Prospective Phase II Study of Post-surgical Adjuvant Chemo-immunotherapy Using Autologous Dendritic Cells and Activated Killer Cells from Tissue Culture of Tumor-draining Lymph Nodes in Primary Lung Cancer Patients

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Abstract. *Background: The efficacy and toxicity of adjuvant chemo-immunotherapy using dendritic cells and activated killer cells are not clear in post-surgical primary lung cancer patients. Patients and Methods: Pathologically diagnosed N2 lung cancer patients were selected for post-surgical adjuvant chemo-immunotherapy. The activated killer cells and dendritic cells (AKT-DC) obtained from tissue cultures of tumor-draining lymph nodes (TDLN) or from TDLN co-cultured with peripheral blood lymphocytes (TDLN-Pb) were used for the adoptive transfer of immunotherapy. The patients received 4 courses of chemotherapy along with immunotherapy every 2 months for 2 years. Results: There were 31 N2 patients eligible for the study. Three cases were excluded because of refusal by the patients after 1-2 courses of immunotherapy. For the 28 cases treated, a total of 313 courses of immunotherapy were administered. The main toxicities were fever (78.0%), chill (83.4%), fatigue (23.0%) and nausea (17.0%) on the day of cell transfer. The 2- and 5-year survival rates were 88.9 % (95.9-81.9; 95% confidence interval, C.I.) and 52.9% (76.4-29.4; C.I.). Conclusion: Adoptive transfer of activated killer cells and dendritic cells from the tumor-draining lymph nodes of primary lung cancer patients is feasible and safe, and a large-scale multi-institutional study is necessary for evaluation of the efficacy of this treatment.*

Lung cancer is the leading cause of cancer death in Japan, its mortality rate having increased four-fold in the past three decades. The five-year survival rate of surgically treated N2 cases is around 20% (1-3), and has not improved despite the progress of treatment modalities such as chemotherapy (4, 5) and radiotherapy (6, 7) as well as of surgical procedures, during this period. Most of the failures in these advanced cases were caused by occult distant metastases at the time of surgical intervention. If 80% of N2 disease patients have occult distant metastases, it is important to deal with these metastases by some treatment modality both before and after surgery. Induction (8, 9) and adjuvant chemotherapy (10, 11) have been used for these N2 cases, but they are not as effective as those employed for relatively early-stage (stage I and II) carcinomas (12-14). We have previously reported a phase III randomized study (15) using lymphokine-activated killer cells aimed at these occult distant metastases and the efficacy of post-surgical chemo-immunotherapy against stage II and III primary lung cancer. In the study reported here, a new technique to cause proliferation of specific activated killer cells and dendritic cells (AKT-DC) from tissue cultures of the tumor-draining lymph nodes (TDLN) of primary lung cancer patients (16) was used. A phase II study of N2 lung cancer patients using these AKT-DC cells obtained from TDLN tissue culture for post-surgical chemo-immunotherapy was conducted.

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Key Words: Lung cancer, immunotherapy, N2 disease, adjuvant therapy, surgery, dendritic cell, regional lymph node.

Patients and Methods

Protocol for the study. This study (153-9-1) was approved by the Ethical Committee of Chiba Cancer Center, and written informed consent was obtained from all the patients before enrollment. The patients were required to have pathologically proven primary non-small cell lung cancer, clinical stage IIIA, be less than 76 years of age, and have a performance status of 0-1 (World Health Organization classification), without evidence of other active malignant disease. Among those who received surgical resection of primary lung cancer and complete resection of mediastinal lymph nodes, pN2 patients were selected as immunotherapy candidates from Oct. 1998 to Dec. 2004.

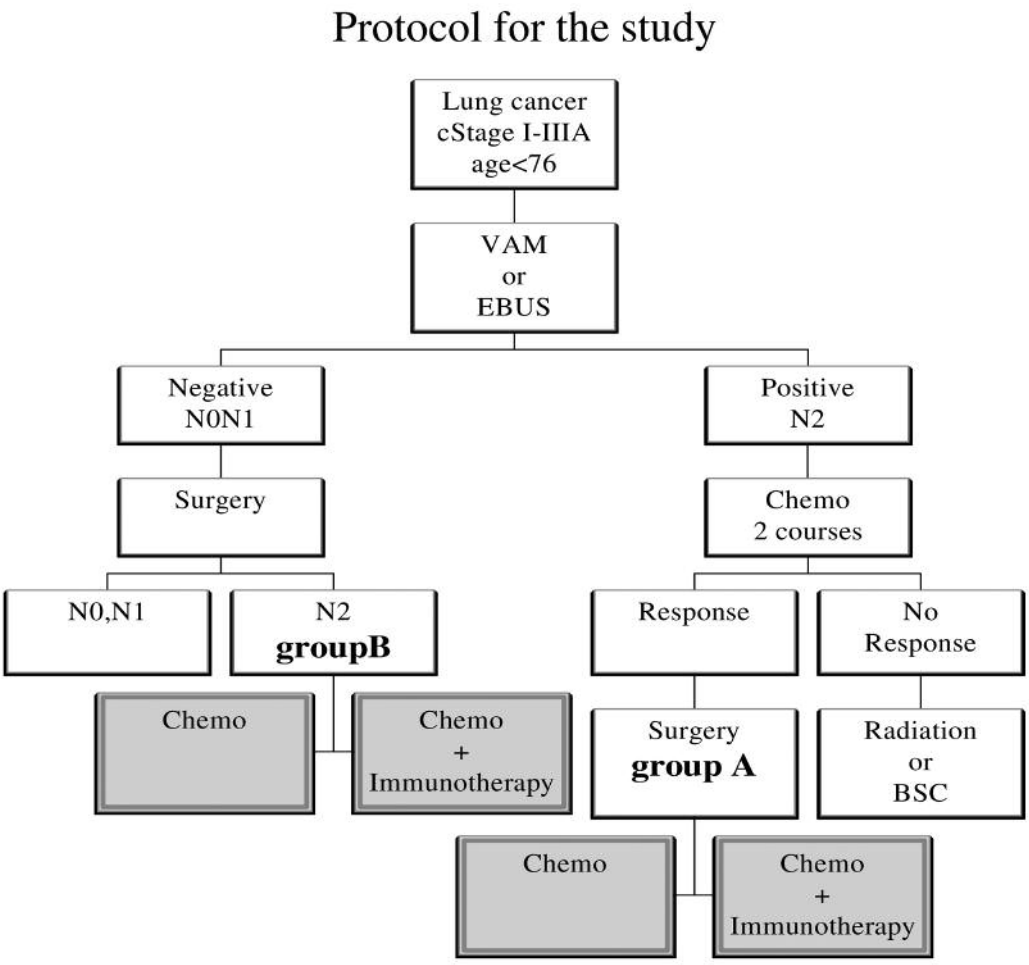

Figure 1. *Study design. Patients with clinical stage I-IIIA surgically resectable primary lung cancer, who were less than 76 years of age, and had WHO PS 0-1, underwent video-assisted mediastinoscopy (VAM), or endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). Negative (N0, N1) patients had surgery and complete resection of the mediastinal lymph nodes. Patients shown to be N2 after surgery (group B) were allocated to an adjuvant chemotherapy or chemo-immunotherapy group according to the indication criteria for immunotherapy. VAM- or EBUS-positive N2 patients received 2 courses of CBDCA+ paclitaxel and those who responded received surgery with dissection of mediastinal lymph nodes. Those in whom curative resection was performed in N2 cases received adjuvant chemotherapy or chemo-immunotherapy. BSC: Best supportive care.*

Both pathologically identified by video-assisted mediastinoscopy (VAM) (17, 18) or endobronchial ultrasound-guided transbronchial needle aspiration (19, 20) (EBUS-TBNA: group A) N2 patients before surgery and N2 patients identified after surgery (group B) were included in the study. The group A patients received 2 courses of induction chemotherapy with calboplatin (CBDCA) (AUC 5-6) and paclitaxel 200 mg/m^2, and those who responded received surgery with mediastinal lymph node dissection *via* median sternotomy (Figure 1).

Eligibility criteria for the immunotherapy. In addition to the criteria cited above, inclusion in the study of the candidates for immunotherapy also required that the AKT+DC from the TDLN grew rapidly (more than 1-3x10^9 cells for each course of therapy) and no recurrence was proved before initiation of the therapy (therapy was usually started 2 months after surgery). Exclusion criteria were; a positive response to HIV, hepatitis C virus, or human T-cell lymphotropic virus antibodies; a positive response for hepatitis B surface antigen, and evidence of another active malignant neoplasm. Those who did not receive chemo-immunotherapy received only chemotherapy or best supportive care. In the early phase of this study, TDLN of some cases did not grow rapidly enough for the treatment or was lost by bacterial or fungal contaminations. Lymphocytes obtained from peripheral blood stimulated in IL-2 (Pb-LAK) were used for the treatment instead of AKT+DC in those patients.

Adjuvant chemotherapy. As post-surgical adjuvant chemotherapy, those who had induction chemotherapy (group A) received 2 courses of 60 mg/m^2 docetaxel, and those who did not received induction chemotherapy (group B) received 2 courses of CBDCA and paclitaxel chemotherapy followed by 2 courses of docetaxel

chemotherapy. When recurrence was observed, 2-4 courses of gemicitabine and vinorelbine chemotherapy or cisplatin (CDDP), irinotecan (CPT11) were administered.

Preparation of killer cells and dendritic cells from lymph nodes. The procedure for the preparation of activated killer T cells and dendritic cells (AKT+DC) has been stated elsewhere (16). Briefly, two to three tumor-draining regional lymph nodes with no tumor metastasis were rinsed with 50 ml RPMI-1640 medium containing antibiotics transferred to a sterile Petri dish and aseptically minced into 1 mm tissue fragments. The cell preparation was then washed with the medium and lymph node tissue was transferred to a 75 cm^2 culture flask suspended in 50 ml of KBM-400 (Kojin Bio Co, Tokyo, Japan) serum-free lymphocyte medium containing 400 IU human recombinant interleukin 2 (Proleukin; Chiron B.V., Amsterdam, Netherlands). A tissue culture of the TDLN was started immediately after resection of the lymph nodes. When the TDLN started to release AKT+DC cells, the tissue and cell preparation was transferred into culture bags containing 800 ml of KBM lymphocyte medium. The generated lymphocytes and DCs were transferred to other bags, and the tissue culture of the TDLN was continued until the release of the cells stopped. The bags containing AKT+DC cells were separated 2-3 times with new bags containing fresh medium every 3-4 days. Usually, the TDLN tissue cultures continued to release killer cells and DCs for 2 to 4 months. When TDLN stopped releasing lymphocytes and DC cells, 1-2x10^9 peripheral blood lymphocytes obtained by lymphocyte apheresis with a COBE Spectra system (COBE BCT, Inc., Colorado, USA) were added. By this procedure, the TDLN resumed the generation of killer cells and DCs (16). When the TDLN was not available for culture by contaminations of bacteria, fungus or tumor cells, peripheral blood lymphocytes cultured in KBM-400 with IL-2 (peripheral blood lymphokine-activated killer cells: Pb-LAK) were used for the immunotherapy (15).

Certification of cells before transfer to patients. As stated elsewhere, the cells were cultured in a sealed bag using serum-free medium, and contamination with bacteria or viruses was prevented, but all the cells received certification of security tests before transfer to the patients. Cell viability was tested by trypan blue dye exclusion and cells of less than 90% viability were discarded. Bacterial contamination was tested just before transfer by two skilled examiners with a light microscope, and an endotoxin test was performed with an endotoxin single test kit (Wako Co., Osaka, Japan) and a toxinometer (MT-358: Wako Co.) when contamination of bacteria was suspected. The cell surface markers were analyzed before initiation of the culture and just before the transfer to the patients using the following two color method: FACS analysis with CD3, 8, 4, 80, 25, 83, B7H1, and HLA-DR monoclonal antibodies (Becton Dickinson Biosciences, CA, USA). Cultures consisting of more than 70% $CD25^+CD4^+$ regulatory T cells were discarded before transfer to the patients.

Contamination of tumor cells in TDLN or TDLN-Pb. Since lymph nodes with no metastasis confirmed pathologically were used and since metastatic lymph nodes do not generate lymphocytes or dendritic cells, contamination by tumor cells was prevented in the lymphocyte preparation for immunotherapy. If the skilled examiners had any suspicion that there might have been contamination by tumor cells, the cells were subjected to cytological examination with Papanicolaou smear stain and FACS analysis with the epithelial cell marker cytokeratin (Cytokeratin LP34 Dako Cytomation Co., Tokyo, Japan) and epithelial membrane antigen (EMA-E29, DakoCytomation Co.). If epithelial cell or tumor cell contamination was suspected, the cells were discarded before transfer to the patients.

Schedule and routes of cell transfer. The patients received 2-4 courses of chemo-immunotherapy after surgery and only immunotherapy every 2 months thereafter for 2 years. The initial treatment was commenced 1-2 months after surgery when the number of cells for adoptive transfer exceeded 1-3x10^9 cells. Usually, the patients received intra-venous infusion of the cells suspended in 50 ml of saline with subcutaneous inoculation of 4.5x10^5 U interleukin 2 (IL2). When distant metastasis to brain, bone or liver tissue, *etc*., was found, the patients received an intra-arterial infusion of cells through a subcutaneous Port-a-Cath catheter indwelling in the ascending aorta, having been inserted from the left subclavian artery. Immunotherapy was administered 5 to 7 days after chemotherapy so that the transferred cells would not be affected by the chemotherapy. When tumor recurrence was found, the therapy resumed even after 2 years and chemo-immunotherapy was stopped when the recurrence of tumors was progressive and not controlled by the therapy.

Immunohistochemical analysis of brain metastasis. The assessment of the immune response included analysis of a brain metastasis surgically removed after intra-arterial infusion of immunotherapy. The specimen was fixed with formalin and stained with CD3 (T cells) and CD20 (B cells) monoclonal antibodies (Becton Dickinson Biosciences).

Statistical analysis. Survival curves were calculated by the Kaplan-Meier method. Statistical comparison was made by the log-rank test.

Results

Treatment and patient distribution. Eighteen patients were shown to have N2 lung cancer before surgery (group A) and received 2 courses of CBDCA + paclitaxel induction chemotherapy and 13 cases were found to have N2 lung cancer after surgery (group B). Three of the 31 patients enrolled for the study dropped out after 1 or 2 courses of therapy, refusing to undergo more. Among the remaining 28 patients, 16 men and 12 women, of mean age 61.7 years (range, 48-75 years), 21 were adenocarcinomas, 5 squamous cell carcinomas and 2 others, 21 were at stage IIIA, 4 at stage IIIB and 3 at stage IV. The reason for inclusion of the N2 patients at stage IIIB or IV was that diagnosis resulted from pulmonary metastasis in the same lobe (PM1: T4 stage IIIB) as the primary cancer or another lobe (PM2:M1 stage IV). There were 13 single-station N2 and 15 multiple-station N2 cases.

Immunotherapy. For the 28 immunotherapy cases, a total of 313 courses of immunotherapy were carried out. TDLN or TDLN-Pb immunotherapy totaled 117 courses and Pb-LAK 196 courses. The mean numbers of cells for a course of

Table I. *Details of cases and numbers of cells transferred in immunotherapy.*

Case No.	Gender	Age	p-Stage	Histology	TNM	Month	Prognosis	TDLN, TDLN-Pb	Pb-LAK	Rec
1	f	66	IIIA	Ad	T1N2M0	18	d	1	29.7	lung
2	m	69	IIIA	Ad	T1N2M0	48	d	5.4	64.8	lung
3	f	48	IIIA	Ad	T2N2M0	28	d	0	51.8	lung
4	m	43	IIIA	La	T3N2M0	46	d	2	43.3	brain
5	m	53	IIIA	Ad	T2N2M0	22	d	12	19	lung
6	m	69	IIIA	Ad	T1N2M0	25	d	14	21	bone
7	f	62	IV	Ad	T2N2M1	50	d	0	39.8	lung
8	f	64	IIIA	Ad	T1N2M0	42	d	3.3	32.3	brain
9	f	51	IV	Ad	T2N2M1	70	a	1.1	15.2	
10	m	67	IIIA	Sq	T1N2M0	66	a	4.8	18	
11	m	70	IIIA	Ad	T1N2M0	61	a	0.46	37	bone
12	m	71	IIIA	Ad	T2N2M0	34	d	13.3	26.2	lung
13	f	73	IIIA	Ad	T3N2M0	63	a	16	58.2	
14	m	48	IIIA	Ad	T1N2M0	36	a	39	38	
15	m	61	IIIA	Sq	T2N2M0	70	a	2	50.2	
16	m	52	IIIA	Sq	T3N2M0	78	a	0	12.5	
17	f	49	IIIB	Ad	T4N2M0	23	a	21.7	13.2	brain
18	f	58	IIIA	Ad	T2N2M0	28	a	31.7	4.6	bone
19	m	67	IIIA	Sq	T3N2M0	63	a	185	36.6	
20	m	57	IIIA	Sq	T2N2M0	20	a	64.2	0	lymph
21	m	67	IIIA	La	T2N2M0	36	a	78	0	
22	f	64	IIIB	Ad	T4N2M0	48	a	12.9	40.7	lung
23	f	51	IV	Ad	T4N2M1	36	a	25.7	0	
24	m	75	IIIA	Ad	T2N2M0	16	a	21.2	0	lung,
25	m	69	IIIA	Ad	T1N2M0	29	a	77.9	0	
26	f	63	IIIA	Ad	T2N2M0	27	a	81.3	0	brain
27	f	68	IIIB	Ad	T3N3M0	34	d	1.2	46.6	lung
28	m	54	IIIB	Ad	T2N3M0	45	a	109.9	0	

Gender: m, male; f, female. Histology: Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma. Month: month after surgery or induction chemotherapy. Prognosis: a, alive; d, dead. TDLN, TDLN-Pb: total number of cells transferred ($x10^9$).Pb-LAK: total number of cells transferred ($x10^9$). Rec: lung, recurrence to lung; brain, recurrence to brain; bone, recurrence to bone; lymph, recurrence to lymph nodes.

immunotherapy were $7.07x10^9$ cells (TDLN-Pb) and $3.71x10^9$ cells (Pb-LAK). Details of the cases and the number of cells transferred are listed in Table I. The mean number of immunotherapy courses for a patient was 10.6 (2-18) and the mean number of cells transferred for a patient was $53.4x10^9$ (12.5-221.6).

Toxicity and side effects of immunotherapy. There was no case with side effects induced by bacterial, viral or tumoral contamination in the preparation of cells for immunotherapy. Chills occurred (83.4% , 261/313), starting 15-30 min after the transfer of the cells and lasting for 30-60 min, and fever (>38.5˚C; 78.0% ; 244/313) starting 2 or 3 hours after the transfer and lasting for 12 hours. Most cases of discomfort, such as arthralgia (15.0% ; 47/313), fatigue (23.0% ; 72/313), nausea (17.0% ; 53/313) and fever, ceased by the next afternoon. There was one case of liver dysfunction in which transaminase increased two weeks after the chemo-immunotherapy. The correlation between the immunotherapy and the liver dysfunction was not clear because the other transfer of cells did not induce liver dysfunction in the same patient. A patient with brain metastasis experienced severe headache and nausea for 2-3 hours after intra-arterial infusion of $7.6\text{-}9.0x10^9$ cells. There was no correlation between the number of cells transferred ($1\text{-}20x10^9$ cells) and the side-effects.

Prognosis. Recurrence: The median follow-up time was 59.6 months after surgery. There were 17 cases of recurrence (9 lung, 4 brain, 3 bone and one mediastinal lymph node), but two of the brain metastasis cases and one bone and one lung metastasis case were treated successfully by irradiation, surgical resection and immunotherapy to attain tumor-free status with long-term survival. Survival rate: The two- and 5-year survival rates of all the cases were 88.9% (95.9-81.9, 95% confidence interval, C.I.) and 52.9% (76.4-29.0, 95% C.I) (Figure 2). The 2-year survival rates of the stage IIIA and IIIB, IV groups were 85.0% (98.5-71.5, 95% C.I.) and 100% respectively. The 5-year survival rates of those groups were 53.2% (79.4-27.0, 95% C.I.) and 41.7% , respectively (Figure 3).

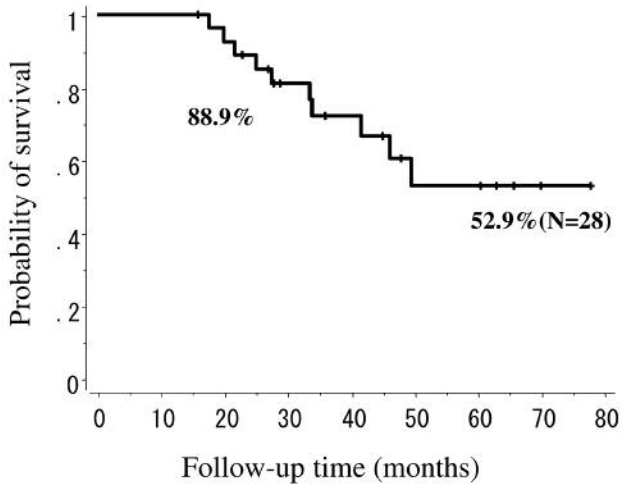

Figure 2. *Survival pattern of all the cases. The solid line indicates survival of the immunotherapy cases after surgery.*

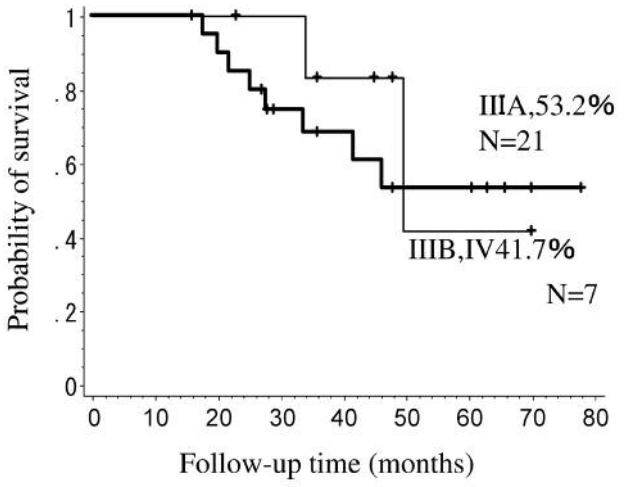

Figure 3. *Survival pattern and pathological stages. The bold line indicates survival of stage IIIA cases, and the fine line, survival of the IIIB and IV cases.*

Numbers of cells transferred and survival. When the total numbers of cells transferred for a patient were compared, the 5-year survival rate of the group that received more than $5x10^{10}$ cells was 80.8% (100-45.2, 95% C.I.), while that of those who received less was 38.5% (66.1-10.9, 95% C.I.) (Figure 4).

Numbers of AKT+DC cells from TDLN or TDLN-Pb and survival. The prognoses of the patients who received more than $2x10^{10}$ cells from TDLN or TDLN-Pb and less than that were compared. The 2- and 5-year survival rates of the former group were 90% (100-71.4, 95% C.I.), and those of

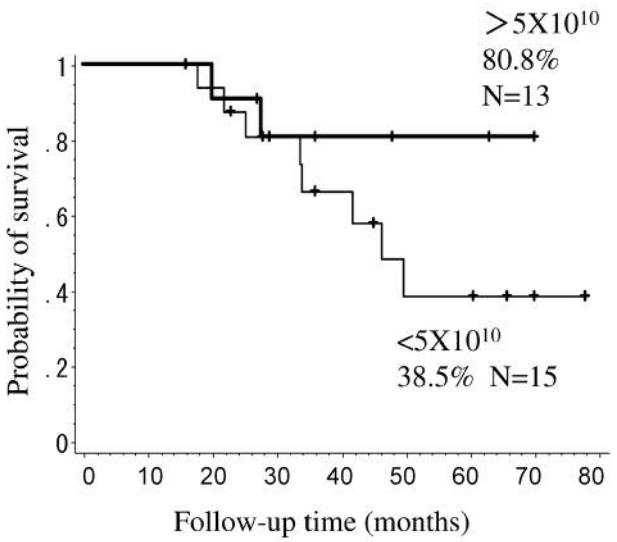

Figure 4. *Survival pattern and the total number of cells transferred per patient. The bold line indicates the survival of the patients who received more than $5x10^{10}$ cells and fine line indicates the survival of the patients who received less than that.*

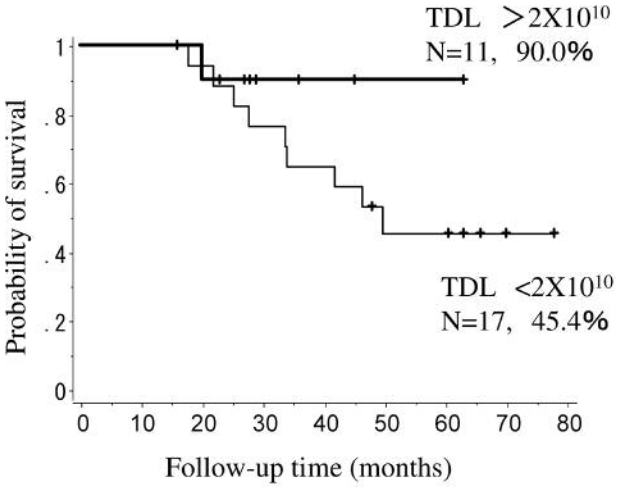

Figure 5. *Survival pattern and total number of cells from TDLN or TDLN-Pb. The bold line indicates the survival of the cases in which more than $2x10^{10}$ cells of TDLN or TDLN-Pb cells were administered, and the fine line, the survival of the patients who received less than that.*

the latter group were 88.2% and 45.4% (69.1-21.7, 95% C.I.), respectively (Figure 5).

Immunotherapy for cases of recurrence. Of the patients treated by immunotherapy, some had recurrence after surgery. In such cases, the interval or route of cell transfer was changed.

Case 1: A 75-year-old man with pT2N2M0 adenocarcinoma underwent surgery after 2 courses of induction chemotherapy (CBDCA+ paclitaxel). He received 2 courses of chemo-immunotherapy, but had a lung recurrence 5 months after the surgery. The interval of immunotherapy was changed from every 2 months to every month and the number of cells was increased. When a total of $10.7x10^9$ cells from TDLN-Pb was transferred, the recurrent tumor in the lung shrank in size (Figure 6), and scar formation continued until the patient died of cerebral infarction.

Case 2: A 63-year-old woman with pT2N2M0 adenocarcinoma had surgery after 2 courses of induction chemotherapy. Brain metastasis was found 3 months after surgery. The route of cell transfer was changed from intravenous injection to intra-arterial injection through a subcutaneous Port-a-Cath catheter indwelling in the ascending aorta from the left subclavian artery. Brain metastasis was controlled after she received irradiation and $4x10^9$ cells of TDLN-Pb (Figure 7). She underwent surgery to remove a frontal lobe metastasis, and a pathological examination revealed that the tumor tissue with extensive necrosis was heavily infiltrated by lymphocytes (Figure 8a), especially in the peri-arterial regions. Immuno-histochemical examination revealed that most of the cells that had infiltrated the site were CD3-positive T cells (Figure 8b,c). The patient continued to maintain tumor-free status for 24 months after successful cerebral irradiation, surgery and immunotherapy.

Discussion

Immunotherapy using effector cells has been widely used for cancer patients in the last two decades (21-23), but has failed to obtain public support as a standard therapy for cancer patients. This is because the effect has been limited and the responses to the immunotherapy were determined by the same methods as those for chemotherapy or radiation therapy. Size reduction has been rare and most immunotherapy has been used for terminal stage patients with no residual immune response who had failed to improve with other conventional treatments. We have previously employed adoptive immunotherapy using LAK cells as an adjuvant for surgery to prevent recurrence and have reported the efficacy of this therapy for stage II and IIIA lung cancer patients (15). In the present study, a new method of effector cell and dendritic cell production from regional lymph nodes was used to deal with the residual occult micro-metastases in post-surgical N2 lung cancer patients.

Immune responses against tumor antigens initially occur in the first tumor draining lymph nodes, the sentinel nodes (24). Effective induction of immune responses can take place only in these secondary lymphoid organs where cell-to-cell interactions are properly guided and cells can meet in an appropriate cytokine-enriched microenvironment (24). At an early stage in the vast majority of carcinomas, TDLN are the primary sites where the specific recognition of tumor antigens and proper activation of the immune system are initiated (24-27). DCs are the specialized antigen-presenting cells of the immune system. To induce an effective immune response, these cells should not only express high levels of major histocompatibility complex (MHC) and co-stimulatory molecules, but should also migrate into the lymph nodes to interact with naive T cells.

We have succeeded in obtaining satisfactory numbers of specific activated killer cells and dendritic cells from the tissue culture of regional lymph nodes (16). The activities of activated killer cells, and cell surface markers as well as the specificities of these killer cells obtained after long term tissue culture of regional lymph nodes of lung cancer patients were tested. The TDLN of lung cancer patients generated killer cells specific to autologous tumor cells when cultured with low-dose IL-2. The majority of the cells from TDLN or TDLN + PBL co-cultures (TDLN-Pb) were CD3-positive T cells (89-99%), and a ^{51}Cr-releasing assay showed that these cells had a strong cytotoxic activity against autologous tumor cells. These results indicated that the killing activity against autologous tumor cells was mediated by cytotoxic killer T cells specific to autologous tumor cells. With this new technique, consistently adequate numbers ($3\text{-}10x10^9$) of specific killer cells and DCs were obtained for each course of immunotherapy every 2 months for 1 to 2 years. When the TDLN stopped generating killer cells or was lost by bacterial contamination, lymphokine-activated killer cells (Pb-LAK) from peripheral blood lymphocytes cultured in IL2 were used.

In the present phase II study, the 2-and 5-year survival rates of the patients were 88.9% and 52.9% while those of N2 patients with tumors of the same clinical stages who underwent surgery and chemotherapy over the same period were 58.4% and 12.5%, respectively. Since this was not a randomized study, it is of no use to compare the survival of the two groups, but the prognoses of the selected N2 patients were comparable to those achieved with chemotherapy (1-6), and immunotherapy using dendritic cells and activated killer cells may be effective for post-surgical lung cancer patients. Furthermore, the prognoses of the patients who received more than $5x10^{10}$ cells were better than those of patients who received less, indicating that the effect is dependent on the number of cells administered.

In the present phase II study, chemo-immunotherapy using killer cells and DCs from the regional lymph nodes of lung cancer patients was a feasible and safe approach. Large-scale phase III studies of this immunotherapy will be necessary before it can be brought into general use.

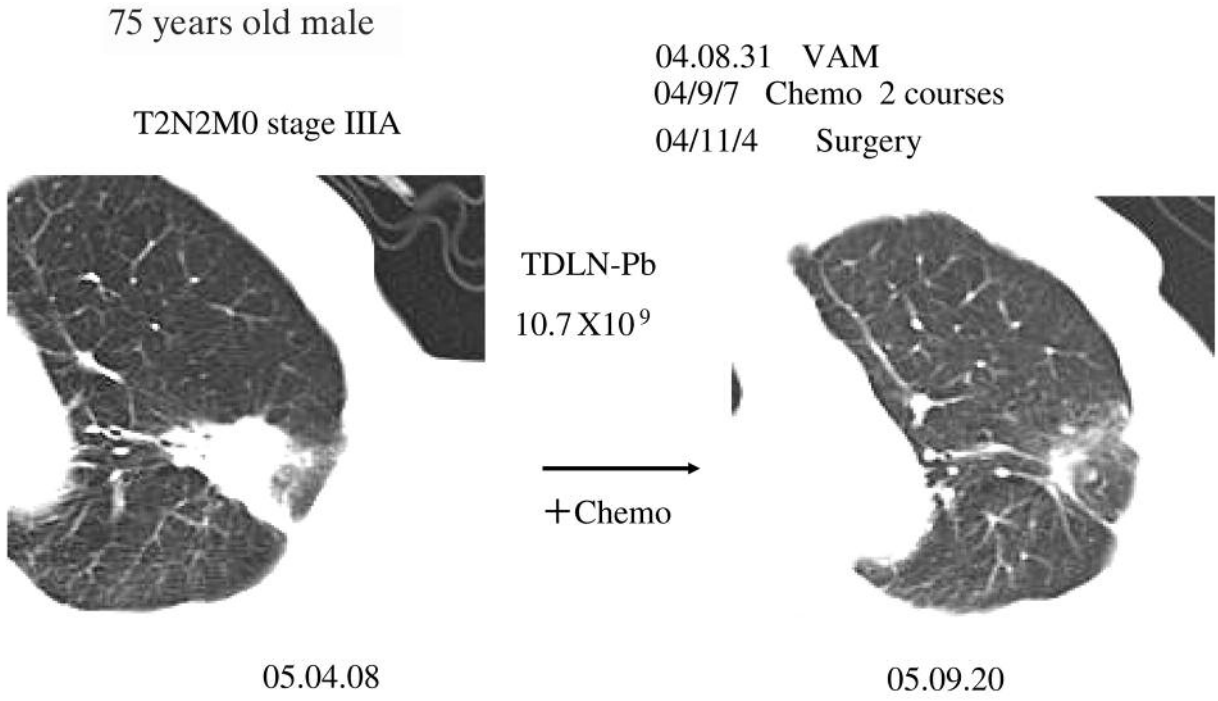

Figure 6. *Chest CT findings in a 75-year-old man (case 1). This stage IIIA T2N2 M0 adenocarcinoma patient had surgery on Nov. 4 2004 after 2 courses of CBDCA+paclitaxel chemotherapy. A lung metastasis was detected in April 2005 by chest CT (left). He received 2 courses of chemotherapy and 10.7x10^9 TDLN-Pb cells. The recurrent tumor in the lung shrank in size (right: Sep 20 2005) and scar formation continued until he died of cerebral infarction.*

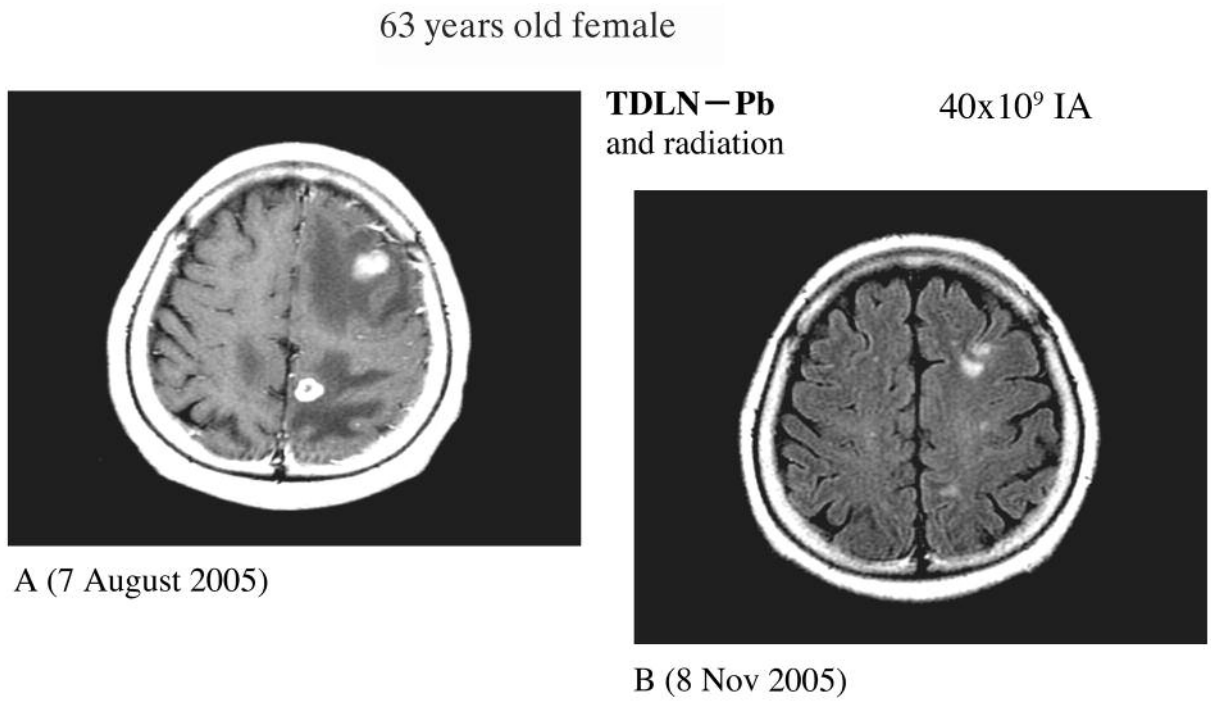

Figure 7. *Brain CT findings in a 63-year-old woman (case 2).The patient with pT2N2M0 adenocarcinoma, had surgery after 2 courses of induction chemotherapy. Brain metastasis was found 3 months after surgery (A: August 7 2005). Brain metastasis was controlled after she received irradiation and 4x10^9 TDL-Pb cells (B: Nov. 8 2005).*

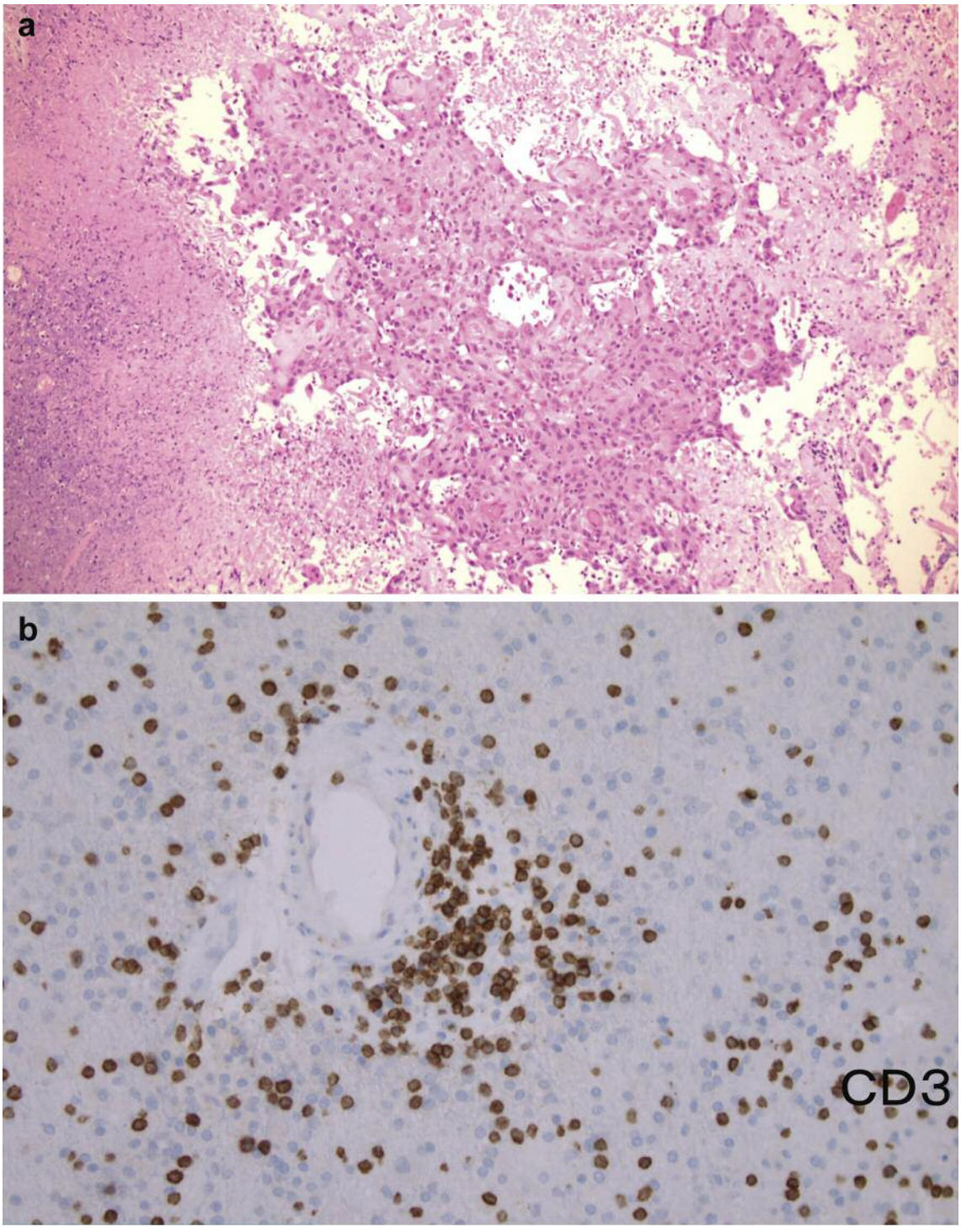

Figure 8. *continued*

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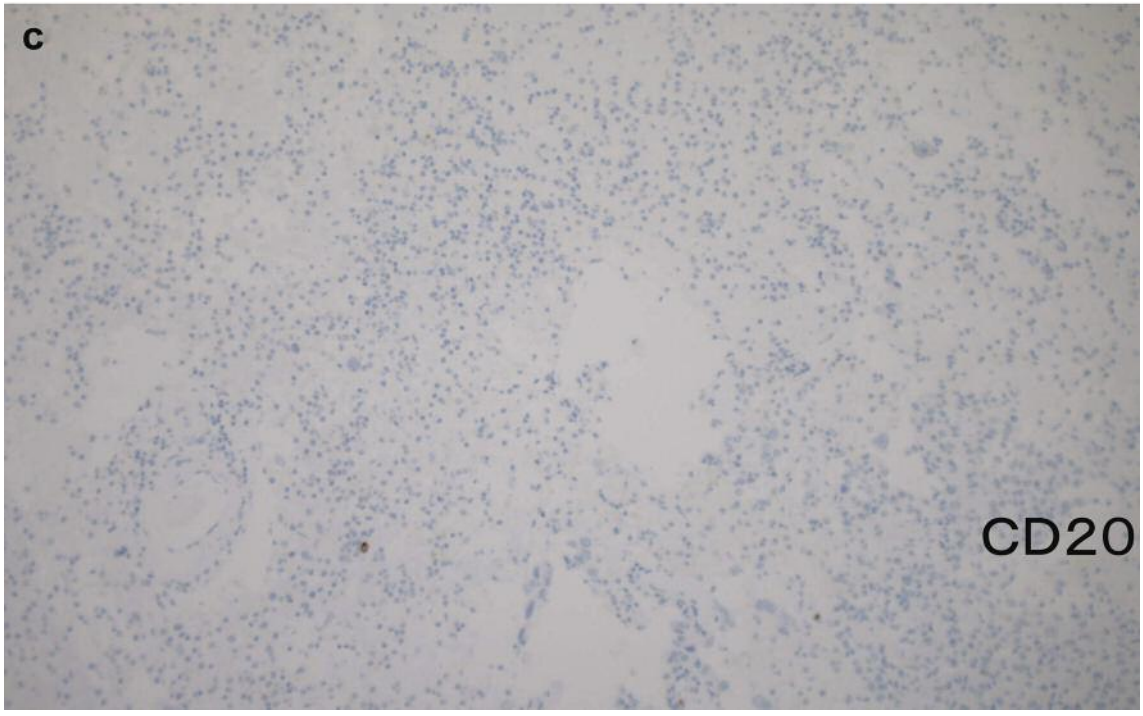

Figure 8. *Histological finding of brain metastasis of case 2. Tumor cells with extensive necrosis are heavily infiltrated by lymphocytes, especially in the peri-arterial region (8a, b). Immuno-histochemical examination revealed that most of the cells that had infiltrated into the site were CD3-positive T cells (8b). No CD20-positive lymphocytes (B cells) were present (8c).*

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