Clinical efficacy of intra-cavitary infusions of autologous dendritic cell/cytokine-induced killer cell products for the treatment of refractory malignant pleural effusions and ascites

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Abstract: To explore the safety and efficacy of intra-cavitary infusions of autologous mixed dendritic cell (DC)-cytokine-induced killer (CIK) cell products in advanced cancer patients with malignant pleural effusions or ascites. DC-CIKs were expanded ex vivo (mean yield of $1.36\times10^9$ cells (range, $0.74~4.98\times10^9$)) from peripheral blood mononuclear cells obtained by repeated venipuncture or apheresis. Patients received at least 1 cycle of 3 infusions of the DC-CIKs administered by indwelling catheter into the pleural or peritoneal cavity every other day. The volume of malignant effusions was assessed radiologically. Peripheral blood lymphocyte populations were enumerated by flow cytometry. Quality of life (QoL) during the DC-CIK infusions was assessed by the EORTC QLQ-30 instrument. ctDNA sequencing was performed to analyze gene clonal load and molecular tumor burden during the infusion treatment. Thirty-seven patients with breast, lung and other malignancies were enrolled. The results showed that intra-cavitary DC-CIK infusions (16 intrapleural and 21 intraperitoneal) were well-tolerated with no grade 3/4 adverse events. There was one complete response with effusion disappearance (CR) (3%), 13 partial responses (PR) (35%), 12 with stable disease (SD) (32%) and 11 with progressive disease (PD) (30%), resulting in a clinical effusion control rate (CCR) of 70% (26/37). The total number of infused CIKs and the CD3+/CD8+ and CD8+/CD28+ T cell frequencies within the CIKs were associated with effusion control (P=0.013). Moreover, increased peripheral blood CD3+/CD8+ (P=0.035) and decreased CD4+/CD25+ T cell frequencies (P=0.041) following the DC-CIK infusions were associated with malignant effusion and ascites control. Reductions in ctDNA correlated with clinical benefit. In conclusion, intra-cavitary autologous cellular immunotherapy is an alternative method to effectively control malignant pleural effusions and ascites. The overall effusion control rate was associated with higher peripheral blood effector T cell frequencies.

Keywords: Autologous cytokine induced killer cell immunotherapy, malignant pleural effusion, malignant ascites, circulating tumor DNA (ctDNA)

Introduction

Malignant pleural effusions (MPE) and ascites (MA) are common complications of epithelial malignancies. Systemic anti-cancer therapy [1-7], indwelling pleural and peritoneal catheter drainage [8-12], and talc or chemical pleurodesis [13] are widely used to treat MPE and MA, but are limited by frequent complications and recurrences requiring retreatment. Autologous adoptive cell immunotherapy with cellular products consisting of mixtures of ex vivo generated dendritic cells and cytokine induced killer cells (DC-CIK) has demonstrated clinical activity across a variety of malignancies [14, 15]. We previously reported our experience with intra-cavitary infusions of DC-CIK into MPE and MA of epithelial origin administered between 2004-2006. The effusion response rate was 54% (14/26) with a median duration of
response of 20 weeks (range 4-45 weeks) [16]. In another study in patients with MPE, we found that intra-cavitary CD3-CD16+CD56+ NK cells, CD3+CD16+CD56+ NK T cells and CD8+/CD28+ T cells were significantly increased after DC-CIK infusions [17]. These clinical data provided evidence that intra-cavitary infusions of DC-CIK were capable of controlling malignant effusions. Our recent studies have also demonstrated the feasibility, safety, and efficacy of combining systemic chemotherapy with intravenous adoptive DC-CIK immunotherapy in patients with advanced breast [18], lung [14], pancreatic [19], and stomach cancers [20].

We now report additional experience with adoptive intra-cavitary DC-CIK therapy, confirming the clinical efficacy and safety of this approach and demonstrating that the relative composition of lymphocyte subsets in the infused product and peripheral blood mononuclear cells correlates with clinical outcome.

Methods
Patients

We performed a retrospective review of data from patients with MPE and MA who were treated with intra-cavitary DC-CIK infusions at Capital Medical University Cancer Center from September, 2012 to August, 2018 under a study approved by the ethics committee of Capital Medical University Cancer Center/Beijing Shijitan Hospital, Beijing, China in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all patients before their entry into the study. Patients were required to meet the following inclusion criteria: pathologically diagnosed solid tumor malignancy, malignant pleural effusions or ascites confirmed by cytology, effusion measurable by ultrasound or CT scan, age ≥18 and ≤ 80 years, Eastern Cooperative Oncology Group performance status (ECOG-PS) [21] of 0-2, life expectancy longer than 3 months, and adequate hematological and organ function.

Expansion of DC-CIK ex vivo

DC-CIK cell products were prepared as described in our previous studies [20]. Briefly, GM-CSF was administered twice a day for two days to mobilize peripheral blood progenitor cells. Peripheral blood mononuclear cells were collected by either repeated venipuncture of 50-60 ml of heparinized peripheral blood over a 2-week period or by apheresis using a COBE Spectra cell separator (COBE BCT, Lakewood, CO, USA). The cells were cultured in plastic flasks in AIM-V medium containing 2% autologous serum and allowed to adhere for 1 h. The non-adherent cells were then collected and induced to become CIK cells using 1000 U/ml rhiFN-γ for the first 24 h followed by stimulation with 100 ng/ml OKT-3, 1000 U/ml rhIL-2 and 100 U/ml IL-1α. The adherent cells were cultured to generate DCs using X-VIVO 15 serum-free medium supplemented with 1000 U/ml GM-CSF and 30 ng/ml IL-4. On the sixth day, another 10 ng/ml of TNF-α was added to the DCs to induce maturation. On the next day, the CIK cells were mixed with DCs (DC-CIK cells) at a ratio of 20:1 and cultured in fresh medium containing 1000 U/ml rhIL-2 for another 7 days. On day 14, the DC-CIK cells were harvested, and their number, viability, and phenotype were analyzed. Lack of bacterial and fungal contamination was confirmed. After meeting lot release criteria, the cultured cells were infused (through the indwelling pleural or peritoneal catheter as appropriate) over 20 minutes.

Intra-cavitary DC-CIK infusions

All patients underwent thoracic or abdominal puncture and drainage of the effusion/ascites for 3 to 5 days through an indwelling catheter. When ultrasonography revealed that the effusion or ascites volume was <50 mL, the DC-CIKs were infused into the pleura or peritoneum respectively through the indwelling catheter every other day for three infusions, which was designated as one cycle. Every patient received at least 1 cycle of cell infusions. Administration of additional cycles was at the discretion of the treating physician and the decision was generally based on continuing stable or improved clinical condition of the patient. Ultrasonic or CT scans of the chest or abdomen were performed 4 and 8 weeks after infusions.

Adverse events and health-related QoL outcome assessments

Adverse events were graded using WHO toxicity criteria. All patients were expected to complete the EORTC QLQ-C30 version 3.0 instrument before and after infusion DC-CIK, which consists of functional (health condition, and physi-
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mental, psychological, and social functions) and symptomatic scales (fatigue, sickness, vomiting, pain, dyspnea, insomnia, loss of appetite, constipation, diarrhea, and economic problems) in order to assess the study health-related QoL (HRQoL). We used the EORTC QLQ-30 measures of mental conception of body, sexual function, attitude toward the future, and side effects of DC-CIK treatment. (EORTC Quality of life. EORTC QLQ-C30. http://groups.eortc.be/qol/eortc-qlq-c30. Accessed 3 Jan 2018).

Flow cytometric analysis of peripheral blood T cell phenotype

T cell subsets in peripheral blood, obtained from each patient before and after the DC-CIK infusions, were enumerated by flow cytometry. Primary antibodies included: anti-CD4-FITC, anti-CD8-PE, anti-CD3-PerCP (Becton-Dickinson), anti-CD4-FITC (Beckman-Coulter), anti-CD25-PE (Beckman-Coulter), anti-CD28-FITC (Beckman-Coulter), anti-CD8-PE (Beckman-Coulter), and anti-CD3-FITC (Beckman-Coulter). The following procedure was based on the instructions of the manufacturer for each antibody: 100 μl blood was incubated in the dark with the primary antibody at 4°C for 15 min. After hemolysis for 10 minutes, samples were centrifuged at 1500 rpm for 10 min at room temperature, and then washed twice in PBS and subjected to flow cytometric analysis using an FC500 (Beckman-Coulter) and CXP software. T cell subpopulations were reported as percentages of the total lymphocyte population.

Response assessments

The response to the treatments was assessed using previously reported criteria [22] as follows: Complete response (CR): the disappearance of effusion (volume <100 ml) and complete remission of symptoms, lasting for more than 4 weeks; Partial response (PR): >50% reduction in effusion volume and an improvement in symptoms lasting for more than 4 weeks; stable disease (SD): the effusion was reduced by <50% but had not increased by more than 25%; Progressive disease (PD): effusion volume increased by ≥25% or the patient required repeated effusion drainage within 4 weeks. The objective response rate (ORR) was the sum of CR plus PR. Patients with a response constituted the “remission group”. The clinical control rate (CCR) was the sum of CR, PR and SD. Another measure of clinical benefit was the total number of days of hospitalization following DC-CIK infusion until 12 months or death, whichever came sooner. One day was defined as a hospital stay crossing midnight and was determined by independent physician review from medical records and patient reports.

DNA extraction, sequencing and processing

Peripheral blood (5 ml) was collected from each patient before and after DC-CIK infusion and, within 4 hours, plasma was separated from the blood samples by centrifugation at 1,600 g at 4°C for 10 min, then retained and stored at -80°C before extraction of cell-free DNA (cf-DNA). The capture region was designed to cover hot exons and hot regions (1041 genes) frequently mutated in solid tumors. Capture hybridization was carried out according to the manufacturer’s protocol. Sequencing libraries were prepared for cfDNA using the Kapa DNA library preparation kit (Kapa Biosystems, Wilmington, MA). DNA sequencing was carried out with the HiSeq3000 sequencing system (Illumina, San Diego, CA) [23].

Gene clonal load and molecular tumor burden analysis

Based on a Bayesian clustering method, PyClone was employed to calculate the allele frequency of mutations (reported as the gene clonal load) in baseline ctDNA and plasma ctDNA samples from patients before and after infusion treatment, respectively, which was then used as an estimate of the cellular percentage of each mutant gene in the tumor. The molecular tumor burden index (mTBI) was calculated using the mean allele fraction of mutations in a mutation cluster with the highest cellular prevalence of ctDNA in a plasma sample. mTBI reflects the percentage of ctDNA detected in cfDNA and ΔmTBI was calculated based on the mTBI of the first ctDNA sample in order to reflect the change of tumor burden at the molecular level [24].

Statistical methods

Continuous variables were expressed as mean ± SD (standard deviation) and compared using a two-tailed unpaired Student’s t test; categorical variables were compared using χ² or Fisher
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analysis. Binary logistic analysis was used to identify the risk factors associated with malignant effusion remission. Results are reported as odds ratios (OR) and their 95% confidence intervals (CI). A OR >1 indicated an elevated risk with respect to the reference category. All statistical evaluations were carried out using SPSS software (Statistical Package for the Social Science, version 15.0, SPSS Inc., Chicago, IL, USA). A value of $P<0.05$ was considered to be statistically significant in all the analyses.

Results

Patient characteristics

From September, 2012 to August, 2018, 37 patients with malignant pleural effusions or malignant ascites received at least 1 cycle of intra-cavitary DC-CIK infusions (16 intrapleural and 21 intraperitoneal). The baseline characteristics of these patients are recorded in Table 1. There were 15 male and 22 female patients with a mean age of 60.5 years (range, 34-90 years). On average, each patient had undergone prior thoracenteses or abdominal paracentesis at least one time (range 1-4 times), and had undergone tube drainage 8 times (range 3-14 times) before DC-CIK treatment. Forty-five percent (45%) of patients received systemic anti-tumor therapy, in the majority, combination chemotherapy, during DC-CIK treatment. The median numbers of DC and CIK cells delivered were $1.01\times10^7$ (range, $0.60$ to $1.35\times10^7$) and $1.36\times10^9$ (range, $0.74$ to $4.98\times10^9$) respectively.

Control of malignant pleural effusions and malignant ascites after DC-CIK treatment

Based on previously reported criteria, the therapeutic response of the effusions to DC-CIK was determined to be 1 CR (3%), 13 PR (35%), 12 SD (32%) and 11 PD (30%), yielding a total clinical control rate of 70.3% (26/37) and objective response rate of 37.8% (14/37) (Table 2). There were no significant differences in clinical control rates observed among the various malignancies (Figure 1A), but there was a numerically higher rate of stable disease for

## Table 1. Demographics and baseline characteristics of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
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<tr>
<td>Sex</td>
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</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>40.50%</td>
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<tr>
<td>Female</td>
<td>22</td>
<td>59.50%</td>
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<td>≥65</td>
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<td>Infusion mode</td>
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<tr>
<td>Peritoneal</td>
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<tr>
<td>Numbers of metastatic sites</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>12</td>
<td>32.40%</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>48.60%</td>
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<tr>
<td>&gt;2</td>
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<td>Cevix</td>
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<tr>
<td>Pancreas</td>
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<td>10.80%</td>
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<tr>
<td>Stomath</td>
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<tr>
<td>Ovary</td>
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<td>8.10%</td>
</tr>
<tr>
<td>Colon</td>
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<td>5.40%</td>
</tr>
<tr>
<td>Breast</td>
<td>3</td>
<td>8.10%</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>19%</td>
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<tr>
<td>Disease control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>14</td>
<td>37.80%</td>
</tr>
<tr>
<td>Non-remission</td>
<td>23</td>
<td>62.20%</td>
</tr>
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</table>

## Table 2. Efficacy of patients with malignant effusions treated with DC-CIK infusion

<table>
<thead>
<tr>
<th></th>
<th>malignant pleural effusions (MPE) (N=16)</th>
<th>malignant ascites (MA) (N=21)</th>
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<tbody>
<tr>
<td>CR</td>
<td>0 (0%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>PR</td>
<td>6 (37.5%)</td>
<td>7 (33.3%)</td>
</tr>
<tr>
<td>SD</td>
<td>8 (50.0%)</td>
<td>4 (19.0%)</td>
</tr>
<tr>
<td>PD</td>
<td>2 (12.5%)</td>
<td>9 (42.9%)</td>
</tr>
</tbody>
</table>

Adoptive T cell intra-cavitary infusions

**A**

Number of patients

![Graph showing distribution of patients](image)

**B**

Combined with systemic anti-tumor therapy

- 64.50% (57/89) PR + SD
- 35.50% (32/89) PD

DC-CIK treatment alone

- 75.00% (13/17) PR + SD
- 25.00% (4/17) PD

**C, D, E, F**

Imaging of malignant pleural effusion and malignant ascites

**G**

Days in hospital until death or 12-month follow-up

- Total Time spent in Hospital during Trial/Days
- Total Time spent in Hospital during Trial/Days

- Median days:
  - DC-CIK + Indwelling Pleural Catheter Treatment: 8.46 days (IQR: 5-12)
  - DC-CIK + Indwelling abdominal catheter Treatment: 10.13 days (IQR: 8-13)
Adoptive T cell intra-cavitary infusions

Figure 1. Control of malignant pleural effusions and malignant ascites after DC-CIK treatment. A. Clinical control rates of pleural effusions and malignant ascites separated by tumor type. B. Clinical control rates for treatment with DC-CIK with or without systemic anti-tumor therapy. C-D. Malignant ascites (MA) detected by Doppler ultrasound before treatment with DC-CIK and after one cycle of treatment. E-F. Malignant pleural effusions (MPE) detected by computed tomography before treatment with DC-CIK and after one cycle of treatment. G. Number of days spent in the hospital over the next twelve months (or until death) from time of DC-CIK administration for patients with malignant pleural effusions and ascites.

MPE than for MA. The clinical control rate for patients receiving concurrent systemic anti-tumor treatment was 64.71% and for patients receiving DC-CIK treatment alone was 75% (Figure 1B). Examples of responses before and after treatment are shown for a patient with a CR and a patient with a PR (Figure 1C-F).

The number of days spent in the hospital over the next twelve months (or until death) from time of DC-CIK administration was determined from medical records and patient reports. The median total hospitalization time for patients with MPE was 8.46 days (IQR: 5-12 days) and for MA was 10.13 days (IQR: 8-13 days) (Figure 1G).

Factors associated with the efficacy of DC-CIK treatment

Binary logistic regression models were used to quantify the factors associated with malignant effusion (ME) clinical control. Number of DC-CIK infusions (P=0.032), ECOG-PS (P=0.010), number of metastatic sites (P=0.001) and infused number of CIKs (P=0.025) were associated with ME response in univariate analysis (Figure 2A-D). A multivariable analysis was then performed to assess the factors that independently predicted response of the effusion. After adjusting for competing risk factors, number of metastatic sites (P=0.007) and infused number of CIKs (P=0.013) remained independent factors associated with therapeutic efficacy. The details are shown in Table 3.

We also analyzed features of the infused CIK cells that were associated with clinical outcome. Multivariable analysis demonstrated that the frequencies of CD3+/CD8+ and CD8+/CD28+ T cells among the infused CIK cells were independent factors associated with ME response (P<0.05) (Figure 2E). As these cells represent the activated, effector T cells, these data suggest that infusion of an activated cell population into the pleural or peritoneal cavities may have direct anti-tumor effects.

Phenotypic analysis of peripheral blood immune cells after DC-CIKs

Phenotypic analysis of peripheral blood mononuclear cells before the treatment and at the end of the first cycle of therapy demonstrated that the CD3+/CD8+ T cell subset was increased (P<0.05) and the CD4+/CD25+ T cell subset was decreased (P<0.05) after DC-CIK cell therapy among the group with response of the effusion compared to the group with no response (Figure 2F).

Adverse events and HRQoL

Overall, the DC-CIK therapy was well tolerated with only mild AEs. The main adverse effects included mild transient fever (<38.5°C), chills, fatigue, bone marrow suppression, grade 1 to 2 chest pain and grade 1 to 3 gastrointestinal reactions. All toxicities were alleviated by symptomatic treatment.

Overall, the EORTC-QOL30 did not detect any differences in HRQoL following DC-CIK treatment; however, the global health and function scales after treatment were slightly worse than before DC-CIK treatment. Some symptom scales, such as pain, insomnia, constipation and diarrhea, deteriorated slightly during DC-CIK treatment. Other symptom scales, such as fatigue, nausea, vomiting, appetite loss and financial difficulties, improved slightly during DC-CIK treatment (Figure 3).

Dynamics of gene clonal load and mTBI value in ctDNA during DC-CIK treatment

Sequencing of ctDNA was performed using peripheral blood specimens obtained before and after treatment from 7 of 37 patients (including 3 PR, 2 SD, and 2 PD patients) who received only DC-CIK infusions. In general, the allele frequency of detectable circulating mutant DNA decreased in patients with partial response or stable disease and increased in those with progressive disease (Figure 4A). Interestingly, one
Adoptive T cell intra-cavitary infusions

Infusion times

- P = 0.032

Number of patients

- Remission group
- Non-remission group
- < 2
- 2

ECOG

- P = 0.010

Number of patients

- Remission group
- Non-remission group
- 0
- 1
- 2

Number of metastasis organs

- P = 0.001

Number of patients

- Remission group
- Non-remission group
- < 2
- 2

CIK Cell number (10^9)

- Remission group
- Non-remission group
- P = 0.025

Multi-factors analysis of infused CIK cells

- CD19+
- CD8+/CD28-
- CD8+/CD28+
- CD4+/CD25+
- CD3+/CD16+CD56+
- CD3-/CD16+CD56+
- CD3+/CD16+
- CD3+/CD4+
- CD3+
patient with a PR had a significant decrease in mutant PIK3CA to the point of undetectability, but a slight increase in mutant TP53. The percentage changes in mTBI values during DC-CIK treatment relative to the baseline mTBI (normalized to 100%) were calculated. In patients with PR and SD, mTBI declined but it increased in the 2 patients with PD (Figure 4B).
Adoptive T cell intra-cavitary infusions

Discussion

Malignant pleural effusions and ascites decrease the quality and quantity of life. Standard treatments such as repeated thoracentesis or paracentesis, closed thoracotomy and pleurodesis with sclerosing agents, diuretics, concentrated ascites reinfusion therapy, and implantation of peritoneal venous shunts have variable effectiveness [25, 26]. Conventional systemic chemotherapy exhibits efficacy in only 9.7% of patients with malignant ascites [27]. Explanations for the poor response rate include malignant cells in pleural and peritoneal effusions with acquired multi-drug resistance and decreased penetration of systemically administered anticancer drugs [28]. Intra-cavitary chemotherapy with cisplatin, carboplatin, bleomycin, mitoxantrone, irinotecan or etoposide has efficacy of up to 40%. However, effusion recurrence, infections, adhesions, pain and fever, which decrease quality of life are common [29].

Immunotherapy is now being applied to a widening group of malignancies, but tumor immune escape remains a common cause of tumor progression and metastasis. Advanced malignancies are often accompanied by immunosuppression with defective macrophage type I and natural killer (NK) cell function [30-32]. Suppression of T cell proliferation and decreased recruitment of CD8+ T cells into malignant pleural effusion or ascites has been reported [33]. Moreover, dendritic cells in malignant ascites, affected by the tumor microenvironment, are characterized by a less mature phenotype with diminished antigen presentation and weaker T-cell stimulation [34]. We hypothesized that intrapleural and intraperitoneal adoptive transfer of ex vivo activated dendritic, NK and T cells would counter these defects.

In the present study, intra-cavitary infusion of mixed DC-CIK cells in patients with MPE and MA resulted in a 38% overall response rate and a 70.3% clinical control rate in 4 weeks. We also collected radiographic data 8 weeks after the treatments. We found that in the remission group (n=14), 3 patients had a malignant effusion recurrence, 5 patients had a sustained malignant effusion decrease, and 6 patients had a stable effusion. In the non-remission group (n=23), 1 patient experienced a remission after he receiving a second cycle of infusion in the 5th week. Seven (7) patients had a stable effusion and 10 patients experienced a continuous progression of their effusion. Five (5) patients were unevaluable because they underwent repuncturing and catheter drainage between the 4th and 8th weeks (Figure 3).
These data support the use of intracavitary autologous cellular immunotherapy as an alternative method to control malignant pleural effusions and ascites. We did not observe a higher tumor control rate when the DC-CIK intra-cavitary infusions were administered concurrent with systemic chemotherapy suggesting that DC-CIK infusions alone may be an adequate strategy for controlling malignant effusions. In an exploratory analysis of ctDNA in a subset of patients, 1041 tumor-related genes were sequenced to assess clonal load and molecular tumor burden before and after treatment as an approach for assessing changes in clinical tumor burden [35, 36]. Previous studies have validated the mTBI as an indicator for evaluating therapeutic response in various malignancies [23, 24]. We observed that patients with partial responses or stability of their effusion following DC-CIK intracavitary infusions had a decrease in the mTBI while those with progressive disease had an increase.
Adoptive T cell intra-cavitary infusions

in the mTBI. These data suggest that a change in circulating tumor DNA may be a useful marker for tumor response in scenarios when measuring tumor burden is more challenging such as malignant effusions.

We also used days of hospitalization prior to death or in the first year after DC-CIK infusions as a metric for clinical benefit as this is an endpoint that has been used in other studies of management of malignant effusions [13]. We observed that the DC-CIK infusions resulted in a shorter hospital stay than reported in the literature for MPE treated with indwelling catheter drainage (8.46 days vs 10 days) [13]. Although the clinical importance of this 1.54 day shorter hospital time is unclear, the expense and inconvenience of hospitalization have driven substantial efforts to decrease hospital stay by even small magnitudes.

We analyzed various clinical factors and DC-CIK features for their association with therapeutic outcomes of DC-CIK intra-cavitary infusions. We found that the number of organs with metastases and the number of infused CIKs were correlated with the response of the malignant effusion. Furthermore, higher frequencies of CD3+/CD8+ and CD8+/CD28+ T cell populations within the DC-CIK product were associated with responses in the malignant effusions, suggesting that these activated cells may have direct anti-tumor activity.

Several studies have shown that regulatory T cells (CD4+CD25+ Tregs) could inhibit the anti-tumor activity of CIK cells [18, 37]. In our study, we have analyzed changes in T-cell subsets in the peripheral blood before and after the DC-CIK therapy. The peripheral blood CD4+/CD25+ T-cell subset was significantly decreased after DC-CIK cell therapy in those with control of their effusions, suggesting that the DC-CIK intra-cavitary infusions favorably modulate the immune milieu of the host.

We were surprised to find that the overall EORTC QLQ-30 score did not improve after the DC-CIK infusions; however, the symptoms associated with malignant pleural effusion and ascites, such as difficulty breathing, nausea and vomiting, and poor appetite, were slightly improved after treatment. This indicates that although DC-CIK local treatment did not improve the overall condition of the patient, it reduced the corresponding symptoms by controlling the production of pleural fluid and ascites.

In summary, we observed that intra-cavitary DC-CIK infusions could control malignant pleural effusions and ascites with mild toxicity and could modulate the systemic immune response. Randomized studies will be required to determine the full contribution of the DC-CIK infusions, independent of the effects of the catheter drainage and subsequent cancer therapies.

Conclusions

Intra-cavitary autologous cellular immunotherapy can control malignant pleural effusions and ascites and is associated with peripheral blood T cell modulation towards a more activated and less immunosuppressive phenotype.

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Disclosure of conflict of interest

None.

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References

Adoptive T cell intra-cavitary infusions


Adoptive T cell intra-cavitary infusions


Adoptive T cell intra-cavitary infusions

Figure S1. Data from radiographic evaluations 8 weeks after the DC-CIK treatments for patient in our trial.