Serous	IV/G2	-	6.4	0	Surgery, TC[c]
C12	49	Adenocarcinoma	IV/G2	+	6.6	0	None
C13	52	Serous	IIIc/G2	+	6	<2	Surgery, PC

Pre-, pre-treatment; post-, post-treatment; TC[a], taxol, cisplatin; PC, pacilitaxel, carboplatin; GP, gemcitabine, cisplatin; DC, docetaxel, cisplatin; CC, cyclophosphamide, carboplatin; TC[b], taxol, caelyx; CD, doxorubicin, cisplatin; EP, etoposide, paclitaxel liposome; TC[c], taxol, carboplatin; CP, cyclophosphamide, cisplatin; VIP, ifosfamide, etoposide, cisplatin.

from ascites of OC patient in contrast to peripheral blood lymphocytes of the same patient. Furthermore, a pilot clinical trial conducted by Freedman and Platsoucas (12) suggested that both 'unselected' TILs and 'unselected' TALs plus rIL-2 could be safely administered intraperitoneally to patients with OC. Similarly, in our study, we collected young (7 to 18 days old) 'unselected' TALs from ascites without screening for the presence of MDSC or macrophages. Our study also confirmed that TALs can be easily expanded to a therapeutic dose after a short incubation with rIL-2. Nevertheless, the data about adoptive cell transfer of TALs therapy in OC is very little. Hence, we hope more clinical trials will be conducted in the future, focusing on the young 'unselected' TALs to treat various solid tumors.

Data from our study corroborated results from Han *et al* (13) and showed that TALs are easily isolated and rapidly expanded from MA before chemotherapy. Under high dose rIL-2 conditions, there was no difference in the percentage of $CD4^+/CD3^+$ or $CD8^+/CD3^+$ TALs in this trial. It is possible that ovarian $CD8^+$ TALs require different growth conditions from those needed for $CD4^+$ TALs or the $CD8^+$ TALs are outgrown by faster-growing $CD4^+$ T cells. Nonetheless, the response rates had no significant

Table II. Characteristics of TALs and survival of patients in TALs group and combination group.

Patient	TALs[a] age (days)	TALs dose (x10^9)	No. of courses	Resp[b]	Resp[c]	% of positive cells			TTP (months)	OS (months)
						CD3	CD4	CD8		
A1	9	3, 4, 2, 2	4	PD	PD	95.5	40.2	47.8	-	6
A2	9	7, 11, 14, 5, 2	5	CR	PR	80.6	41.2	22.9	5	5
A3	8	10, 7	2	SD	PD	90.0	40.5	60.5	2	12
A4	7	11, 9, 10, 8	4	PD	PD	87.4	21.3	40.2	-	8
A5	10	12, 10, 7	3	PD	PD	84.5	44.8	37.1	-	4
A6	14	13, 10, 6	3	CR	PR	86.4	40.4	39.8	2	7
A7	10	9, 13	2	SD	SD	93.3	54.2	51.1	1	13
A8	15	6, 3	2	PR	SD	96.0	20.4	64.3	3	9
B1	11	12, 10, 7	3	CR	PR	90.6	40.2	66.8	17	23
B2	11	9, 8, 11	3	CR	PR	87.0	18.9	54.6	10	18
B3	18	14, 14, 10, 8, 8	5	CR	PR	79.6	26.3	50.5	17	68
B4	8	8, 12, 11, 11	4	PR	SD	80.4	29.3	47.5	14	25
B5	9	14, 11, 10	3	PR	PD	87.4	29.9	50.0	6	16
B6	10	7, 7	2	CR	CR	82.3	37	56.5	15	36
B7	16	13, 12, 12	3	CR	PR	75.7	49.2	18.7	12	25
B8	8	9, 7, 8, 3	4	SD	SD	94.7	37.2	49.3	8	22
B9	10	1, 1, 1	3	PR	CR	87.5	41.4	41.2	13	32
B10	7	1, 1, 1, 1	4	PR	SD	84.3	14.8	44.6	9	13
B11	7	1, 1, 1, 1	4	CR	PR	78.7	52.1	21.0	32	43

TALs, tumor-associated lymphocytes; TALs[a], the mean days of TALs cultured; Resp[b], ascites response; Resp[c], tumor response; TTP, time to progression; OS, overall survival; PD, progressive disease; CR, complete remission; SD, stable disease; PR, partial remission.

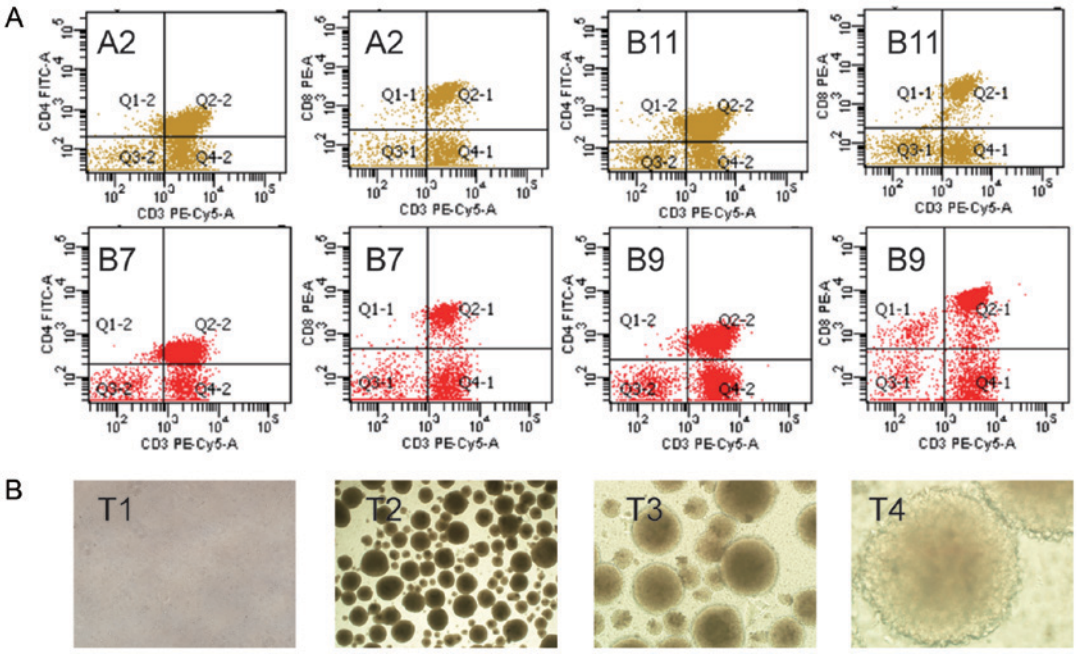

Figure 1. The immune phenotype and morphological observation of TALs. (A) The $CD3^+$, $CD4^+/CD3^+$ and $CD8^+/CD3^+$ T lymphocyte populations of TALs were detected by flow cytometry in A2, B7, B9 and B11 patients when they received TALs therapy. (B) The morphological observation of TALs in patient A2. TALs, tumor-associated lymphocytes; T1, the TALs when first separation, x100; T2, the TALs after 10 days' cultivation, x100; T3, the TALs after 10 days' cultivation, x200; T4, the TALs after 10 days' cultivation, x400.

difference in this study whether patients received TALs that contained predominantly $CD4^+$ or $CD8^+$ T cells. However, the critical point of adoptive transfer of tumor-reactive lymphocytes was not reasonable in combination with chemotherapy

Table III. Treatment effects on patients between the three groups.

	KPS score (mean ± SD)			CA125 (U/ml) (mean ± SD)			ALB (g/l) (mean ± SD)		
	Pre-	Post-	P-value	Pre-	Post-	P-value	Pre-	Post-	P-value
Combination group	60.9 (9.4)	81.8 (7.5)	<0.001	477.2 (370.5)	250.2 (279)	0.061	31.2 (4.0)	36±5.3	0.033
Chemotherapy group	63.1 (14.4)	74.6 (16.1)	0.012	1,294.9 (1,399.7)	559.3 (917.8)	0.021	35.1 (3.6)	37.7±7.7	0.175
TALs group	57.5 (12.8)	60.0 (15.1)	0.626	428.3 (282.6)	375.9 (354.1)	0.393	34.2 (4.4)	32.7±3.9	0.279

KPS, Karnofsky performance status; CA, cancer antigen; SD, standard deviation; ALB, albumin; pre-, pre-treatment; post-, post-treatment; TALs, tumor-associated lymphocytes.

Table IV. Comparison of patient outcomes between the three groups.

	TTP (months)		OS (months)		Acites response		Tumor response	
	Median (95% CI)	P-value	Median (95% CI)	P-value	CR+PR (%)	P-value	CR+PR (%)	P-value
Combination group	13 (8.7-17.3)	NA	25 (21.9-28.1)	NA	10 (90.9)	NA	7 (63.6)	NA
TALs group	1 (0-2.8)	<0.001	7 (4.2-9.8)	<0.001	3 (37.5)	0.009	2 (25.0)	0.002
Chemotherapy group	6 (2.5-9.5)	0.027	18 (13.3-22.7)	0.135	7 (53.8)	0.047	4 (30.8)	0.107

TTP, time to progression; OS, overall survival; CR, complete remission; PR, partial remission; NA, non-available; TALs, tumor-associated lymphocytes.

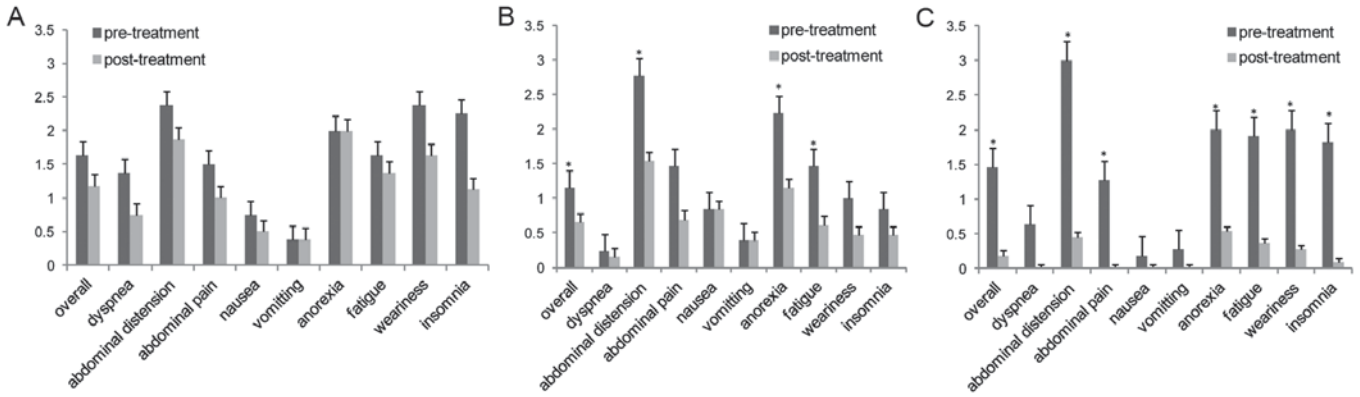

Figure 2. Comparison of ascites-associated symptoms between pre-treatment (black bars) and post-treatment (gray bars) in three groups. Each symptom was graded on a 5-point scale of increasing severity (0, not at all; 1, very little; 2, somewhat; 3, moderately; 4, very much). Bars represent mean scores of corresponding symptoms. *P<0.05. (A) TALs group, (B) chemotherapy group, (C) combination group.

and other treatment methods. Although chemotherapy had an immunosuppressive effect on immunotherapy, it can reduce the number of MDSCs, activate dendritic cells and cytotoxic T cells (30,31) Previous studies also indicated that immune ablation is an effective preconditioning regimen that can increase T-cell responses after adoptive transfer (32-36) and suggests that chemotherapeutic agents can be used in combination with adoptive cell therapy. Furthermore, most adoptive TILs therapies used to treat melanoma patients occurs after lymphodepleting chemotherapy (5,22,23).

Adoptive cell transfer of TALs followed by chemotherapy demonstrated higher response rates and longer OS than use of TALs or chemotherapy alone. We found that a single use of TALs therapy provided a short duration response that lasted 1 to 5 months and was slightly less effective at controlling MA and improving quality of life. Compared with a single use of chemotherapy, the combination TALs therapy and chemotherapy not only showed higher response rates and longer OS, but also induced fewer side-effects in OC patients. TALs may play a role in reducing the toxicities associated with chemotherapy and help rebuild the immune system.

However, the inadequacy of this study was lack to detect the percentages of MDSCs or $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) cells in TALs, and further analysis their clinic effect on OC patients. Whatever, there is little study to support that the TILs or TALs which had no MDSCs, macrophages and Tregs have more clinic effect on patients than the 'unselected' lymphocytes. Although MDSCs are a heterogeneous group

of immature myeloid cells that negatively regulate the immune responses during tumor progression, inflammation and infection, it is still unclear what subset of MDSC may be responsible for T cell suppression and what the specific nature of MDSC-suppression is, i.e., antigen dependent or independent. To the best of our knowledge, the low reactivity of TALs can be due to suppressive cytokine environment within the ascites (37). The lower reactivity of TALs can be also caused by higher DNA damage which occurs in those lymphocytes more than that in peripheral blood lymphocytes (18). In addition, mature macrophages do not play a major role in the low reactivity of the TALs (29). However, some groups have identified Treg infiltration to be a biomarker of good clinical outcome in ovarian carcinoma (38), highlighting the complexity of Tregs as biomarker. Other studies also demonstrated that $CD4^+$ T lymphocytes increases proportionally to the effector T cells in cancer, thus Tregs could be associated with improved outcome (39,40). Furthermore, the help given by given $CD4^+$ T lymphocytes during the priming of $CD8^+$ cytotoxic T lymphocytes confers a key feature of immunological memory (41). Pace *et al* also suggested that Tregs are important regulators of the homeostasis of $CD8^+$ T cell priming and played a critical role in the induction of high-avidity responses and effective memory (42). According to the clinical results, Hinrich and Rosenberg suggest that Treg cells may be important in TILs therapy but that Treg cells from the reconstituting host rather than from the infused cell product may suppress antitumor responses (43). Although MDSCs are a heterogeneous group of immature myeloid cells that negatively regulate the immune responses during tumor progression, inflammation and infection, it is still unclear what subset of MDSCs may be responsible or the dominant mechanism for T cell suppression. So, there remains a significant gap in our understanding of their phenotypical and functional heterogeneity.

Overall, the data demonstrate that chemotherapy can be safely administered before TALs therapy and provide impressive response rates in the treatment of MA. However, more studies are needed to combine a variety of non-proven modalities in an effort to find an effective combination to combat OC.

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