Case Report

Immunotherapy with lymphocytes derived from banked tumor tissue in two refractory NSCLC patients with leptomeningeal metastases: a report of two cases

Ai-Hong Zheng[1,2#], Chong Yu[2#], Yong-Rui Su[3], Min Peng[4], Jian-Yuan Chen[3], Fu-Wei Wang[2], Xiu-Ming Zhu[1], Pei-Yuan Yan[1], Hai-Tao Wang[5], Jian Shen[5], Wei-Jun Chen[6], Qiang Li[6], Yuan Chen[7], Yi Chen[7], Yin-Shuang Wang[8], Hang-Yu Gu[1], Zhuo-Nan Meng[3], Jing-Wen Zhao[9], Wan-Mao Ni[7], Tian-Hua Wang[10], Sheng-Lian Wu[10], Min Li[11], Zheng Wang[11], Ai-Ping Cheng[12], Xiao-Xian Huang[13], Zi-Yan Yang[1], Jia-Hong Jiang[1], Qun-Jiang Wang[1], Jing Qu[1], Shi-Tai Zhang[3], Ke-Ke Shi[1], Hua-Xin Zhang[1], Da-Hong Zhang[2], Guo-Qing Wu[1,2]

[1]Cancer Center, Department of Medical Oncology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [2]Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [3]The Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, China; [4]Department of Hospital Infection Management and Preventive Medicine, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [5]Cancer Center, Department of Cardio-Thoracic Surgery, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [6]Cancer Center, Department of Radiation Oncology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [7]Cancer Center, Department of Pathology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [8]Department of Ultrasound Medicine, the First Affiliated Hospital of Ningbo University, Ningbo, China; [9]Department of Pathology, Jinhua Municipal Central Hospital, Jinhua, China; [10]Cancer Center, Department of Neurosurgery, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [11]Cancer Center, Gamma Knife Treatment Center, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [12]Cancer Center, Department of Nuclear Medicine, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China; [13]Cancer Center, Department of Hematology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China

Contributions: (I) Conception and design: GQ Wu, DH Zhang, AH Zheng, C Yu; (II) Administrative support: GQ Wu, DH Zhang, AH Zheng, M Peng; (III) Provision of study materials or patients: GQ Wu, AH Zheng; (IV) Collection and assembly of data: GQ Wu, DH Zhang, AH Zheng, C Yu, YR Su, JY Chen, FW Wang, PY Yan, HT Wang, TH Wang, M Li, AP Cheng, WM Ni; (V) Data analysis and interpretation: C Yu, YR Su, ST Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Guo-Qing Wu, MD, PhD. Cancer Center, Department of Medical Oncology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, No. 158, Shangtang Road, Hangzhou 310000, China; Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China. Email: gqwuzsu@hotmail.com; Da-Hong Zhang, MD. Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, No. 158, Shangtang Road, Hangzhou 310000, China. Email: zhangdahong88@126.com.

Background: Cancer patients relapsing with leptomeningeal metastases (LM) but without extracranial lesions are usually unsuitable for cellular immunotherapy with tumor-infiltrating lymphocytes (TILs) owing to lack of tumor tissue. TILs generated from heavily pretreated patients, especially those with non-melanoma cancer often have anergic effects and are less toxic to tumors, limiting the antitumor efficacy of lymphocyte-based therapy. Whether using autologous tumor tissue banked in advance addresses the dilemma has not been explored.

Case Description: We present two cases of non-small cell lung cancer (NSCLC) who relapsed with LM but without extracranial lesions for whom TIL therapy is otherwise unsuitable. Using autologous tumor tissue banked in advance when they initially underwent tumor resection, we successfully generated

therapeutic TILs of which the enhancer of zeste homolog 2 (EZH2) activity was further inhibited in regulatory T cells (Tregs). One case received autologous TILs prepared from a cryopreserved pathological complete response lesion and achieved a complete remission of LM that was ongoing till the preparation of this manuscript. The other case was treated with autologous TILs derived from a cryopreserved treatment-naïve tumor tissue and only achieved a transient response manifested by short-term decrease of circulating tumor deoxyribonucleic acid and serum carcinoembryonic antigen.

Conclusions: TILs generated from treatment-responsive lesions and underwent inhibition of EZH2 activity in Tregs have high antitumor efficacy and the banking in advance of treatment-responsive tumor tissue potentially provides a safe and effective adoptive cell therapy (ACT) with TILs for refractory NSCLC patients with LM for whom TIL therapy is otherwise unsuitable.

Keywords: Tumor-infiltrating lymphocyte; non-small cell lung cancer (NSCLC); leptomeningeal metastases; adoptive cell therapy (ACT); case report

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Introduction

The incidence of leptomeningeal metastases (LM) in advanced non-small cell lung cancer (NSCLC) has been reported to be 3–5% and has increased over the past 10 years (1). However, the actual incidence is likely underestimated (2).

Despite the application of molecularly targeted treatments and immune checkpoint inhibitors, the standard of care for LM remains to be established (2,3). The median overall survival (mOS) of NSCLC patients with LM is 4.2–8.1 months (4,5). Although some NSCLC patients treated with third-generation epidermal growth factor receptor-tyrosine kinase inhibitors were reported to have a longer mOS of 17.0 months, those without a targetable mutation have much shorter survival times (4).

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TILs) is a promising immunotherapy for cancer, but tumor tissues need to be obtained from patients at the refractory stage of disease, which makes this approach inappropriate for LM patients without extracranial solid lesions. In addition, TILs generated from heavily pretreated patients often have anergic effects and are less toxic to tumors especially in patients with non-melanoma cancers (6), which limits the antitumor efficacy of lymphocyte-based therapy. Induction chemotherapy and/or immunotherapy significantly promote lymphocyte infiltration in responders (7,8). Hence, we presume that tumor tissues harvested in advance from patients who respond to induction systemic therapies contain highly active and toxic TILs that could be prepared for ACT in refractory NSCLC patients with LM, even those without measurable lesions for whom TIL therapy is otherwise unsuitable.

In addition, regulatory T cells (Tregs) mediate immune suppression, and enhancer of zeste homolog 2 (EZH2) is extensively involved in the regulation of cell activity, including Tregs (9). Immunotherapy by targeting Tregs is potentially an effective strategy against cancer (10). Our unpublished data suggested that TIL populations containing EZH2 activity-inhibited Tregs showed much stronger antitumor efficacy in both *in vitro* and *in vivo* studies.

Whether using autologous tumor tissue banked in advance to generate therapeutic TILs with EZH2 activity inhibited specifically in Tregs addresses the dilemma has not been explored. Here we report two cases with LM who received TILs prepared from banked tissue in a clinical trial at Zhejiang Provincial People's Hospital (www.chictr.org.cn, ChiCTR2100054429). We present this article in accordance with the CARE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2025-274/rc).

Case presentation

Case 1

A 59-year-old man (Patient ChiCTR-101) was diagnosed with squamous NSCLC in the upper lobe of the right lung at 57 years of age (*Figure 1A-1D*). A solitary nodule in the posterior horn of the right lateral ventricle was found during the initial evaluation (*Figure 1E*). The disease stage

was IVA (cT3N1M1b). In 2022, he underwent gamma knife radiosurgery (GKR) of the intracranial nodule and right superior lobectomy plus mediastinal lymph node dissection after two cycles of chemoimmunotherapy that contributed to sharp shrinkage of lung lesion (*Figure 1F*); the Tumor, Node, Metastasis (TNM) stage after neoadjuvant treatment was ypT0N0Mx. The primary lung lesion exhibiting a pathological complete response (pCR) was frozen in liquid nitrogen right after resection. The patient received two additional cycles of chemoimmunotherapy, followed by immunotherapy with antiangiogenic therapy. He was disease-free until April 2023 when he complained of headache; a diagnostic lumbar puncture cerebrospinal fluid (CSF) cytology detected tumor cells with elevated opening pressure of lumbar puncture (245 mm H_2O) that confirmed LM, after which ventriculoperitoneal shunt (VPS) and inline Ommaya reservoir implantation were performed. In addition, test for circulating tumor cells (CTCs) was positive.

In 2023, he participated in a clinical trial, during which TILs were generated from the above-mentioned cryopreserved (for 14 months) pCR lesion. In August 2023, the patient received a single infusion of 5×10^9 autologous TILs with EZH2 activity inhibited in regulatory T cells that consisted mainly of $CD4^+$ T cells (*Figure 2A*), of which more than 80% were central or effector memory cells (*Figure 2B*). Compared with their peripheral counterparts, the infused immune cells expressed higher levels of proinflammatory and antitumor cytokines (*Figure 2C*).

T-cell receptor (TCR) clones were detected in 70% of the infusion product, which was much greater than the percentage detected in pre-infusion peripheral blood mononuclear cells (PBMCs) (*Figure 2D*). The top three TCR clones constitute approximately 27% of all the functional TCR clones detected in the manufactured TILs (*Figure 2E* and *Table 1*), and T cells expressing these TCR clones, especially clonotype 3, exhibit a proinflammatory phenotype (*Figure 2F*).

Following the infusion of TILs, the patient experienced resolution of the headache and normalization of CSF pressure even after percutaneous drainage tube removal (*Figure 3A*). The response was sustained at the latest follow-up, 20 months after TIL infusion.

CSF cytology was performed before TIL infusion (*Figure 3B* top) and repeated every 20 to 30 days after cell infusion; the CSF was negative for tumor cells as quickly as 20 days post-TIL infusion and thereafter (*Figure 3B* bottom).

CTC analysis showed 5 and 3 CTCs per milliliter at 122 and 3 days before TIL infusion, respectively. Surprisingly, the CTC count decreased gradually after the infusion of TILs, with 3, 2, and 1 CTCs per milliliter at 20, 47, and 75 days after cell infusion, respectively. From 89 days after TIL transfer, no CTCs were detected (*Figure 3C*).

At 19 days post-TIL infusion, the absolute number of immune cells in the peripheral blood markedly increased. The numbers of Th1, Th2 and Th17, and $CD4^+$ T cells increased after TIL infusion (*Figure 4A*,*4B*). Similarly, the numbers of naïve $CD8^+$ and effector $CD8^+$ T cells slightly increased from baseline, whereas those of central memory and effector memory $CD8^+$ T cells dramatically increased after TIL infusion (*Figure 4C*). TIL infusion also increased the number of natural killer (NK) cells (*Figure 4D*).

Highlight box

Key findings

- A banked treatment-responsive lung lesion-derived tumor-infiltrating lymphocytes (TILs) with enhancer of zeste homolog 2 activity inhibited in Tregs induced remission in a patient with leptomeningeal metastases (LM) only for whom TILs are otherwise unavailable.
- It's feasible to generate therapeutic TILs from long-time cryopreserved treatment-responsive tumor tissue, which enables more refractory patients to benefit from adoptive cell therapy (ACT) with TILs.

What is known and what is new?

- The Food and Drug Administration (FDA) approved lifileucel for the treatment of unresectable or metastatic melanoma previously treated with a programmed death 1 blocking antibody, and BRAF inhibitor with or without mitogen-activated protein inhibitor if BRAF V600 mutation positive. TILs have shown antitumor efficacy in and are potentially an effective treatment for advanced patients with solid tumors beyond melanoma. Currently, autologous tumor tissue has to be obtained from heavily pretreated patients who are planning TIL therapy, which makes those who developed LM only but without extracranial lesions unsuitable for ACT with TILs owing to lack of tumor tissue.
- Banking tumor tissues in advance of disease relapse so that TIL therapy can be applied to more patients who have advanced or metastatic diseases and become resistant to standard therapies. TILs prepared from treatment-responsive lesions are potentially an effective treatment for refractory non-small cell lung cancer patients with LM.

What is the implication, and what should change now?

- Our cases imply the significance of banking treatment-responsive tumor tissue in advance of disease recurrence to facilitate ACT with TILs. Clinical studies are urgently warranted to substantiate this strategy of TIL therapy.

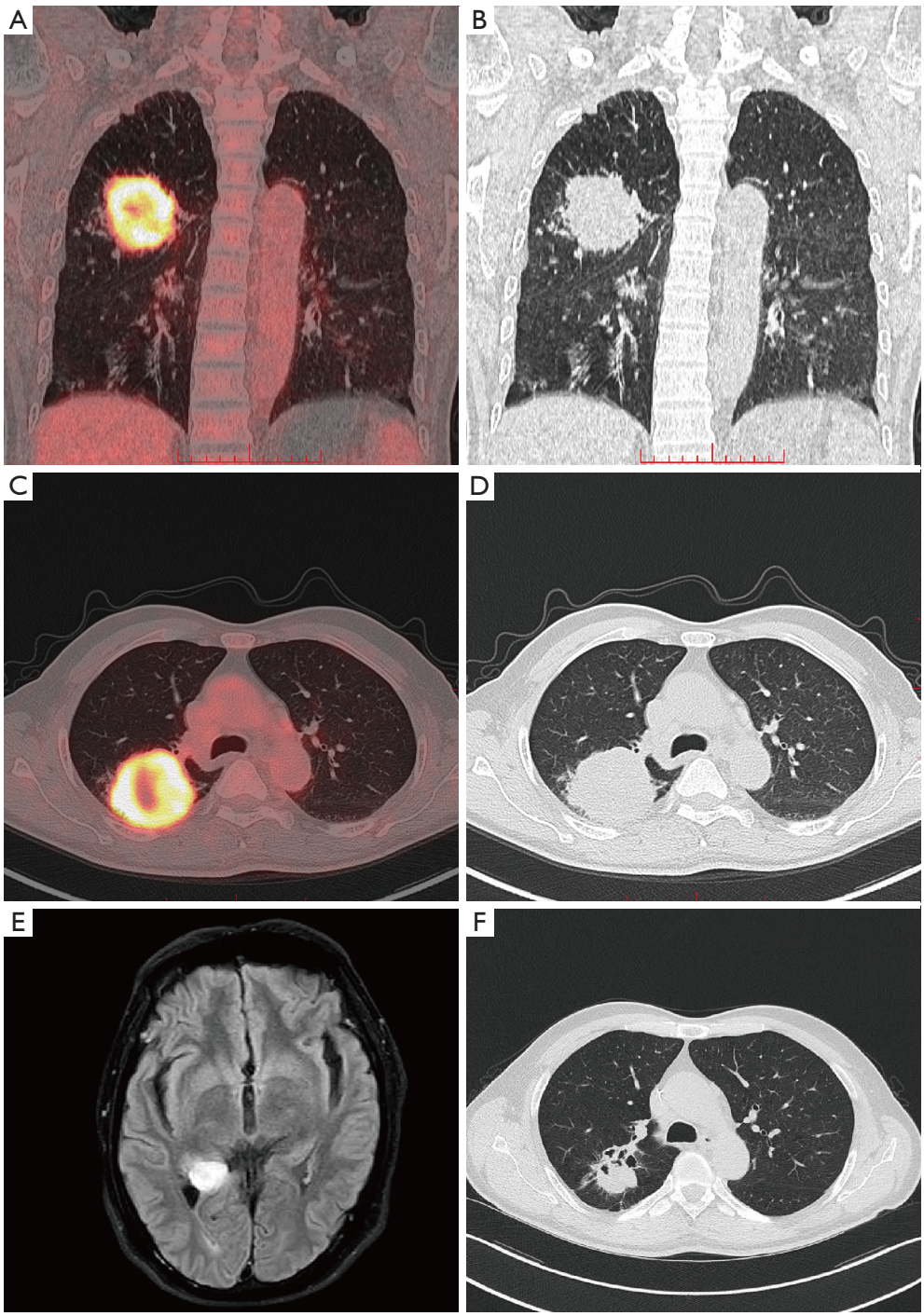

Figure 1 Lung and intracranial lesions in Patient ChiCTR-101. (A-D) Positron emission tomography/computed tomography imaging of pulmonary lesion upon diagnosis. (E) The intracranial lesion determined by fluid attenuated inversion recovery sequence of magnetic resonance imaging. (F) The decreased pulmonary lesion after two cycles of systemic therapy.

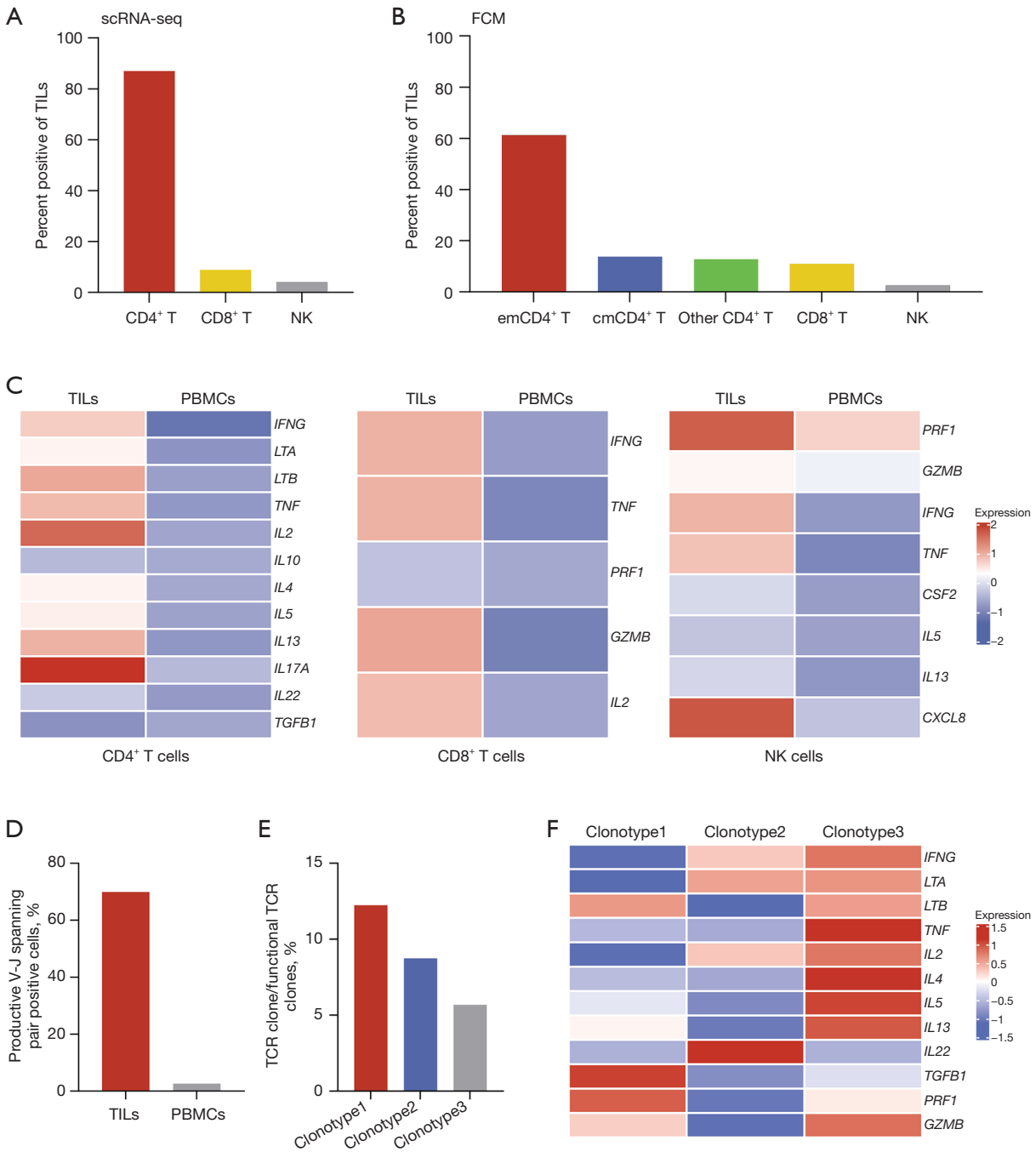

Figure 2 Characterization of the infusion product in Patient ChiCTR-101. (A) The proportions of CD4$^+$ T, CD8$^+$ T, and NK cells among the manufactured bulk TILs as measured by scRNA-seq. (B) FCM showed that CD4$^+$ T cells in manufactured TILs mainly consist of effector and central memory phenotypes. (C) The differential expression of selected cytokine and chemokine genes in immune cells derived from manufactured TILs and paired PBMCs. (D) The percentage of cells identified with productive V-J spanning pairs of TCRs among manufactured TILs and paired PBMCs. (E) The proportions of the top 3 clonotypes of all the TCR clones with productive V-J spanning pairs among the manufactured TILs. (F) The expression levels of selected key cytokine genes in T cells with the top 3 clonotypes. cmCD4$^+$ T, central memory CD4$^+$ T cells; emCD4$^+$ T, effector memory CD4$^+$ T cells; FCM, flow cytometry; NK, natural killer; PBMCs, peripheral blood mononuclear cells; scRNA-seq, single-cell RNA sequencing; TCRs, T-cell receptors; TILs, tumor-infiltrating lymphocytes.

Table 1 Details of top 15 TCR clones in manufactured TILs

Clonotype ID	Frequency	Proportion	CDR3 AA
Clonotype1	612	12.235	TRA:CAATNQAGTALIF;TRB:CASSLSLSEAFF
Clonotype2	437	8.737	TRA:CAVREGSYQLTF;TRB:CASSHKQGGGEKLFF
Clonotype3	284	5.678	TRA:CALSVSGGYQKVTF;TRB:CASSLQTGTDTQYF
Clonotype4	242	4.838	TRB:CASSLSLSEAFF;TRA:CAATNQAGTALIF;TRA:CVVSDHRPGAGSYQLTF
Clonotype5	208	4.158	TRA:CATDNNNDMRF;TRB:CASSLGPGYGSPLHF
Clonotype6	204	4.078	TRB:CASSWSDTQYF;TRA:CAMSANFQGGSEKLVF
Clonotype7	179	3.579	TRA:CAANDYKLSF;TRB:CASSYSADSPLHF
Clonotype8	166	3.319	TRB:CASTNPGTGELFF;TRA:CAVNALTWGNTGKLIF
Clonotype9	165	3.299	TRB:CASSQDRGRTLTDTQYF;TRA:CAGATGANSKLTF
Clonotype10	128	2.559	TRB:CASSETLAGVADTQYF;TRA:CAFIGNNNDMRF
Clonotype11	98	1.959	TRB:CASEWENEQFF;TRA:CLAVFSGGYNKLIF
Clonotype12	85	1.699	TRB:CASSLAGTAADTQYF;TRA:CAENEGGRDDKIIF
Clonotype13	84	1.679	TRA:CAFMRAAGNMLTF;TRB:CASSRHYEQYF
Clonotype14	78	1.559	TRB:CAWSVASYGYTF;TRA:CAVDVSASGYALNF
Clonotype15	64	1.279	TRA:CAVRSSNTGKLIF;TRB:CASRYRNVGYTEAFF

Frequency refers to the number of cells expressing the specified TCR clonal type. Proportion refers to the percentage of each clonotype. Only cells with productive V-J spanning (TRA, TRB) pair are included in the analysis. CDR3 AA, amino acid sequence of complementarity determining region; TCR, T cell receptor; TILs, tumor-infiltrating lymphocytes; TRA, T-cell receptor alpha-chain; TRB, T-cell receptor beta-chain.

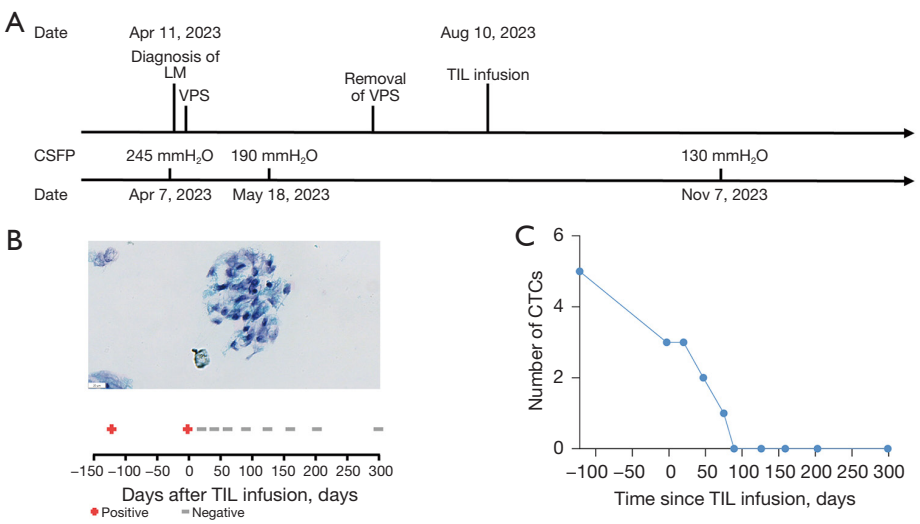

Figure 3 TIL infusion depleted tumor cells in both CSF and PB in Patient ChiCTR-101. (A) Timeline of treatments and CSF pressure. (B) Representative cancer cells stained with Papanicolaou method in CSF before the infusion of TILs (top) (scale bar 20 μM) and CSF cytology analysis results both pre- and post-cell infusion (bottom). (C) CTC evaluation in the PB. CSF, cerebrospinal fluid; CSFP, cerebrospinal fluid pressure; CTC, circulating tumor cell; LM, leptomeningeal metastases; PB, peripheral blood; TIL, tumor-infiltrating lymphocyte; VPS, ventriculoperitoneal shunt.

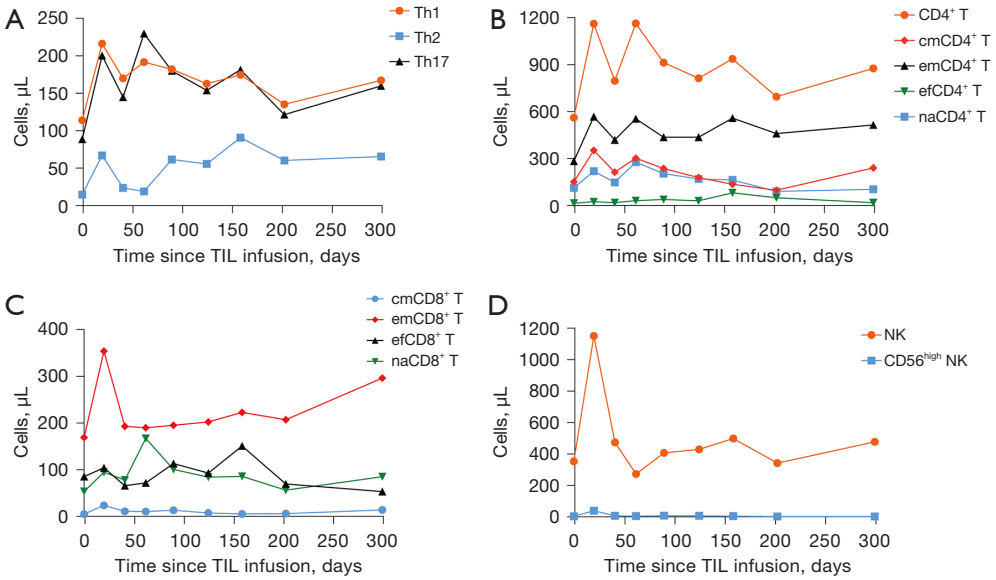

Figure 4 TIL infusion increased the numbers of immune cells in PB in Patient ChiCTR-101. (A) The numbers of Th1, Th2, and Th17 cells increased dramatically after cell infusion and the elevation of Th1 and Th17 cells was maintained during the follow-up. (B) The numbers of $CD4^+$, $cmCD4^+$, and $emCD4^+$ T cells increased dramatically after cell infusion and the level of effector memory $CD4^+$ T cells remained at a high plateau during the follow-up. (C) The number of $emCD8^+$ T cells increased dramatically after cell infusion and the level of $emCD8^+$ T cells stayed high during the follow-up. (D) The number of NK cells increased dramatically after cell infusion. $cmCD4^+$ T, central memory $CD4^+$ T cells; $cmCD8^+$ T, central memory $CD8^+$ T cells; $efCD4^+$ T, effector $CD4^+$ T cells; $efCD8^+$ T, effector $CD8^+$ T cells; $emCD4^+$ T, effector memory $CD4^+$ T cells; $emCD8^+$ T, effector memory $CD8^+$ T cells; $naCD4^+$ T, naïve $CD4^+$ T cells; $naCD8^+$ T, naïve $CD8^+$ T cells; NK, natural killer; PB, peripheral blood; Th, T helper; TIL, tumor-infiltrating lymphocyte.

Case 2

A 70-year-old man (Patient ChiCTR-102) underwent lobectomy plus mediastinal lymph node dissection without neoadjuvant therapy after being diagnosed with relapsed lung adenocarcinoma at 67 years of age (*Figure 5A*). The resected treatment-naïve tumor tissue was cryopreserved. The patient received 6 cycles of chemoimmunotherapy plus bevacizumab, followed by maintenance therapy first with pemetrexed plus bevacizumab combined with tislelizumab and then with bevacizumab and tislelizumab because of intolerance to pemetrexed. He developed LM (Figure S1) 27 months after lobectomy and received an infusion of 1.4×10^{10} autologous TILs that were prepared from the above-mentioned cryopreserved (for over 2 years) tumor tissue (*Figure 5B*). A marked increase of immune cells was shown 16 days after TIL infusion (*Figure 6A-6D*). Circulating tumor DNA (ctDNA) level decreased 36 days after cell infusion (*Figure 6E*). The level of the serum carcinoembryonic antigen (CEA) decreased 43 days after cell infusion (*Figure 6F*). However, both the CEA and ctDNA levels increased to even greater levels before infusion, as determined 66 days after cell infusion (*Figure 6E,6F*) and the patient died 6 months after receipt of the TIL therapy. Additional analysis of the immune cells both before and post cell infusion is provided in Figure S2.

Detailed methods are shown in Appendix 1.

All procedures performed in this study were in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Medical Ethics Committee of Zhejiang Provincial People's Hospital (No. 2021KY053). Written informed consent was obtained from the patients and their families for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

NSCLC patients who experienced pCR after neoadjuvant systemic therapy have much better event-free survival than those without pCR (11). However, data from prospective

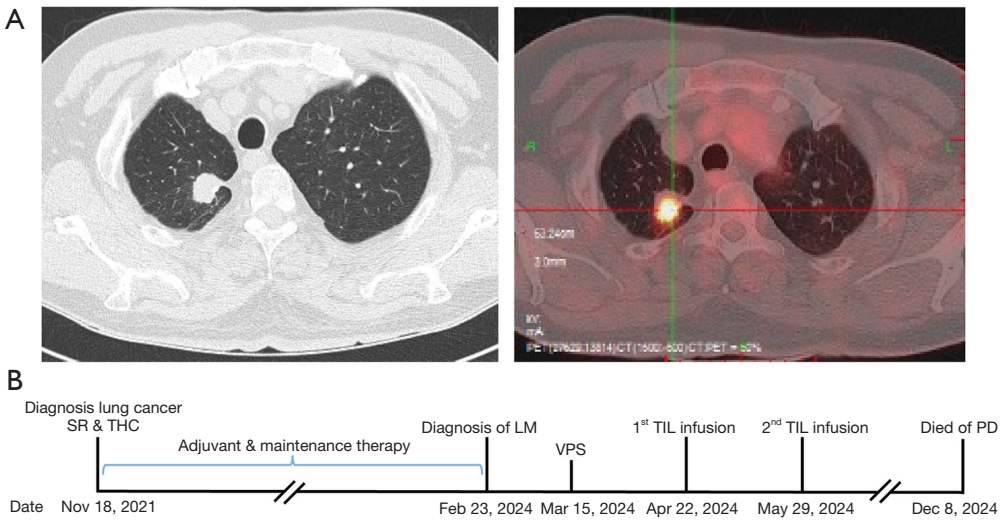

Figure 5 Pulmonary lesion and treatment overview for Patient ChiCTR-102. (A) PET/CT views of the pulmonary lesion in Patient ChiCTR-102. (B) Treatment overview for Patient ChiCTR-102. CT, computed tomography; LM, leptomeningeal metastasis; PD, progression disease; PET/CT, positron emission tomography/computed tomography; SR, surgical resection; THC, tissue handling and cryopreservation; TIL, tumor-infiltrating lymphocyte; VPS, ventriculoperitoneal shunt.

studies are currently lacking for the exact incidence of and mechanism underlying LM in NSCLC patients who achieved pCR during neoadjuvant.

We report a sustained response of a squamous NSCLC patient with LM who was treated with TILs derived from a treatment-responsive (pCR) lesion and a transient response of a lung adenocarcinoma patient with LM who received immunotherapy with TILs prepared from a treatment-naïve tumor tissue.

The first patient received TILs that consisted of $CD4^+$ T and $CD8^+$ T cells at an approximate 8:1 ratio. Although tumor rejection was previously attributed to cytotoxic $CD8^+$ T cells, $CD4^+$ T cells that target major histocompatibility complex class II epitopes on antigen-presenting cells have been recently considered to play a vital role in antitumor responses (12-14), which underscores the essential role of $CD4^+$ T cells in antitumor immunity.

The gold standard imaging method for the diagnosis of LM is cerebrospinal magnetic resonance imaging (MRI) (15). However, cerebrospinal MRI can be normal in patients with LM (16). A retrospective study of 171 lung cancer patients with LM suggested that 26% of patients had normal MRI at the time of LM presentation (5). Tumor cells were detected both in CSF (cytology) and peripheral blood (liquid biopsy) at the time of LM diagnosis in first case. Although the potential value of peripheral blood liquid biopsies in the diagnosis and management of LM has not been established (15), repeated evaluation of tumor cells in CSF during follow-up might help to estimate the response to treatments (17). The patient's CSF cytological analysis was negative for tumor cells at 19 days post-TIL infusion and in monthly and quarterly (from 6 months on) tests thereafter. In addition, peripheral blood liquid biopsy, which showed a conversion to negative results for CTCs starting 75 days after cell infusion, also suggested a response to TIL infusion.

Most patients who respond to TIL therapy received a single infusion of TILs at a dose above 5×10^{10} cells (18,19). However, the main challenge in developing effective TILs for cancer treatment may not be the number of bulk TILs but rather the number of tumor-reactive T cells with high toxicity toward cancer cells (20). Notably, the first case in current report received only a single dose of 5×10^{9} TILs, which is much lower than previously reported doses, possibly suggesting high tumor reactivity of the TILs in the pCR lesion.

The antitumor efficacy of immunotherapy and/or chemotherapy is at least partly dependent on immune cells. Chemotherapy increases TIL infiltration, the degree of which is correlated with the therapeutic response (21,22). Neoadjuvant chemoimmunotherapy markedly improved $CD8^+$ T-cell proliferation and activation, especially in patients with a major pathologic response and a pCR (7,8), which implies that tissues from lesions exhibiting a partial or complete response possibly contribute to easier and more

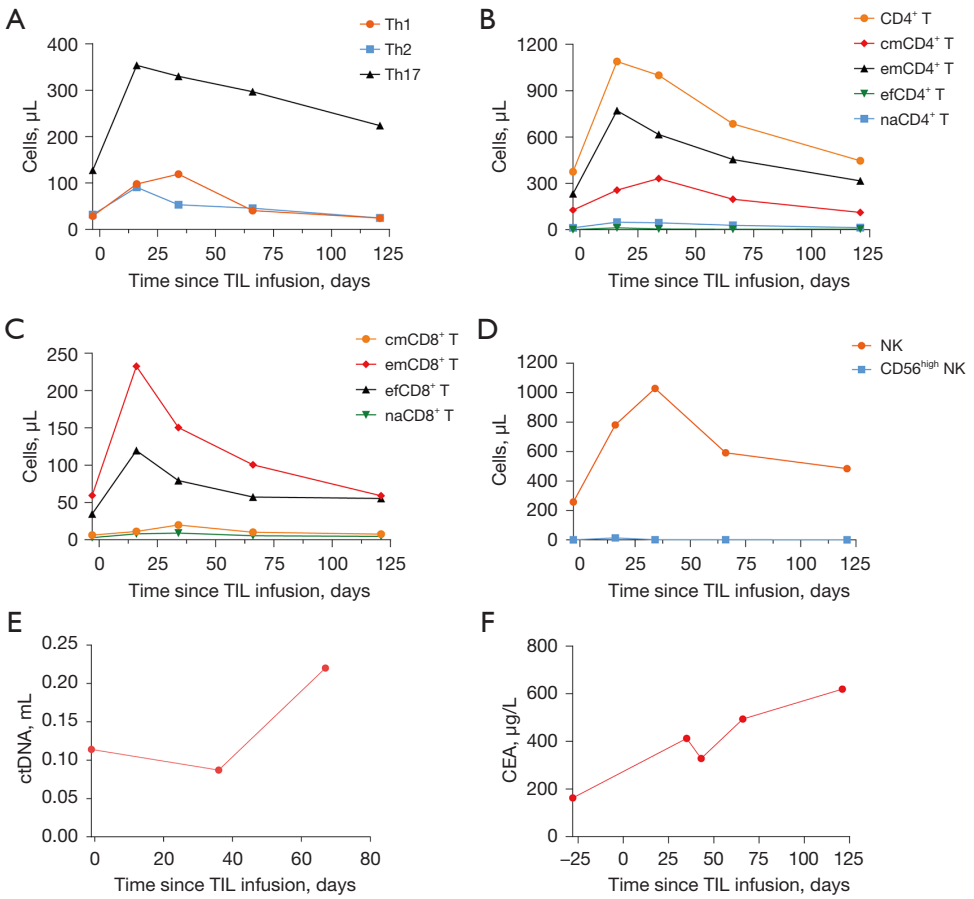

Figure 6 TIL infusion increased the numbers of immune cells and induced transient response in Patient ChiCTR-102. (A) The numbers of Th1, Th2, and Th17 cells increased dramatically after cell infusion and decreased gradually during the follow-up. (B) The numbers of CD4$^+$, cmCD4$^+$, and emCD4$^+$ T cells increased dramatically after cell infusion and decreased gradually during the follow-up. (C) The numbers of efCD8$^+$ T and emCD8$^+$ T cells increased dramatically after cell infusion and decreased gradually during the follow-up. (D) The number of NK cells increased dramatically after cell infusion. (E) The number of ctDNA per milliliter plasma in Patient ChiCTR-102. (F) The concentration of serous CEA in Patient ChiCTR-102. CEA, carcinoembryonic antigen; cmCD4$^+$ T, central memory CD4$^+$ T cells; cmCD8$^+$ T, central memory CD8$^+$ T cells; ctDNA, circulating tumor DNA; efCD4$^+$ T, effector CD4$^+$ T cells; efCD8$^+$ T, effector CD8$^+$ T cells; emCD4$^+$ T, effector memory CD4$^+$ T cells; emCD8$^+$ T, effector memory CD8$^+$ T cells; naCD4$^+$ T, naïve CD4$^+$ T cells; naCD8$^+$ T, naïve CD8$^+$ T cells; NK, natural killer; Th, T helper; TIL, tumor-infiltrating lymphocyte.

successful ACT. These findings prompted us to presume that neoplastic lesions that respond to systemic therapy serve as better sources of therapeutic TILs than resistant tumors that fail to respond to multiple lines of therapy.

Findings in our center indicate that Tregs may account for as many as 15% of TILs and that EZH2 maintains its stability as well as immune suppressive function (9), which justifies the inhibition of EZH2 in Tregs to enhance the antitumor effects of TILs. The long-term durability of the response in Case 1 strongly suggests that TILs generated from the pCR lesion cryopreserved in advance, with EZH2 inhibited specifically in regulatory T cells, can mediate the regression of LM in squamous NSCLC patients.

TIL infusion is commonly "sandwiched" with nonmyeloablative lymphodepleting chemotherapy and a high-dose bolus of interleukin 2 (IL-2). Nevertheless, serious adverse events, including life-threatening toxicities associated with nonmyeloablative lymphodepleting preconditioning, have been reported (7,23), which presents challenges for some advanced-stage cancer patients who have already undergone multiple lines of therapy. The toxicities related to high-dose IL-2 after cell infusion limit

the implementation of ACT with TILs, and the infusion of TILs followed by attenuated, even very low-dose, IL-2 has been reported to successfully induce disease remission (8,24). Recently, patients with advanced solid tumors who did not undergo lymphodepletion were reported to respond better to ACT with neoantigen-specific T cells than those who received higher-intensity preconditioning chemotherapy (25). In addition, the breakthrough of immune checkpoint inhibitors without IL-2 in solid tumors inspired us to reason that nonmyeloablative chemotherapy and high-dose IL-2 are not indispensable for ACT with TILs as long as the infused cells are sufficiently strong and cytotoxic against tumor cells. Consequently, nonmyeloablative lymphodepleting preconditioning chemotherapy was skipped in light of the risk of inducing an infection, which had resolved a few days before the indicated infusion date and low-dose IL-2 was administered following TIL infusion in Case 1.

Owing to a long duration from the initial radical excision (tissue banking) to the enrollment when patients experience relapse and become resistant to standard therapies, most candidates do not have banked treatment-naïve or -responsive lesions, which contributes to the major limitation of this study as small number of participants.

Conclusions

These results argue the reasonability of TIL therapy by leveraging autologous banked treatment-responsive tumor tissue obtained at patients' initial surgery, which potentially provides a safe and effective ACT for refractory NSCLC patients with LM for whom TIL therapy is otherwise unsuitable. However, prospective studies are warranted to evaluate this new modality of ACT with TILs.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2025-274/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Medical Ethics Committee of Zhejiang Provincial People's Hospital (No. 2021KY053). Written informed consent was obtained from the patients and their families for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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