

ORIGINAL ARTICLE

Experience of Autologous Immunotherapy for Non-Small Cell Lung Cancer Using Zoledronate-Activated Gammadelta T Cells

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SUMMARY

Background: Several nations around the world have utilized *autologous* immune enhancement therapy in the treatment of cancer, with initial positive outcomes. This study describes our experience with autologous gamma delta T cell immunotherapy for the treatment of non-small cell lung cancer patients in Vietnam, a developing nation.

Methods: Five patients with non-small cell lung cancer at stages III – IV were enrolled in the study. Each patient received six infusions of autologous $\gamma\delta$ T cells, separated by two weeks. Before, during, at the end of treatment, and three and six months after treatment, a comprehensive evaluation of clinical, laboratory, quality of life, and adverse events related to the method was conducted.

Results: At the time of culture seeding, the total number of cells ranged from 2.9 to 18.2×10^6 , with $\gamma\delta$ T cells ranging in number from 10.7 to 19.6×10^4 . On day 14 of the culture, the number of $\gamma\delta$ T cells ranged from 3.1 to 8.3×10^8 . Regarding the safety of therapy in a total of 30 infusions, two (fever), one (myalgia), and one (joint pain) were graded as 1 by CTCAE criteria. After the course, no toxicity was observed in the hematopoietic system, kidney function, or liver function. Evaluation of the patient's response in accordance with the RECIST 1.1 criteria: 20% of patients (one patient) had partial response disease, and 80% of patients (four patients) had stable disease at the end of treatment. During the follow-up period of the study, three patients were still alive, and the disease remained stable. The patient's quality of life improved after treatment in most functional measures (activity, cognitive, and social), but physical and emotional scores decreased slightly. Two patients' fatigue symptoms increased, but after six months of treatment, the average value dropped from 25.3 to 8.3. Dyspnea symptoms decreased gradually from 33.3 at the start of treatment to 8.3 six months later.

Conclusions: The initial results we obtained regarding the efficacy and safety of autologous $\gamma\delta$ T cell immunotherapy for patients with non-small cell lung cancer are extremely encouraging and comparable to those of previous studies.

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KEYWORDS

non-small cell lung cancer, $\gamma\delta$ T cell, autologous immunotherapy, zoledronate

LIST OF ABBREVIATIONS

SCLC - Small Cell Lung Cancer
 NSCLC - Non-Small Cell Lung Cancer
 $\gamma\delta$ T - Gamma delta T
 TCR - T cell receptor
 IFN- γ - Interferon gamma
 TNF- α - Tumor necrosis factor-alpha
 RECIST - Response Evaluation Criteria in Solid Tumours
 PBMNCs - Peripheral Blood Mononuclear Cells
 ECOG - Eastern Cooperative Oncology Group
 EORTC QLQ - European Organization for Research and Treatment of Cancer Quality of Life Questionnaire
 QoL - Quality of Life
 CTCAE - Common Terminology Criteria for Adverse Events
 RBC - Red Blood Cell
 HGB - Hemoglobin
 WBC - White Blood Cell
 PLT - Platelet
 AST - Aspartate Transaminase
 ALT - Alanine Transaminase
 MHC - Major Histocompatibility Complex
 FACT-G - Functional Assessment of Cancer Therapy - General
 CT - Computed Tomography
 MRI - Magnetic Resonance Imaging
 SD - Standard deviation
 EU/mL - Endotoxin Unit/milliliter
 G/L - Giga/Liter
 T/L - Tera/Liter
 U/L - Unit/Liter
 g/L - gram/Liter
 μ mol/L - micromol/Liter
 mmol/L - millimol/Liter

INTRODUCTION

Globally, lung cancer is the second most commonly diagnosed cancer and the leading cause of cancer death, with an estimated 2.2 million new cases (11.4% of total cancer cases) and 1.8 million deaths (18.0% of total cancer deaths) in 2020 [1]. Lung cancer includes two subtypes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), of which NSCLC accounts for 80 - 85% of the cases and has a 5-year survival rate of only 15% [2]. Recently, in addition to traditional therapy for lung cancer, immunotherapy has become a revolutionary cancer treatment that employs several immune system components, including cytokines, antibodies, and effector immune cells [3]. Autologous im-

munotherapy, utilizing immune cells extracted from the patient's body with a high degree of safety and the prevention of immunological rejection, has been successful in lung cancer treatment studies [4]. Gamma delta T cells ($\gamma\delta$ T cells) are T lymphocytes that express a specific T cell receptor (TCR) and are utilized in autologous immunotherapy. $\gamma\delta$ T cells have antitumor efficacy against tumors due to their powerful cytotoxicity, and the release of IFN- γ and TNF- α contributes to antitumor immunity [5]. A number of clinical trials using these ligands for $\gamma\delta$ T cells are being conducted and have initially shown the safety and effectiveness of $\gamma\delta$ T cells in the treatment of lung cancer [6]. Based on the foregoing, we undertook this pilot research to investigate the efficacy as well as safety of $\gamma\delta$ T cells for NSCLC patients, as measured by adverse effects, quality of life, and treatment response. The study's preliminary findings can assist clinicians and researchers in developing novel cancer treatment techniques in Vietnam, a developing nation.

MATERIALS AND METHODS

Subjects

This research, including all of the methods and experiments, was carried out in compliance with the ethical standards outlined in the Helsinki Declaration of 1975, as revised in 2008, as well as national law. In addition, Ethics Committee for Biomedical Research at Hanoi Medical University approved the study (code 1818/HM-UIRB, date 03/08/2018).

Five patients aged 20 to 80 years old were diagnosed with NSCLC stages III - IV based on clinical, laboratory, and histopathological findings. All patients had a performance status of 0, 1, or 2 and a life expectancy of at least six months; normal or almost normal renal, hepatic, and hematologic function; and possession of target lesions for evaluating response according to Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 criteria. Patients with significant co-morbidities, including liver failure, renal failure, heart failure, active infection, autoimmune illness, and immunosuppressive drug use, were excluded from the study.

Clinical protocol and study design

The study was carried out at the Cell Therapy Unit, Hanoi Medical University Hospital, from January 2021 to February 2023.

Before undergoing a screening evaluation to determine eligibility, written informed consent was obtained from all of the patients. Patients were clinically examined and evaluated prior to treatment, which involved patient histories such as drug allergies, cardiovascular disease, and current medications, a comprehensive physical examination, standard laboratory values, and a lesion size assessment. Patients who met the criteria and were assigned to receive autologous $\gamma\delta$ T cell immunotherapy would progress to the subsequent phase, which included

peripheral blood collection, cell extraction, cell culture, and cell harvesting. The harvested cell mass was evaluated for safety and quality before being transferred back to the patient. Each autologous $\gamma\delta$ T cell immunotherapy cycle consisted of six infusions administered every two weeks for three months. A specific course of one infusion is illustrated in Figure 1. At the end of the course, and three months and six months after the infusion of immune cells, the clinician evaluated the patient's treatment efficacy. This evaluation included a clinical examination, questionnaire-based information extraction, laboratory tests, and diagnostic imaging.

Isolation and large-scale expansion of gamma delta T cells from peripheral blood

The method for immune cell expansion has been optimized for available laboratory conditions. Ten milliliters of peripheral blood from lung cancer patients were collected in a tube containing heparin, an anticoagulant. The blood samples were transferred to the cell therapy unit for cell separation and cultivation. Blood samples were processed within 6 - 8 hours from the time of collection. Peripheral blood mononuclear cells (PBMNCs) were separated by density gradient centrifugation using Ficoll 1.077 (GE Healthcare Life Sciences, USA). After isolation, cells at a density of 1.10^6 cells/mL were cultured in AIM-V medium (Gibco, USA) containing 10% of the patient's serum supplemented with the cytokine IL-2 (600 IU) (Peprotech, USA) and zoledronate (5 mM) (Novartis, Switzerland). Every 2 - 3 days, the culture medium was replaced with a subculture medium containing IL-2, but the old medium was not discarded. As the number of cultured cells started to grow logarithmically, they were transferred to a culture bag (Corning, USA) and maintained there until the end of the culture. After 14 days of cultivation, the cells were harvested, washed three times, and resuspended in 100 mL of 5% albumin in saline. The average number of viable cells per infusion per patient obtained was greater than 5×10^8 , with $\gamma\delta$ T cells comprising more than 80% of the total. Criteria for the administration of activated $\gamma\delta$ T cells included a negative bacterial culture 48 hours prior to $\gamma\delta$ T cell injection, a negative mycoplasma culture, and an endotoxin test 48 hours prior to cell injection with a result < 0.015 EU/mL. To ensure maximum viability, the cells had to be administered within 14 hours of processing and transported from the facility to the Ha Noi Medical University Hospital for infusion.

Flow cytometry was used to examine the phenotype of expanded cells at the end of culture and PBMNCs at baseline (day 0). The corresponding isotypes of monoclonal antibodies specific for CD45, CD3, CD8, and $\gamma\delta$ T were conjugated with Peridin chlorophyll (PerCP), fluorescein isothiocyanate (FITC), phycoerythrin (PE), Allophycocyanin (APC) and used to characterize cell populations. According to the manufacturer's instructions, cells were analyzed using a NovoCyte Flow Cytometer (ACEA Biosciences, Inc., USA), and data was collected using NovoExpress software, version 1.3.

Dosage and duration

The patient received six infusions of expanded $\gamma\delta$ T cells with a cell survival rate of over 90%, each separated by two weeks. The specific cell count was dependent on the severity, stage, spread, and general health of the patient. Patients may be treated with autologous immune cells alone or in conjunction with other interventional therapies, such as chemotherapy and radiotherapy, for optimal results. For cancer patients receiving chemotherapy, this therapy must be discontinued three days prior to the immune cell infusion and resumed three days after. Prior to beginning radiotherapy, peripheral blood had to be collected. It was planned to administer the cell infusions an hour before and after radiotherapy. The expanded immune cells were infused intravenously into the patient. Approximately 100 mL of immune cell solution was infused within 15 - 60 minutes. Throughout and after the infusion, patients were monitored for adverse events.

Clinical assessment

We evaluated the patients who took part in autologous $\gamma\delta$ T cell therapy to determine whether or not it was safe and whether or not it was effective.

With regard to the safety of the treatment, we evaluated the adverse effects that occurred after autologous immune cell mass infusion. These adverse effects included vital signs (pulse rate, body temperature, and blood pressure), as well as side effects related to intravenous administration, such as itching, urticaria, edema, vomiting, abdominal pain, diarrhea, and so on.

To determine the efficacy of the treatment, we conducted evaluations at the beginning of treatment, at the end of treatment, three months, and six months after autologous immune cell infusion. According to the Eastern Cooperative Oncology Group (ECOG) performance status, a patient's level of functioning is represented by their capacity for self-care, performance of daily tasks, and physical capacity (walking, working, etc.). Assessment of treatment response in solid tumors according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria includes evaluation of tumor size and characteristics; non-target lesions, and new lesions. Advanced disease is defined as a clearly progressive lesion with an increase of at least 20% of the total measured diameter (at least 5 mm) and tumor markers that continue to rise above the normal limit. The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30, version 3.0, was used to assess the quality of life of lung cancer patients. Thirty items in the EORTC QLQ-C30 describe functional and symptom dimensions. Physical scales, as well as emotional, cognitive, social, and professional activity, make up the functional dimensions. The scales for fatigue, pain, and nausea or vomiting make up the symptom dimension. A scale measuring the perceived financial impact of the illness is also included, along with five scales describing basic symptoms (dyspnea, insomnia, loss of appetite, consti-

pation, and diarrhea). Scores on the items are scaled from 0 to 100 in accordance with EORTC regulations. High scores on the functional scale indicate a healthy level of functioning, while high scores for general health status indicate a high quality of life. However, high scores on the symptoms scale indicate a high level of symptomatology.

Statistical analysis

STATA 14.0 software (StataCorp LLC) was used for statistical analysis. Comparisons between groups were determined using the Wilcoxon test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Patients' profiles

This study enrolled a total of five cancer patients, including three men and two women. The average participant age was 53 ± 6.2 years (ranging from 45 to 60 years old). The study included five patients with adenocarcinoma. Four patients with stage IV disease were evaluated, but only one patient had stage IIIA disease. In this patient cohort, four individuals were diagnosed with tissue metastasis, and one was diagnosed as non-metastatic. Prior to enrollment in this study, all patients had received prior treatment, including three patients who had undergone surgery at the time of diagnosis, two patients who had received radiation therapy, three patients who had received > 4 weeks of chemotherapy, and four out of five patients who used targeted therapy (Table 1).

Immune cell expansion ability

All study participants received one course of treatment consisting of six infusions administered approximately every 14 days. In this study, the total number of cells from each expansion was utilized, achieving a range of 3.8 to 9.7×10^8 cells per patient. On the same day, peripheral blood was extracted from cancer patients and cultured. The mean number of white blood cells at the time of collection was 6.15 ± 2.3 G/L, with lymphocytes accounting for $23.6 \pm 8.3\%$ and monocytes for $6.7 \pm 2.1\%$. At the time of culture seeding, the total number of cells ranged from 2.9 to 18.2×10^6 , with $\gamma\delta$ T cells comprising 2.4% and ranging in number from 10.7 to 19.6×10^4 . At day 14 of culture, the number and composition of cells in each condition had changed significantly. The expansion of $\gamma\delta$ T cells was remarkable, with an 8,676-fold increase compared to the initial number of $\gamma\delta$ T cells. The number of $\gamma\delta$ T cells ranged from 3.1 to 8.3×10^8 after culture. Mycoplasma and bacteria or fungi were absent at all times in the culture medium. The endotoxin concentration was < 0.015 EU/mL. Immune cell expansion and the number of cell infusions are described in Table 2 and Figure 2.

Adverse events

The general status indicators (pulse, temperature, blood pressure, respiration rate, and SpO₂) were recorded prior to the patient's autologous immune cell infusion and re-evaluated if there were any abnormal manifestations after the infusion. During cell mass infusion, anticipated early adverse effects such as rash, itching, allergic reactions, etc. are closely monitored, assessed according to symptom severity, and treated in accordance with Hanoi Medical University Hospital protocol. The patient's interaction with the clinical staff revealed the possibility of future side effects.

Out of a total of 30 cell transfusions administered to patients, the most frequent side effects were fever (2/30), arthralgia (1/30), and myalgia (1/30). All of these side effects were mild (grade 1 according to the Common Terminology Criteria for Adverse Events - CTCAE) and appeared within 24 hours of cell infusion. After continued surveillance at home without medical intervention, the patient's side effects returned to normal, and there were no adverse effects of a serious nature.

On the hematopoietic system, adverse events were evaluated based on the analysis of the results of the complete blood analysis such as red blood cell count (RBC), hemoglobin (HGB) index, number of white blood cell count (WBC) and platelet count (PLT). Adverse effects on liver and kidney function are mainly expressed in liver enzyme tests (AST, ALT) and glomerular filtration function tests (urea, creatinine). These indices were assessed with each infusion of immune cells to the patient and re-evaluated at 3 and 6 months. The study results showed that no adverse effects were observed on the monitored functional indices (Figure 3 and Figure 4).

Clinical outcome

Table 3 demonstrates that, prior to treatment, functional criterion scores were high, with average values ranging from 80 to 92. Following completion of one course of autologous immune cell therapy, most functional measures (activity, cognitive, and social) showed an improvement in scores. There was a slight decrease in the patient's physical and emotional scores at the end of treatment; however, the change was not statistically significant, and these scores remained at a very high level with average values of 86.6 and 75, respectively. Interestingly, all functional parameters at 6 months after treatment were significantly enhanced, with mean values ranging from 75 to 100.

In terms of symptoms, fatigue and dyspnea were the most prevalent among this group of patients. After one course of treatment consisting of six $\gamma\delta$ T cell mass infusions, fatigue symptoms tended to increase in two patients; however, after six months of treatment, the average value for this symptom decreased from 25.3 to 8.3. On the other hand, dyspnea symptoms tended to diminish gradually from the beginning of treatment with a mean value of 33.3 to six months after treatment with a mean value of 8.3.

Table 1. Clinicopathological data of the lung cancer patients enrolled in the study.

Patients No.	Age/Gender	PS	Clinical Stage	Histological Type	Metastatic site organ	Previous treatment
GD01	49/M	1	IV	Ad	Lung	S, C, M
GD02	45/M	1	IV	Ad	Bone, kidney	C, R, M
GD03	53/FM	1	IV	Ad	Brain	S, M
GD04	60/FM	1	IV	Ad	Brain	M
GD05	58/M	1	IIIA	Ad		C, S, R

F - female; M - male; PS - performance status, Ad - adenocarcinoma, M - molecular-targeted therapy, C - chemotherapy, R - radiotherapy, S - surgery.

Table 2. Analysis of immune cells expansion and number of cell infusions.

Patients No.	No. of infusion	Total Cells Infused ($\times 10^6$)/infusion (Mean \pm SD)	$\gamma\delta$ T Cells Number ($\times 10^6$)/infusion (Mean \pm SD)	Rage dose of $\gamma\delta$ T Cells/infusion ($\times 10^6$)	Total dose of $\gamma\delta$ T Cells ($\times 10^6$)
GD01	6	747.7 \pm 85.9	622.9 \pm 104.9	508 - 749.9	3,737.5
GD02	6	653.5 \pm 115.3	520.8 \pm 102.3	412.8 - 645.4	3,125.1
GD03	6	805.8 \pm 148.4	685.9 \pm 126.5	492.9 - 822.9	4,115.8
GD04	6	639.6 \pm 185.4	529 \pm 160	307.3 - 806.5	3,174
GD05	6	729.2 \pm 44.5	4,405.4 \pm 50.2	341.2 - 461.4	2,432.7

SD - Standard deviation.

Financially, the financial impact of treatment did not fluctuate significantly during the follow-up period, with the exception of a slight increase at the end of treatment compared to the beginning. Meanwhile, the overall health score at the observation points improved, with the mean score increasing steadily from 63.3 to 75.

Evaluate the patient's response in accordance with the RECIST 1.1 criteria, which are based on the measurement of target lesions, non-target lesions, and new lesions both before and after treatment, thereby evaluating the tumor's response and evolution. Complete response, partial response, disease stability, and disease progression are the four levels of response. In this study, 20% of patients (one patient) had partial response disease, and 80% of patients (four patients) had stable disease at the end of treatment. During the follow-up period of the study, three patients were still alive, and the disease remained stable.

DISCUSSION

With its high morbidity and mortality, lung cancer imposes a burden not only on medicine but also on the economies and societies of numerous nations. Traditional treatment methods, including surgery, chemother-

apy, and radiation, have proven ineffective, especially for patients with advanced disease when the lesion has invaded and spread. Conversely, chemotherapy and radiotherapy are frequently associated with undesirable side effects. The development of new treatments that combine and supplement traditional methods is urgently required.

Among several novel treatments, autologous immune cell therapy is emerging as an essential alternative in cancer treatment, including lung cancer [7,8]. Among the currently used immune cell therapies, autologous $\gamma\delta$ T cells have the advantage of being able to recognize target cells, being independent of MHC (Major Histocompatibility Complex), and migrating to the site of the target cell, which allows them to be used to treat metastatic tumors. In order to conduct clinical trials on humans, the scientists conducted tests on mice and discovered that $\gamma\delta$ T cell-deficient mice were extremely vulnerable to agents that cause skin cancer [9,10]. In addition, numerous in vitro studies have demonstrated that $\gamma\delta$ T cells are highly toxic to cancer cell lines (breast cancer, liver cancer, and colorectal cancer) due to their ability to secrete cytokines that kill cancer cells [11]. For these reasons, we were motivated to conduct this study to evaluate the initial safety and efficacy of autologous $\gamma\delta$ T cell therapy in patients with advanced lung cancer.

Table 3. Quality of life improvement after immune cells transplantation.

Items	Means ± Standard Deviation			
	Baseline	End of treatment	3 months	6 months
Global Health Status	63.3 ± 13.9 (50 - 83.3)	66.6 ± 13.6 (50 - 83.3)	68.7 ± 14.2 (50 ± 83.3)	75 ± 9.6 (66.6 - 83.3)
Physical functioning	92 ± 7.3 (86.6 - 100)	86.6 ± 9.4 (80 - 100)	90 ± 8.6 (80 - 100)	93.3 ± 9.4 (80 - 100)
Role functioning	86.6 ± 18.2 (66.6 - 100)	87.5 ± 15.9 (66.6 - 100)	91.6 ± 16.6 (66.6 - 100)	100
Emotional functioning	80 ± 12.6 (66.6 - 91.6)	75 ± 16.6 (66.6 - 100)	83.3 ± 18 (58.3 - 100)	97.9 ± 4.1 (91.6 - 100)
Cognitive functioning	86.6 ± 7.4 (83.3 - 100)	91.6 ± 9.6 (83.3 - 100)	91.6 ± 16.6 (66.6 - 100)	100
Social functioning	83.3 ± 16.6 (66.6 - 100)	79.1 ± 15.9 (66.6 - 100)	83.3 ± 33.3 (33.3 - 100)	100
Symptom scales				
Fatigue	15.5 ± 9.9 (11.1 - 33.3)	25 ± 24.6 (0 - 55.5)	16.6 ± 19.2 (0 - 44.4)	8.3 ± 9.6 (0 - 16.6)
Nausea and Vomiting	0	12.5 ± 25 (0 - 50)	8.3 ± 16.6 (0 - 33.3)	0
Pains	10 ± 9.1 (0 - 16.6)	16.6 ± 13.6 (0 - 33.3)	16.6	8.3 ± 9.6 (0 - 16.6)
Dyspnea	33.3 (33.3 - 33.3)	16.6 ± 19.2 (0 - 33.3)	8.3 ± 16.6 (0 - 33.3)	8.3 ± 16.6 (0 - 33.3)
Insomnia	6.6 ± 14.9 (0 - 33.3)	16.9 ± 18.9 (0 - 33.3)	16.6 ± 19.2 (0 - 33.3)	8.3 ± 16.6 (0 - 33.3)
Appetite loss	6.6 ± 14.9 (0 - 33.3)	33.3 ± 27.2 (0 - 66.6)	8.3 ± 16.6 (0 - 33.3)	8.3 ± 16.6 (0 - 33.3)
Constipation	0	8.3 ± 16.6 (0 - 33.3)	0	0
Diarrhea	0	8.3 ± 16.6 (0 - 33.3)	0	0
Financial difficulties	26.6 ± 36.5 (0 - 66.6)	41.6 ± 41.9 (0 - 100)	25 ± 31.9 (0 - 66.6)	25 ± 31.9 (0 - 66.6)

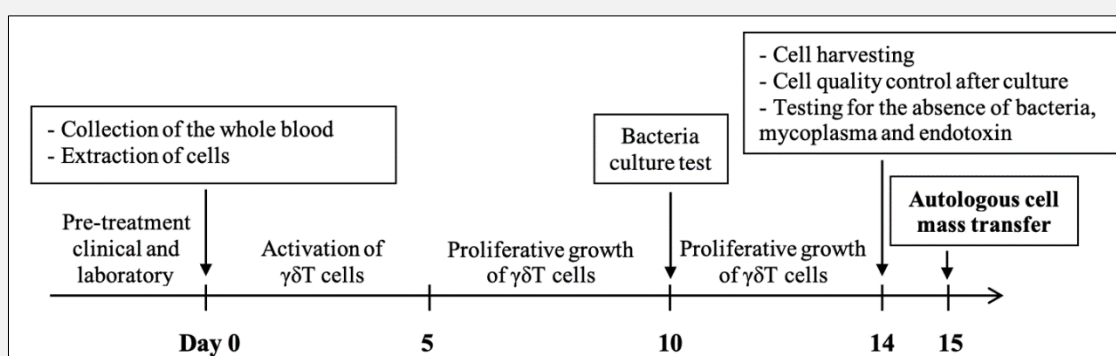


Figure 1. Specific procedure for one infusion.

Every two weeks, peripheral blood samples were collected for $\gamma\delta$ T cell culture. $\gamma\delta$ T cells were incubated in vitro for two weeks and then administered intravenously six times to the patient.

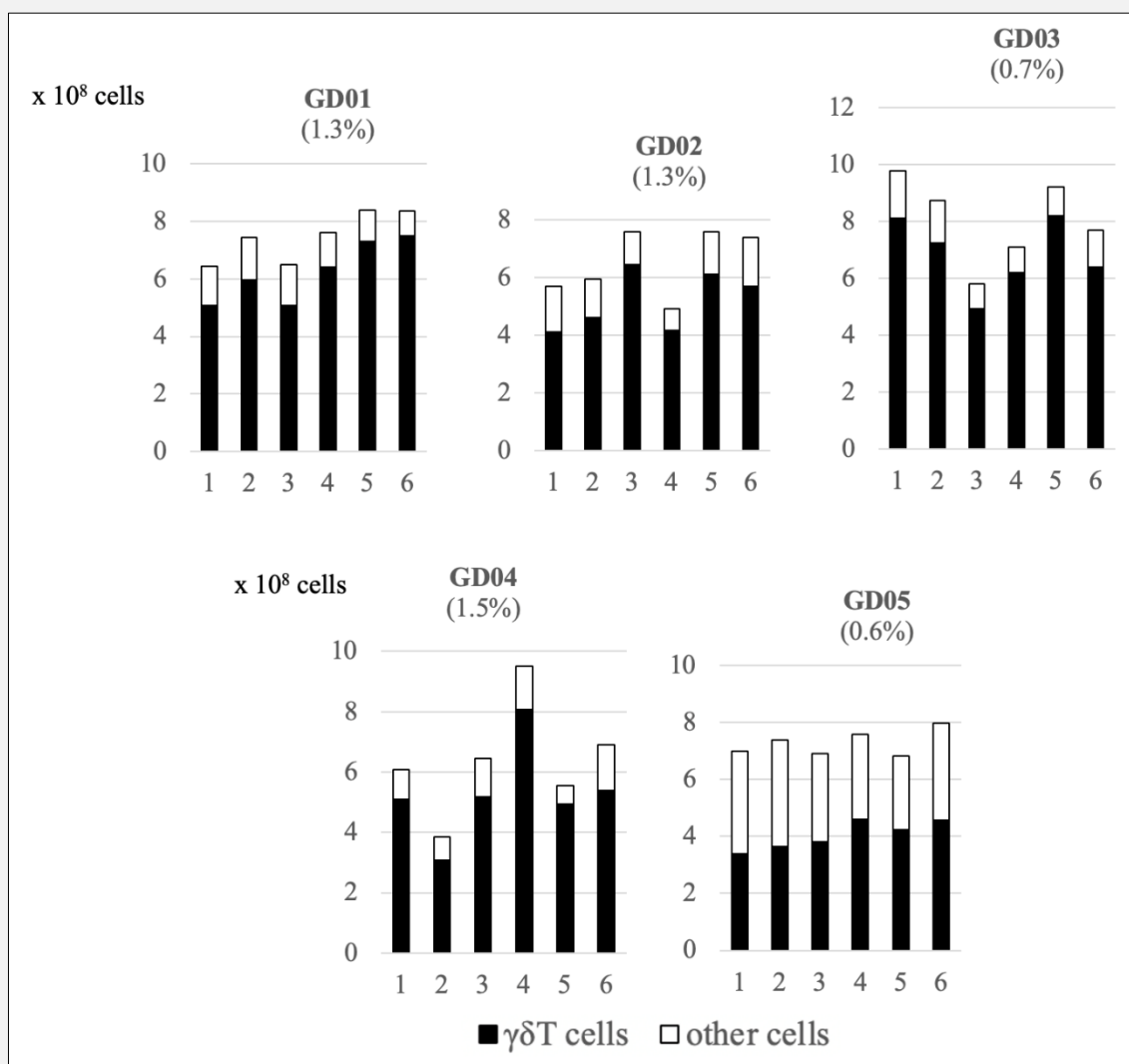


Figure 2. The composition of each patient's harvested and adoptively transferred cells.

For each patient, the total number of cells infused at each of the six infusions is depicted as separate bars numbered 1 through 6. Each bar is stratified into blue and orange to represent the proportions of $\gamma\delta$ T cells and other cells (TCD8 cells, TCD4 cells, and CD56 cells) in the cultures. Under each patient's number, the frequency of $\gamma\delta$ T cells in peripheral blood mononuclear cells prior to treatment initiation is indicated in parentheses.

In the treatment of lung cancer, the standard treatment methods continue to be surgery, chemotherapy, and radiation therapy; additionally, targeted therapy is administered to patients with the appropriate disease characteristics. Immunotherapy is an adjuvant treatment that bolsters and enhances the patient's immunity. When combined with other, more conventional treatments for cancer, such as surgery, chemotherapy, or radiation therapy, this method has not only been shown to be risk-free, but it also contributes to a thirty percent in-

crease in the treatment's overall efficacy [12]. A total of fifteen patients with recurrent or advanced NSCLC participated in a phase I study to examine the safety and potential antitumor effects of re-infusing ex vivo expanded $\gamma\delta$ T cells. The cumulative number of $\gamma\delta$ T cells that were transferred ranged from 2.6 to 45.1×10^9 , with the median value being 15.7×10^9 . There were no serious side effects from the treatment. The median survival time for all patients was 589 days, and the median progression-free survival time was 126 days [13]. Atsutaka

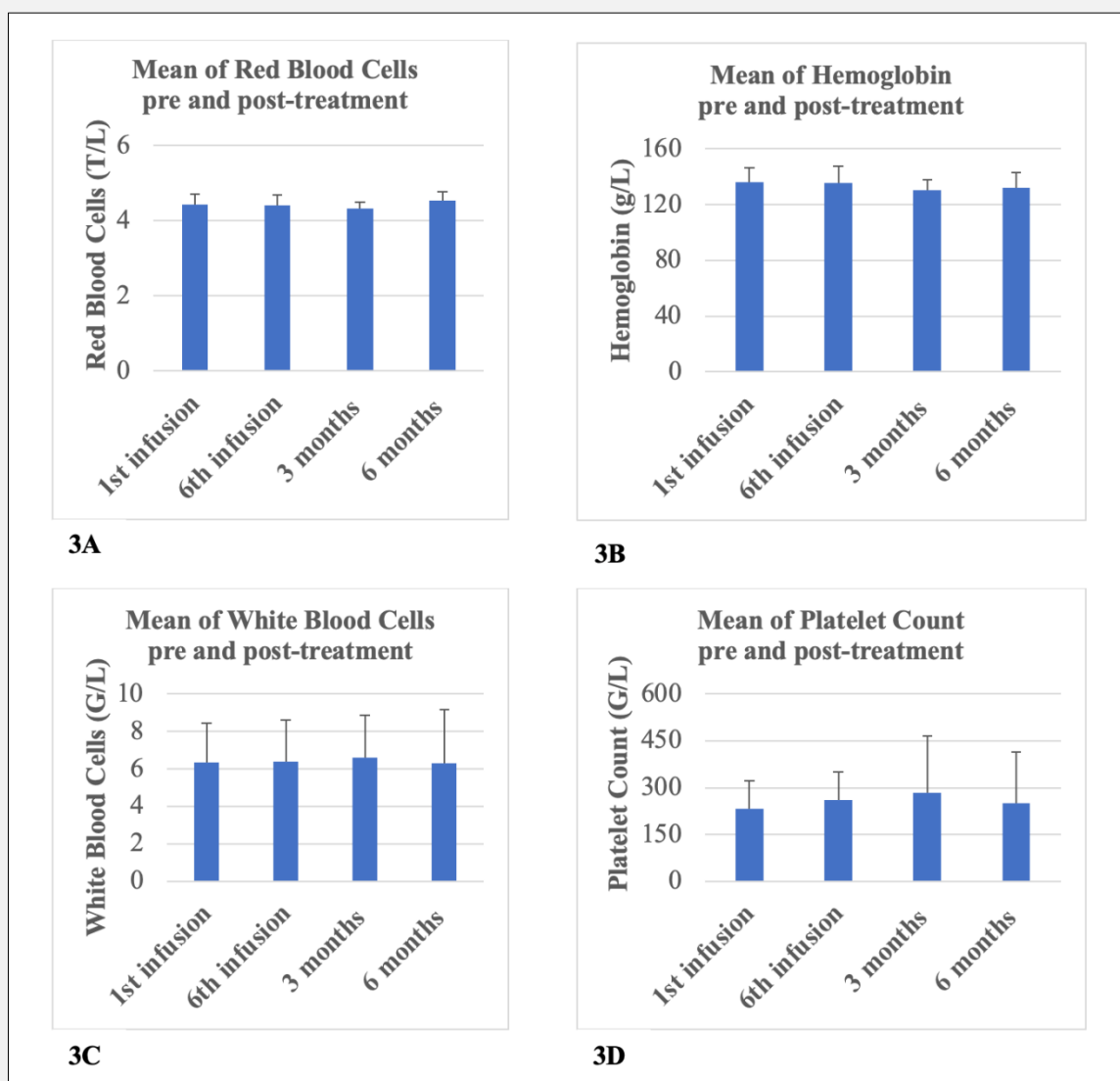


Figure 3. Hematopoietic system pre and post autologous $\gamma\delta$ T immune cell infusion.

3A - Red Blood Cells, 3B - Hemoglobin, 3C - White Blood Cells, 3D - Platelet Count.

Noguchi, et al. assessed the safety, immunologic effect, and practicability of zoledronate-activated $V\gamma 9\gamma\delta$ T cell-based immunotherapy in a clinical trial involving 25 patients with various solid tumors. The total cell number, frequency, and number of CD3 $V\gamma 9\gamma\delta$ T cells in the cells used for the first treatment were $409 \pm 284 \times 10^7$ cells, $56 \pm 33\%$, and $255 \pm 244 \times 10^7$ cells, respectively. No significant toxicity was detected. Four out of five patients treated with immunotherapy based on $V\gamma 9\gamma\delta$ T cells alone were evaluated for objective tumor response. During the six infusions, disease progression was observed in two patients, while disease

stability was observed in two. Ten of the twenty patients treated with a combination of treatments were evaluated. Three cases of partial response, one case of stable disease, and six cases of progressive disease were observed among these ten patients [14]. A phase I study reported that ten patients with recurrent non-small cell lung cancer underwent immunotherapy with $\gamma\delta$ T cells. The intravenous administration of $\gamma\delta$ T cells ranged from three to twelve (median = six). The total number of transferred $\gamma\delta$ T cells varied between 2.6 and 31.4×10^9 . Except for one patient who had pneumonia, the Functional Assessment of Cancer Therapy-Biologic

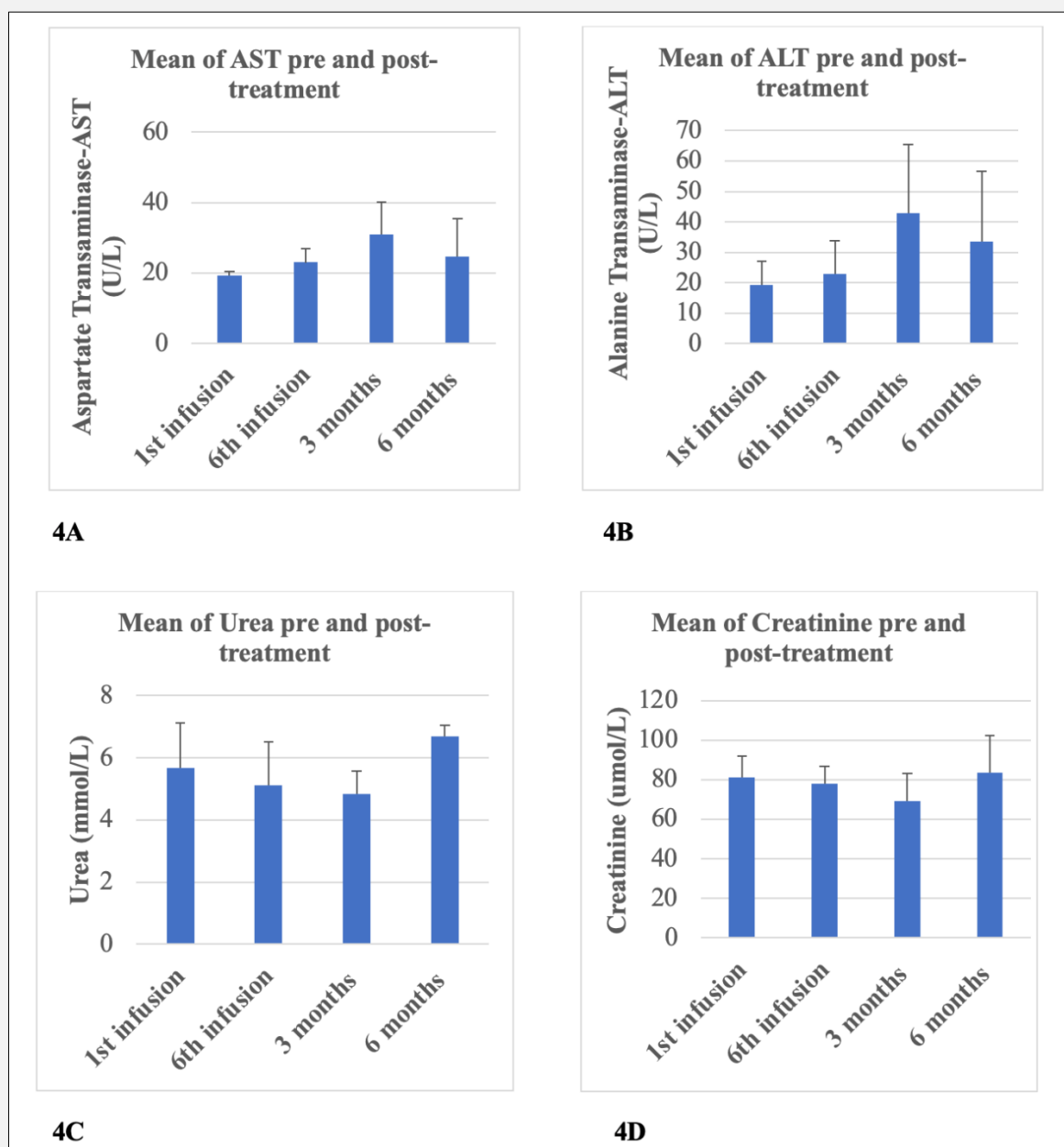


Figure 4. Liver and kidney function pre and post autologous $\gamma\delta$ T immune cell infusion.

4A - AST, 4B - ALT, 4C - Urea, 4D - Creatinine.

Response Modifier scores of the immunotherapy patients remained stable or improved. Following immunotherapy, patients were monitored for 240 - 850 days (median = 401 days). At the conclusion of the study, six patients were still alive [6]. In our study, the total number of transmitted $\gamma\delta$ T cells varied between 2.4 and 4.1×10^9 . The most frequent adverse effects were

fever, joint pain, and myalgia; however, they were all mild and resolved without medical intervention. Three patients were alive at the conclusion of the follow-up period.

Alongside traditional outcomes such as survival time and disease control, quality of life (QoL) has recently emerged as an increasingly important factor in cancer

treatment. QoL is a subjective, multidimensional concept that evaluates the correlation between physical, mental, and social factors. Understanding and evaluating QoL is essential for the holistic management of the patient and can aid clinicians in determining the most effective treatment regimen for the patient. EORTC and FACT-G (Functional Assessment of Cancer Therapy - General) are the two most widely used QoL assessment questionnaires for cancer patients at present. For this study, we used the EORTC-QLQ questionnaire because it is a scale that focuses more on assessing symptoms and physical problems in patients, whereas FACT-G emphasizes more on assessing the patient's emotional and social functioning. According to Table 1 regarding the characteristics of study participants, the majority of patients in our study were in the advanced stages of the disease. All functional scales (physical, cognitive, emotional, and social) improved over time, as measured by higher scores at the end of the study (Table 3). This result can be explained by the fact that autologous immune cell infusion stimulates the immune system and enhances health. Moreover, as a therapy that gives patients more confidence in their treatment, this has resulted in significant improvements in the patient's social and psychological aspects. This is consistent with reports by other authors of a significant improvement in life quality during the follow-up period [15].

Iyer et al. analyzed the symptom scale of lung cancer patients and discovered that fatigue (98%), appetite loss (98%), dyspnea (94%), cough (93%), and discomfort (90%) were the most prevalent symptoms. Analysis of correlations reveals that the severity of symptoms was inversely proportional to the QoL, and that symptoms such as fatigue, pain, and dyspnea were the most detrimental to the quality of life. In addition, fatigue, dyspnea, and pain decreased emotional scores, whereas sleep disturbances diminished cognitive function [16]. In our study, pain, fatigue, and dyspnea were the most prevalent symptoms. Moreover, a positive correlation was observed between the improvement in symptom severity (dyspnea, cough, fatigue, and diarrhea) and the improvement in patients' overall quality of life at the conclusion of autologous $\gamma\delta$ T cells immunotherapy.

In addition to evaluating the patient's QoL, we evaluated the patient's response using RECIST 1.1 criteria. After the course of treatment, four patients in this study had stable disease, while one patient had a partial response. Typically, patient GD04 had a significant improvement after three autologous immune cell infusions; CT and MRI scans revealed that the primary tumor shrunk by more than half compared to its initial size, supraclavicular lymph nodes disappeared, and metastatic lesions in the frontal lobes were significantly diminished. This finding is consistent with a case report describing the treatment of cholangiocarcinoma with autologous $\gamma\delta$ T cell immunotherapy. This patient received eight infusions, the first four times at two-week intervals and the last two times at one-month intervals. The results of the patient's MRI after treatment showed

that the lymph node size decreased sharply and was almost eliminated after the increased number of cell transfusions [17].

To evaluate the safety of the therapy, we based it on the adverse effects as well as the toxicity assessment on the hematopoietic system, liver and kidney of the patient after each infusion. During and after the infusion for all 30 infusions, we monitored whether there were cases of fever, joint pain or muscle pain, but all were at grade 1 and all returned to normal after the patient continued monitoring at home without medical intervention. In addition, no side effects have been recorded in patients such as: chills, rash, hypotension/hypertension, vomiting/nausea, diarrhea. Moreover, the paraclinical indicators of toxicity on hematopoietic system, liver and kidney were all within normal limits at the end of the course. This result demonstrated the safety of the therapy for the patient and was an advantage of this therapy in comparison to other conventional treatments such as chemotherapy and radiation therapy.

CONCLUSION

In this study, we carried out autologous $\gamma\delta$ T cell immunotherapy for the first time in five patients with non-small cell lung cancer. Despite a small sample size, clinical, subclinical, and quality-of-life results indicated that autologous $\gamma\delta$ T immunotherapy for lung cancer patients was safe and yielded positive results. Hopefully, this study will pave the way for future trials of autologous $\gamma\delta$ T immunotherapy for the treatment of cancer in Vietnam.

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Declaration of Interest:

All authors declare no conflict of interest regarding this article.

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