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Cellular immunotherapy combined with platinum-based chemotherapy prolongs survival for non-small cell lung cancer patients

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Platinum-based chemotherapy is the primary treatment option for advanced non-small cell lung cancer (NSCLC) patients without a driver gene mutation, but its efficacy is still modest. Through a potential synergistic effect, autologous cellular immunotherapy (CIT) composed of cytokine-induced killer (CIK), natural killer (NK), and T cells might enhance it. NK cells exhibited in vitro cytotoxicity toward lung cancer cells (A549 cells) following platinum therapy. Using flow cytometry, the expression of MICA, MICB, DR4, DR5, CD112, and CD155 on lung cancer cells was assessed. In this retrospective cohort study, there were included 102 previously untreated stage IIIB/IV NSCLC patients ineligible for tyrosine kinase inhibitor (TKI) target therapy who received either chemotherapy alone (n=75) or combination therapy (n=27). The cytotoxicity of NK cells for A549 cells was increased obviously and a time-dependent enhancement of this effect was also observed. After platinum therapy, the levels of MICA, MICB, DR4, DR5, CD112, and CD155 on the surface of A549 cells were increased. In the combination group, the median PFS was 8.3 months, compared to 5.5 months in the control group (p=0.042); the median overall survival was 18.00 months, compared to 13.67 months in the combined group (p=0.003). The combination group had no obvious immune-related adverse effects. The combination of NK cells with platinum showed synergistic anticancer effects. Combining the two strategies increased survival with minor adverse effects. Incorporating CIT into conventional chemotherapy regimens may improve NSCLC treatment. However, additional evidence will require multicenter randomized controlled trials.

Key words: chemotherapy; non-small cell lung cancer; combination therapy; adoptive immunotherapy

As the leading cause of cancer death and the most common cancer worldwide, with 1.8 million (18%) deaths predicted for 2021, lung cancer poses great challenges to both clinicians and patients [1]. Approximately 85% of lung cancers are non-small cell lung cancers (NSCLCs). The major treatment option for advanced patients who are ineligible for checkpoint blockade immunotherapy and tyrosine kinase inhibitor (TKI)-targeted therapy is platinum-based cytotoxic chemotherapy. Even with more advanced agents, the 5-year survival rate is still below 20% [2, 3]. Thus, modifications of the present treatment strategies are urgently needed to increase survival while minimizing the impacts on quality of life.

Immunotherapy represents the most promising novel strategy for lung cancer, considering the recent evidence regarding the clinical efficacy and basic research on immunotherapeutic approaches for cancer [4]. Improvements in lung cancer treatment have likely contributed to the decline in mortality, such as novel targeted therapy immune checkpoint inhibitors (ICIs) [5]. NSCLC patients were found to have a variety of abnormal immune cells, based on quantity and quality, such as dendritic cells (DCs), natural killer cells (NKs), natural killer T (NKT) cells, and T cells [6, 7]. Therefore, cellular immunotherapy (CIT), which involves the *ex vivo* transfusion of immune cell growth and activation, may be a feasible and effective method for eliminating tumor cells and treating immunological dysfunction.

It is unknown, however, which cell types are required for optimal CIT anti-tumor effects. According to previous reports, several mechanisms or phenomena might be

involved in the dysfunction of immunocytes and immune evasion in NSCLC; these include: i) reduced expression of IFN-γ, granzyme B, and perforin by infiltrating T, NK cells, and NK-like T cells [8, 9]; ii) insufficient NK and T lymphocytes cells to kill the tumor cells [10, 11]; iii) T cells are evaded by tumor cells without HLA-class I antigens [12]. Innate immunity requires NK and γδT cells, which exhibit anti-tumor properties that are not MHC-restricted. The CIK is a lymphocyte that is activated *ex vivo* and consists of $CD3^-CD56^+$ (NK cells), $CD3^+CD56^+$, and $CD3^+CD56^-$ (typical T cells) cells. It is $CD3^+CD56^+$ cells that primarily show anticancer activity, which exhibit NK-like, non-MHC-specific cytolytic abilities. In light of these considerations, CIT comprising of CIK, NK, and γδT cells, might be a reasonable option for cancer therapy.

In addition to covalent binding to DNA leading to tumor cell apoptosis, the anti-tumor effects of platinum-based agents might be partly due to their immune potentiating mechanisms, such as regulation of immune-associated receptor expression [13]. It also has been shown that i) cisplatin exposure may enhance immune effector cell recruitment and proliferation, ii) adoptively transferred effector cells have an enhanced lytic activity when treated with cisplatin, and iii) both cisplatin alone and in combination with new immunotherapies can decrease immunosuppressive factors [14]. Our hypothesis was that combining CIT with platinum-based chemotherapy would improve NSCLC patients' prognosis.

We thus explored the immune stimulatory effect of platinum on adoptive immunocytes in our laboratory study. Considering that CIK, NK, and γδT cells share similar immunostimulatory mechanisms, which mainly function through its activated receptors binding ligands expressed on tumor cells, only NK cell was selected to detect synergistic effects in our study. In the retrospective cohort study, CIT (a combination of autologous γδT, NK, and CIK cells) was also evaluated for effectiveness and safety.

Patients and methods

Cytotoxic effects of NK cells combined with platinum *in vitro*. Cytotoxicity of NK cells combined with platinum to lung cancer cells was detected *in vitro* by performing Calcein-AM release assays. The NSCLC-derived cell line A549 was employed as the target cells in this assay, and 5×10^4 cells were used to investigate the *in vitro* cytotoxicity of NK cells. Effector cell to target cell ratios (E/T) of 30:1, 10:1, and 3:1 were examined. After pre-treating A549 cells with 0.5 μg/ml cisplatin for 12, 24, 48, and 72 h, target cells were collected.

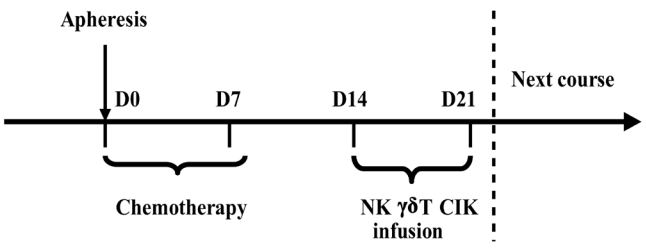

Figure 1. Schedule for autologous cellular immunotherapy (CIT) combined with chemotherapy.

The expression of surface molecules on A549 cells before and after platinum treatment analyzed by flow cytometry. At a density of 5×10^5 cells/dish, A549 cells in the logarithmic growth phase were seeded on 10 cm culture dishes. 24 h after cultivation, platinum was added at different times. According to the treatment schedule, 0.5 μg/ml cisplatin was added for 12, 24, 48, and 72 h, while vehicle treatment served as a control. After culturing, we collected and washed A549 cells with phosphate-buffered saline (PBS) before and after platinum treatment. After being counted, the cells were divided across several tubes. Monoclonal antibodies (mAbs) against MICA, MICB, CD112, CD155, DR4, and DR5 were added at a concentration of 1 μg/10^6 cells and incubated at 4°C for 30 min. Samples were examined after PBS washing. The identical IgG1 antibody served as the oppositional control. Positive cells were analyzed by flow cytometry among 1×10^4 cells, and percentages were computed. These tests were carried out three times.

Study designs and patients. The effectiveness and safety of CIT consecutive platinum-based chemotherapy for NSCLC patients were assessed in a retrospective cohort study. The Jilin University First Hospital Ethical Committee examined and approved this study (Approval No. 2016-297) based on the Declaration of Helsinki. Before enrollment in the study, all patients signed an informed consent form.

From June 1, 2009 to January 1, 2021, study participants met the following criteria: i) NSCLC diagnosed histologically or cytologically; ii) ineligible for TKI target therapy in stage IIIB/IV; iii) at least 18 years of age; iv) without congestive heart failure or cardiac arrhythmias; v) normal or mildly abnormal kidney and liver function (less than three times of the upper limit); vi) more than 3 months' expected life; vii) East Cooperative Oncology Group (ECOG) performance equal to or below 2. The exclusion criteria were as follows: i) previous history of autoimmune diseases or organ transplantation; ii) clinically serious infection status; iii) pregnant or lactating women; iv) received other immunotherapies in the last three months.

Participants in the study had the option of receiving chemotherapy alone (control group) or chemotherapy in combination with CIT (study group). Patients received up to six cycles of gemcitabine plus platinum (GP) or gemcitabine plus carboplatin (GC) as platinum-based chemotherapy regimens. Radiotherapy was administered according to disease status. In the case of recurrence or progression, patients were given 2nd or 3rd line chemotherapy or the best supportive treatment. The treatment schedule is summarized in Figure 1.

In general, follow-up was performed after two courses of chemotherapy during treatment or every 3 months for outpatients. Basic serum chemistry as well as chest and

abdominal computed tomography were done at every follow-up. Bone scans and magnetic resonance imaging (MRI) of the brain were used according to clinical needs. In order to determine response, we used Response Evaluation Criteria in Solid Tumors (RECIST 1.1) guidelines. Progression-free survival (PFS) is defined as the duration of the initiation of treatment up until the onset of the first progressive illness or death. Overall survival (OS) is the number of days between the onset of treatment and the death, regardless of the reason. PFS was the main endpoint and OS and CIT safety were the secondary endpoints.

Immune cells preparation. The Jilin Provincial Center for Sanitation Inspection and Test has approved the production of autologous immunocytes in accordance with Good Manufacturing Practices (GMP) (Certificate ID: A20090047). Standard operating procedures (SOPs) were strictly followed for the production and control of all immunocytes. Based on previously published protocols [15], we modified our previous study's procedures for preparing immune cells. We obtained PBMCs (peripheral blood mononuclear cells) ($1.0–2.0\times10^9$ cells) from patients using a Cobe Spectra Apheresis System (Gambro BCT, Inc., USA). A 50 ml centrifuge tube was used to centrifuge separated PBMCs for 10 min at 3,000×g. After removing the supernatant, using 30 ml PBS, we re-suspended the cell pellets and placed them over 15 ml Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden). Ficoll-Hypaque density centrifugation was used to isolate lymphocytes from PBMCs (Ficoll separation). A total of 1.3×10^9 ($1.0–1.8\times10^9$) mononuclear cells were obtained using the procedure. A variety of cytokines were used to stimulate NK, T, and CIK cells (described below).

We cultured PBMCs in AIM-V medium (Invitrogen, Carlsbad, CA, USA) with 700 U/ml of IL-2 (Miltenyi, Cologne, Germany) and 1 ng/ml of OK432 (Shandong Lu Ya Pharma) for 24 h at 37°C in a flask coated with mouse anti-human CD16 mAb (Beckman Coulter, Marseille, France) to increase NK cells. After centrifuging, the supernatant was discarded. In an AIM-V medium with 700 U/ml IL-2, the cells were again cultured at 37°C for two to three weeks. NK cells were stimulated by adding IL-15 to the medium (Miltenyi, Cologne, Germany). PBMCs in the AIM-V medium were stimulated with 1 μM zoledronate (Zometa®, Novartis, Beijing, China) and 400 U/ml of IL-2 to generate γδT cells. We added fresh medium every 3 days containing 400 IU/ml of IL-2. We cultivated PBMCs in the AIM-V medium with 1,000 U/ml IFN-γ (Miltenyi, Cologne, Germany) to produce CIK cells. The last stage included the addition of 1,000 U/ml of IL-2, 1,000 U/ml of IL-1 (both from Miltenyi, Cologne, Germany), and 100 ng/ml of a mouse anti-human CD3 mAb (Peprotech, Rocky Hill, NJ, USA).

IL-2 was supplemented with 1,000 U/ ml in a fresh complete medium every 3 days.

Cells were enumerated, tested for phenotype and viability (described below), and inspected for contamination before transfusion.

Pre-infusion analysis of immunocytes. Immunocytes were identified using the FACSCalibur (BD Biosciences, San Diego, CA, USA) and 4-color flow cytometry. mAbs derived from mice against human CD3-PerCP, CD56-APC, and CD69-PE (BD Biosciences) were incubated with NK cells for 15 min, V9-FITC and CD3-APC were used to incubate γδT cells, while CD3-PerCP, CD4-FITC, CD8-PE, and CD56-APC (BD Biosciences) were applied to incubate CIK cells. As controls, isotype-matched antibodies were used.

Immune cell reinfusion. In order to assess the viability of the cells, the dye-exclusion test was performed. For checking for potential immune cell contamination, PCR-based methods were used to detect mycoplasma, bacteria, endotoxins, and fungi 24 h before and on the day of the product's release. There are several criteria that immunocytes must meet in order to be released: i) Over 95% viability; ii) The two assessments did not reveal any contamination from bacteria, endotoxins, fungi, or mycoplasma; iii) A minimum of $1.2–2.0\times10^9$ of every kind of cell should be infused; iv) More than 50% of the cells exhibiting the NK ($CD3^-CD56^+$) or γδT ($CD3^+V\gamma9^+$) phenotype, and more than 20% of cells with the $CD3^+CD56^+$ CIK phenotype in NK, γδT, and CIK cell culture systems, respectively, as detected by flow cytometry. Immunocytes were to be washed three times in normal saline and then reconstituted in 50 ml of normal saline before reinfusion. Patients received the transfusions intravenously over 30 minutes. $2.4–4.0\times10^9$ cells were transfused per transfusion.

Peripheral blood immune cell proportion analysis. Before apheresis and one week after the final course of CIT, the number of immune cells in the blood was monitored. Using a FACSCalibur, populations of B cells ($CD19^+$), T cells ($CD3^+CD4^+$, $CD3^+CD8^+$), NKT cells ($CD3^+CD56^+$), NK cells ($CD3^-CD56^+$), monocytes ($CD14^+$), and regulatory T (Treg) cells ($CD4^+CD25^+Foxp3^+$) were examined. All of the antibodies were bought from BD Biosciences.

Statistical methods. SPSS 17.0 software was used to conduct all computations (SPSS, Chicago, IL, USA). In addition to calculating PFS and OS using the Kaplan-Meier method, we used the log-rank test to compare PFS and OS across groups. And then used a multivariate Cox proportional hazard model to assess parameters that univariate analysis determined to be statistically significant. Fisher's exact test was applied to binary data, and the Mann-Whitney test was used to compare medians. A p-value <0.05 was considered statistically significant.

Results

Quality of the cultured immune cells. Over 95% of immune cells in our culture system were viable. There were no signs of contamination in the cultured immune cells, and the preparations were in accordance with the release specifications. Before and after induction, the percentages of

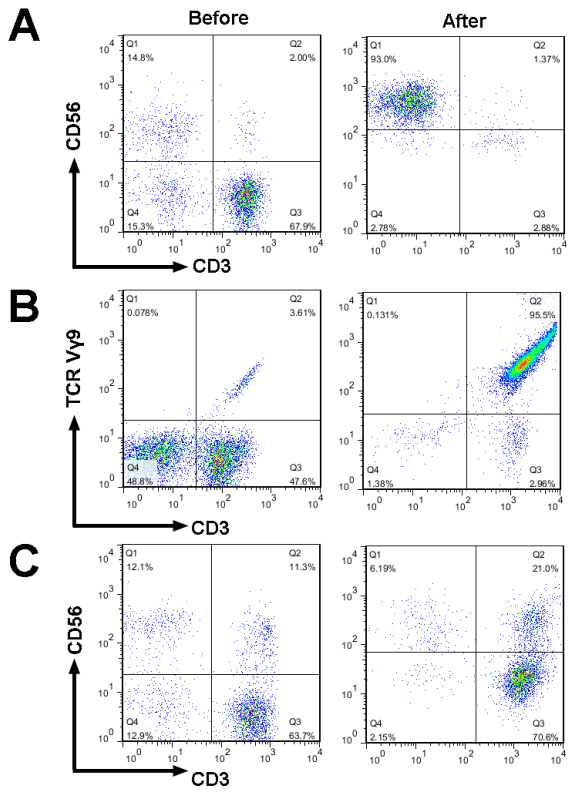

Figure 2. Percentage of natural killer (NK), γδT, and cytokine-induced killer (CIK) cells before and after induction. Representative results from a single patient are shown. The percentages of NK cells (A), γδT cells (B), and CIK cells (C) before and after induction were 14.8% vs. 93%, 3.61% vs. 95.5%, and 11.3% vs. 21.0%, respectively.

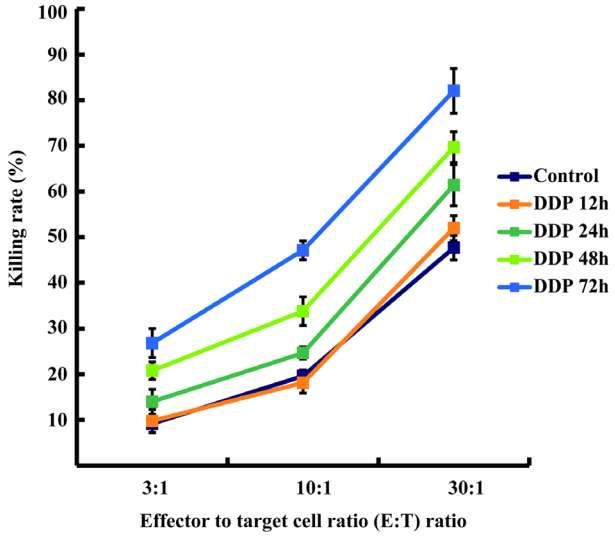

Figure 3. The cytotoxic effect of natural killer (NK) cells toward A549 non-small cell lung cancer cells. NK cells exerted a significant cytotoxic effect on A549 cells, and this cytotoxicity was increased further as the effector to target cell ratio increased. In the presence of platinum, A549 cells showed enhanced susceptibility to NK cell-mediated cytotoxicity. Platinum combined with NK cells showed a synergistic anti-tumor effect on A549 cells in a time-dependent manner.

NK ($CD3^-CD56^+$), CIK ($CD3^+CD56^+$), and γδT ($CD3^+V9^+$) cells were 8.01% (4.12–17.35%) vs. 85.32% (61.33–99.61%), 4.51% (1.62–7.96%) vs. 34.52% (17.25–47.28%), and 4.22% (2.79–11.26%) vs. 82.63% (63.72–98.21%), respectively. These cell types were significantly enriched when induced. Thus, activated NK cells ($CD56^+CD69^+$) were significantly increased. Figure 2 shows a representative case.

Cytotoxic effect of NK cells towards tumor cells. Based on Calcein-AM release assays, NK cells were tested for cytotoxicity. As the ratio of E/T cells increased, NK cells showed a significant cytotoxic effect on A549 cells. A549 cells exhibited enhanced NK cell cytotoxicity when platinum was present. Further, platinum combined with NK cells displayed a synergistic anti-tumor effect on A549 cells in a time-dependent manner (Figure 3).

Platinum-regulated expression of surface molecules in A549 cells. The expression of CD112, CD155, MICA, MICB, DR4, and DR5 was increased after platinum treatment in a time-dependent manner. After a 48 h incubation with platinum, levels of MICA, MICB, CD112, CD155, and DR4 were significantly enhanced ($p<0.05$; Figures 4A, 4B).

Characteristics of patients. Between June 1, 2009 and January 1, 2021, 102 eligible patients were enrolled. The study group consisted of 27 patients, and the control group consisted of 75 patients. Patients were followed up for an average of 27 months (range, 4–93 months) to November 18, 2021. The median of CIT treatment in the study group was 3 rounds (range, 1–18). In both groups, the demographics of the patients were well-balanced, such as tumor stage, sex, age, ECOG performance status and smoking index ($p>0.05$). Nevertheless, there was not a balanced distribution of histological types, with 31/75 (41.3%) squamous carcinomas in the study group and 4/27 (14.8%) in the control group ($p=0.032$; Table 1).

Table 1. Characteristics of study and control patients.

Clinical features	Control group	Study group	p-value
Sex			
Male	48	17	0.923
Female	27	10	
Age, years			
Median (range)	58 (24–76)	59 (42–79)	0.785
Smoking index			
Median (range)	0 (0–1500)	0 (0–800)	0.861
ECOG			
≤1	70	23	0.240
2	5	4	
Histology			
Squamous carcinoma	31	4	0.032*
Adenocarcinoma	41	21	
Adenosquamous carcinoma	3	2	

Abbreviation: ECOG-Eastern Cooperative Oncology Group

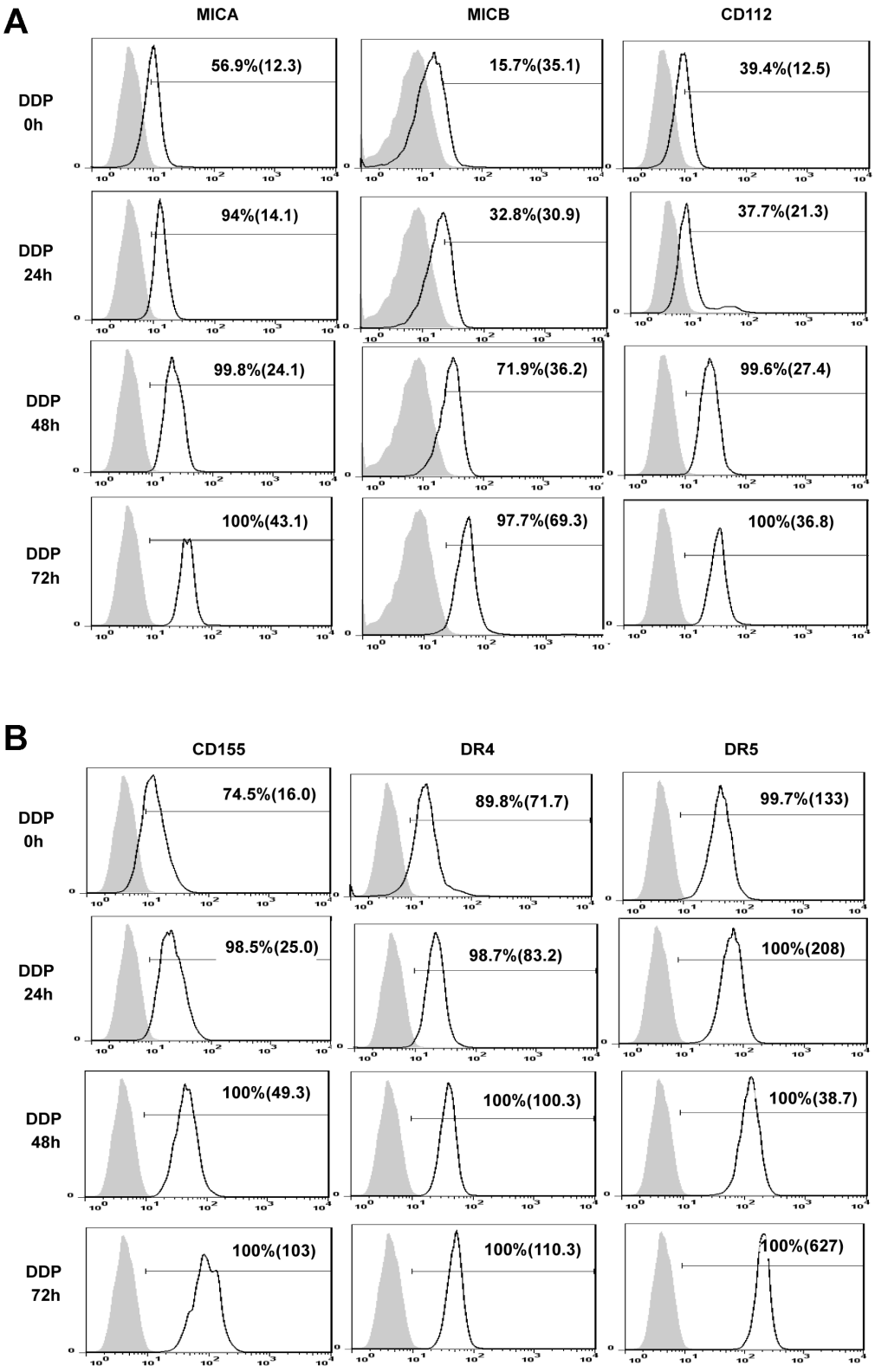

Figure 4. Platinum upregulates the surface expression of NKG2D ligands, CD226 ligands, and TRAIL receptors. A) Showed that levels of MICA, MICB (both NK2GD ligands), and CD112 (CD226 ligands) were increased after treatment with platinum in a time-dependent manner; B) Showed that CD155 CD226 ligands, DR4 and DR5 (TRAIL receptors) were increased after treatment with platinum in a time-dependent manner.

Patient survival and side effects of CIT infusion. For the study and control groups, the median PFS was 8.3 months and 5.5 months, respectively (HR = 0.582, 95% CI 0.36–0.94; p=0.042), the median OS was 18.00 months and 13.67 months, respectively (HR = 0.579, 95% CI 0.358–0.934; p=0.003) (Figures 5A, 5B).

CIT infusion led to mild fatigue in four patients. In two patients, a transient fever of 38.2°C was experienced after infusion, but they recovered within two hours. No other side effects were reported.

Prior and post-CIT immune cell percentage in peripheral blood. Pre- and post-CIT, the percentage of T, B, NK, NKT, and monocytes, as well as Treg cells, were analyzed. There were more $CD3^+CD8^+$ cells and fewer Treg cells after CIT ($p<0.05$; Figures 6A, 6B). The percentage of $CD3^+CD4^+$, $CD3^+CD56^+$, and $CD3^-CD56^+$ cells was not significantly different before and after CIT ($p>0.05$).

Discussion

In the past years, platinum-doublet chemotherapy alone or combined with bevacizumab was the main approach for most advanced NSCLC patients with no sensitive gene as the first-line therapy [16]. Whereas, with the development of cancer immunotherapy, ICIs (such as anti-PD-L1 antibodies atezolizumab and anti-PD-1 antibodies pembrolizumab) alone or combined with chemotherapy, have been the standard first-line therapy at present [17]. The application of PD-1/PD-L1 inhibitor demonstrates the feasibility of immunotherapy and the potential synergy between immunotherapy and chemotherapy for lung cancer. Although its application has improved survival and changed the treatment pattern for NSCLC, only a minority of patients can benefit from it and the efficacy still needed to be improved [18]. Besides, it is also limited by immune-related adverse events, especially for

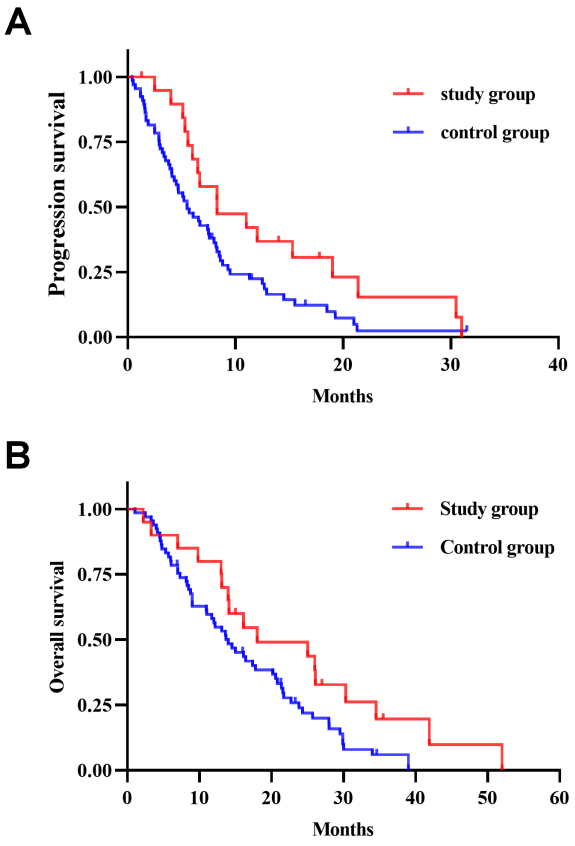

Figure 5. Comparison of PFS and OS in the chemo/CIT and chemo groups. PFS and OS were significantly longer in the CIT combination group than in the control (chemotherapy only) group ($p<0.05$).

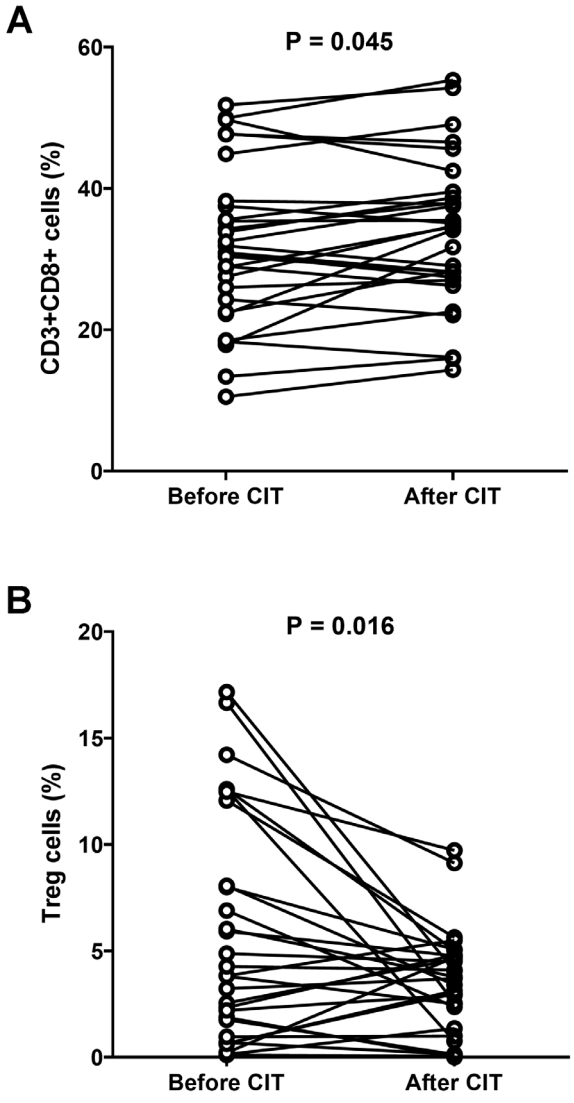

Figure 6. Proportion of Treg and $CD3^+CD8^+$ cells in the peripheral blood before and after CIT. More $CD3^+CD8^+$ cells (A) and fewer Treg cells (B) were identified after CIT ($p<0.05$) in patients with non-small cell lung cancer.

some rare and potentially life-threatening side effects, such as pneumonitis and myocarditis [19, 20]. Considering the safety of autologous CIK and the effect of the immune modulation of the platinum-containing chemotherapy regimen, we explored the feasibility of the combination regimen and potential mechanism.

Firstly, we measured the expression levels of NKG2D ligands (MICA and MICB), CD226 ligands (CD112 and CD155), and TRAIL receptors (DR4 and DR5) on NSCLC cells after treating them with cisplatin to investigate the potential mechanisms associated with the synergetic anti-cancer effects of NK cells combined with platinum. According to previous studies, normal cells rarely express major histocompatibility complex class I chain-related (MIC) proteins; however, they are broadly expressed by a variety of tumors. MIC proteins bind to the stimulatory NKG2D receptor on NK, $CD8^+$, αβ, and γδT cells [21]. The binding of MIC to NKG2D could underlie the killing of NK and T cells on tumor cells [22]. The overexpression of MIC can contribute to the cytotoxicity of CIK cells, resulting in superior OS for lung cancer patients [21]. Death receptor 4 (DR4; also known as TRAIL-R1) and death receptor 5 (DR5) can induce apoptosis after binding to their ligands, including FAS ligand (FASL), TNF-related apoptosis-inducing ligand (TRAIL), and APO3 ligand [23]. Reports have demonstrated that some anti-cancer drugs can augment T-cell-mediated killing by upregulating death receptors [24, 25]. CD112 (also known as Nectin-2) and CD155 (also known as PVR) have been found as unique ligands for the human DNAM-1-activating receptor and are abundantly expressed in tumor cell lines with neuronal or epithelial origin. Almost all human NK and T cells express DNAM-1. Activating signals are transduced between DNAM-1 and its ligands, which lead to DNAM-1 tyrosine phosphorylation, enhanced cytokine production, and cytotoxicity by both T and NK cells [26]. Our study showed that the expression of MICA, MICB, CD112, CD155, DR4, and DR5 was increased after treatment with platinum in a time-dependent manner. We can speculate, therefore, that upregulation of these molecules after platinum treatment could enhance NK cell cytotoxicity and augment the effect of CIT in our study. Considering that these three types of nonspecific immunocytes share similar mechanisms of activation, such as the interaction between MICA and its ligand, we only detected the synergistic effect of NK and cisplatin. However, for further confirmation, the synergistic effect of this platinum agent with CIK and γδT cells should be evaluated in the future.

The positive results of these preliminary experiments provided the foundation for the subsequent clinical study design. As expected, our study showed that combining CIT with chemotherapy results in superior survival for advanced NSCLC patients. However, we still wonder if any other potential mechanisms contribute to the result. We speculate that another two potential mechanisms according to present reports: i) directly killing mediated by any of these cell types: both preclinical and clinical studies have shown that NSCLC cells are sensitive to CIK, NK, and γδT cell-mediated killing [27]; ii) cross-talking among these cells: for example, γδT cells are capable of triggering NK cell-mediated killing of tumor cells, which are normally insusceptible to cytolysis by NK cells, and can also cross-present tumor antigens to $CD8^+$ T cells in order to trigger adaptive immune responses [28]. As well known, cancer cells employ a variety of mechanisms to evade the immune response, and thus a single type of activated immunocyte or CIT alone is unlikely to produce optimal anti-cancer effects. The success may be based on the basic characteristics of immune functions and the potential immune-promoting effect of chemotherapy.

The effect of $CD3^+CD4^+$ T, B, NK, Treg, and NKT cells on systemic immunity was analyzed in peripheral blood before and after CIT. Compared to the baseline, $CD3^+CD4^+$ T cells were significantly increased after CIT, while Treg cells were relatively decreased. Although it is not clear what indicators should be used to assess the immune response after CIT, these results indicate that the percentage of immunocytes in the peripheral blood might be considered a parameter to evaluate immune status. Systemic immune status might be related to the patient's prognosis after treatment with CIT. However, other mechanisms need to be addressed in future studies.

Patients who have been administered more than three courses of CIT seemed to have better OS and PFS, although this result was not statistically significant (data not shown). It could be partially explained by the small number of samples. Similar conclusions were obtained for SCLC patients in our previous study. According to our findings and the findings of previous studies, more CIT courses improve patient outcomes. However, it is still unclear how many treatment courses and how long the treatment should last.

At present, more combinations of ICIs and chemotherapy have been proven for advanced NSCLC in clinical practice, with obviously positive results obtained from some famous studies, such as Keynote 189, Keynote 407, and IMPOWER 131. The KEYNOTE-189 study, a phase 3 randomized, double-blind study comparing pembrolizumab to placebo in combination with pemetrexed and platinum in patients with metastatic NSCLC, has shown significantly longer overall survival (median OS: 22.0 m vs. 10.7 m) and progression-free survival (median PFS: 9.0 m vs. 4.9 m) than chemotherapy alone according to the updated data in 2020 [29, 30]. For advanced squamous NSCLC, Keynote 407 study (PD-1 antibody plus chemotherapy) also showed the combination group has the benefit, with OS (17.1 vs. 14.6 months) and PFS (median, 8.0 vs. 5.1 months) [31]. According to IMPOWER 131 (PD-L1 antibody plus chemotherapy), a similar phase 3 trial for advanced squamous NSCLC, the combination group had a longer PFS (6.3 vs. 5.6 months) and a similar OS (14.0 vs. 13.9 months). Our retrospective results showed a

significant improvement in combined treatment. Compared to these combination strategies, the combination of CIT and platinum-based chemotherapy seems to be promising. However, retrospective study without high-quality evidence leads to less persuasive.

Safety is another factor to consider when optimizing CIT applications. There were no significant side effects observed after CIT. In a meta-analysis, mild fever, shivering, nausea, and fatigue were among the adverse effects, which were self-limited [32]. While, adverse events mediated by ICIs have a broad spectrum, such as rash, colitis, hepatitis, endocrinopathies, and pneumonitis, and some toxicity may be life-threatening [33, 34]. Thus, the combination of CIT and chemotherapy was well-tolerated and thus it might be suitable for integration into first-line NSCLC therapy.

This study has the limitation of assigning patients to groups based on their individual therapeutic needs. To verify the efficacy of this treatment modality, additional multicenter randomized clinical trials are required.

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