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T Cell-Mediated Immunotherapy Boosts Clinical Efficacy in the Treatment

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Abstract

To investigate the anti-tumor efficacy and safety of adoptive cell therapy for the treatment of relapsed, refractory or chemotherapy-resistant lymphoma, we conducted a pilot clinical study using autologous anti-CD3 expanded T cells derived from patients with the diseases. A total of 12 patients, who were pathologically diagnosed with malignant lymphoma at various stages were enrolled in the study. PBMCs from the immunotherapy group were collected and expanded by anti-CD3 in the presence of IFNγ and IL2. The expanded T cells were then infused back to the patients, who were assessed for changes in lymphocyte subgroups, tumor-related biological parameters, imaging characteristics, and the condition of remission and survival. The overall response rate (ORR) to the therapy was 66.7% of the treated patients achieving the complete response (CR) and partial response (PR), with a median progression-free survival (PFS) of 43 months and median overall survival (OS) of 54 months separately. No severe toxicity or side effects were observed. Also, we found new factors influencing the clinical outcomes. The higher percentages of $CD4^+$ T cells and $CD4^+HLA\ DR^+$ T cells, but the lower $CD8^+HLA\ DR^+$ T cells in the blood was positively correlated to the therapeutic outcomes achieving CR as compared to the patients still having stable disease (SD) or partial disease (PD).

Précis: The early form of adoptive T cell therapy still provides a valuable option in the treatment of relapsed, refractory, or chemotherapy-resistant lymphoma by improving progression-free survival (PFS) and overall survival (OS).

Keywords: Refractory lymphoma; Adoptive T cell immunotherapy; CD4 T cells; Progression-free survival; Overall survival

Abbreviations: ABVD: Adriamycin, Bleomycin, Vinblastine, Dacarbazine; BEACOPP: Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Oncovin (vincristine), Procarbazine, Prednisone; CHOP: Cyclophosphamide, Hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), Prednisone; CIK: Cytokines-induced killer cells; CR: Complete response; DHAP: Dexamethasone, High-dose cytarabine, Cisplatin; DHAX: Dexamethasone, High-dose cytarabine, and Oxaliplatin; ORR: Overall Response Rate; OS: Overall Survival; PBMC: Peripheral Blood Mononuclear Cells; PD: Progressive Disease; PFS: Progression-free Survival; PR: Partial Response; R-CHOP: Rituximab, Cyclophosphamide, Hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), Prednisone; R-DHAP: Rituximab, Dexamethasone, High-dose cytarabine, Cisplatin; SD: Stable Disease

Introduction

Lymphoma is a malignant tumor derived from hematopoietic and lymphoid tissues. About 20-30% of malignant lymphomas eventually develop into refractory and relapsed lymphomas in the early stage and during treatment [1]. Refractory lymphoma is defined as treatment ineffective after at least two cycles of chemotherapy, or relapse occurs shortly after withdrawal [2]. Therefore, relapsed and refractory lymphoma continues to pose the most significant challenge in the treatment, and the prognosis after relapse remains relatively poor [3].

Over the past several decades, considerable effort and progress have been made in the treatment of various hematologic malignancies. Chemotherapy and radiotherapy are among the first choices of treatment options. However, the long-term efficacy remains disappointing, since most patients might eventually relapse or suffer from severe side effects caused by chemo drugs and radiation. Especially for elderly patients, who are most vulnerable to hematological malignancies, treatment-related adverse reactions are a significant cause of drug discontinuation and reduced dose-intensity, thus aggravating treatment efficacy [4-7].

Recently, *ex vivo* harvested tumor-infiltrating lymphocytes (TILs) [8], sentinel lymph node (SLN)-T cells [9], *in vitro* constructed chimeric antigen receptor (CAR) T cells [10] and TCR transgenic T cells [11], PD1 antibody [12] and NK cells [13] have emerged as innovative approaches in the treatment with cancers and malignant lymphomas. The immunotherapy aims at stimulating the patients' immune system to trigger an anti-tumor immune response, eventually enabling the body's immune cells to better recognize and ultimately kill

cancer cells. These anti-tumor effects have been well-documented in the animal models and clinical trials [14,15]. However, as an alternative to the antigen-targeting adoptive cellular immunotherapy, cytokine-induced killer (CIK) cell therapy was developed in the early '90s [16], in which peripheral blood mononuclear cells were activated and grown in the presence of IFNγ, anti-CD3 mAb, and IL2. It represents a heterogeneous cell population, including $CD3^+CD56^+$, $CD3^+CD56^-$ and $CD3^-CD56^+$ cells. These CIK cells are thought to be adjuvants and have potent cytotoxicity against cancerous tissues and cells [17]. CIK cell therapy still offers a relatively effective option in the treatment of solid tumors by significantly improving progression-free (PFS) and overall survival (OS) [18-21].

In this study, we investigated the potential anti-tumor efficacy and safety of autologous tumor-reactive T cell immunotherapy as a standalone treatment for refractory lymphomas after the failure of standard chemotherapy. Here, we randomly selected admitted and treated 12 cases of patients with relapsed and refractory lymphomas, who had prior treatment of multiple cycles of chemotherapy, such as R-CHOP, in our clinical center between 2011 and 2016. The clinical efficacy and safety results from this study are presented. Our data suggest this safe, efficient and feasible approach accelerates immune reconstitution that may also contribute to enhance immune recovery and improve antitumor immunity.

Materials and Methods

Patient characteristics and selection criteria

This pilot study was conducted at the Affiliated Hospital of Guiyang Medical University between August 2011 to April 2016. A total of 12 eligible patients who were diagnosed with malignant lymphomas (4 Diffuse large-B-cell lymphomas, 3 Hodgkin's lymphomas, 2 non-Hodgkin's lymphomas, 2 Mantle-cell lymphomas and 1 Follicular lymphoma) were selected based on the following inclusion criteria: (1) pathologically confirmed lymphoma; (2) previously administered with ABVD, BEACOPP, CHOP, DHAP, DHAX, R-CHOP, and R-DHAP for 1-6 cycles and had no remission; (3) must be over 12 years old and had normal heart, lung, liver and kidney functions; (4) had no infection or infection under control; (5) no hemorrhage and abnormal clotting function. Our exclusion criteria eliminated patient who had (1) T cell lymphoma; (2) post organ transplantation with long-term immunosuppressive regimen; (3) autoimmune diseases under immunosuppressive drugs; (4) HIV-positive; (5) severe liver and kidney malfunction; (6) pregnant and nursing women. Table 1 showed the patient baseline characteristics.

	ACT Group* (n=12)
Age (Mean)	57 (32-82)
Gender (Male/Female)	9/3
Disease stage	
I -II	7
III-IV	5
Bone marrow involvement	3
Diffuse large-B-cell lymphoma	4
Non-diffuse large-B-cell lymphoma	
Mantle-cell lymphoma	2
Follicular lymphoma	1
Hodgkin's lymphoma	3
Non-hodgkin's lymphoma	2
Other lymphoma near lymph nodes	

Table 1: Patient baseline characteristics (*ACT: Adoptive Cell Therapy by the infusion of anti-CD3 expanded autologous T cells Stage is defined by the Ann Arbor staging system)

The mean age of those patients was 57 years old (a range of 32-82). The Ann Arbor Staging System was applied to determine the disease stage [22].

The study protocol and informed-consent forms were reviewed and approved by the hospital ethical committee. All patients must give their written informed-consents before entering the study. The study was under supervision by an independent data and safety monitoring committee.

Study design

Autologous CIK cells were prepared followed by the previous reports [23,24] with some modifications. In brief, about 50 ml-100 ml of heparinized peripheral blood from refractory lymphoma patients were collected on day 0 and separated using Ficoll-Paque (Amersham) as described previously [25]. The peripheral blood mononuclear cells (PBMCs) harvested at the interface layer were mixed and washed twice with phosphate-buffered saline (PBS) by centrifuging at 300 g for 10 min at 4°C. Single PBMC suspensions obtained were re-suspended and activated with OKT3 (500 ng/ml) in AIM-V serum-free cell culture medium (Gibco) at a density of 2×10^6 cells/ml in the presence of 500 IU/ml recombinant human IL2 (Shuanglu, China) for first 24 hrs. The cells were followed by adding 500 IU/ml each of recombinant human IL2, human IFNγ (Shuanglu, China) and human IL1γ (Shuanglu, China) for 2 to 3 days. These cells were incubated and expanded in flasks in a humidified atmosphere containing 5% CO_2 at 37°C for 12-14 days to induce CIK cells. The cell cultures had periodically received a fresh medium containing 500 IU/ml each of recombinant human IL2, human IFNγ, and human IL1γ. The corresponding cell density was maintained at 2×10^6 cells/ml. At the end of the culture, the expanded cells were harvested, washed, and transferred to a sterile plastic bag containing 200 ml of saline solution and 1% human serum albumin (CSL Behring GmbH, Germany). Before infusion, a portion of cells was collected for further characterization to evaluate the number, viability, phenotype, and possible contamination.

	Cell Therapy	
	n(%)(N=12)	95%CI**
Overall response rate	8 (66.7)	39.1, 86.2
Complete response	6 (50)	25.4, 74.6
Partial response	2 (16.7)	3, 44.8
Stable disease	3 (25)	8.9, 53.2

Progressive disease	1 (8.3)	0.4, 35.4

Table 2: Clinical outcomes in patients evaluated for efficacy: response to treatment (*Tumor responses were classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) per the Cheson revised response assessment criteria [26]; **CI: Confidence Interval)

The treatment schedule is shown in Figure 1. After 14 days of expansion, cytokine-induced killer cell infusions were given to the patients in 200 ml volume a day for six consecutive days per course. The cells were intravenously transfused over a 60 min interval by the guidelines in the hospital. During the infusion, heart rate, respiratory rate, blood pressure, and temperature were carefully monitored and recorded. Each patient received a total of $2^{-11} \times 10^9$ cells per transfusion. The infusion protocol was repeated every four weeks for additional 1-5 courses depending on patient conditions. Transfusion-related toxicity was assessed after the cell transfusion using the Common Terminology Criteria for Adverse Events (CTCAE) 3.0 criteria.

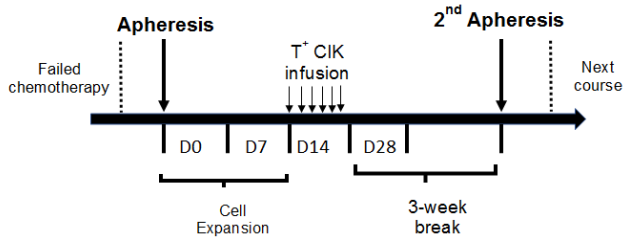
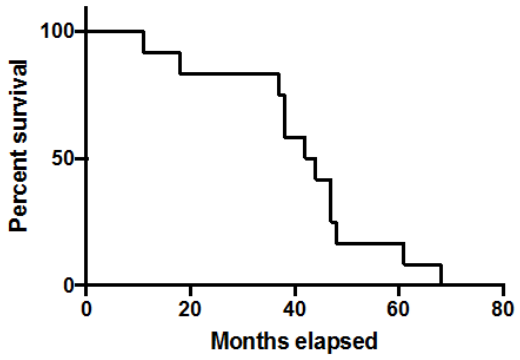


Figure 1: The schematic diagram of autologous cellular immunotherapy schedule. Autologous peripheral blood mononuclear cells (PBMCs) were collected by apheresis on D0 and induced into T^+CIK cells. The expanded T cells were then infused back into patients 14 days later (D14) as the initial transfusion. That was done for six consecutive transfusion days (D14–D20), and each course was completed within four weeks of apheresis. The second course of PBMCs collection was started one week after the end of the first course.



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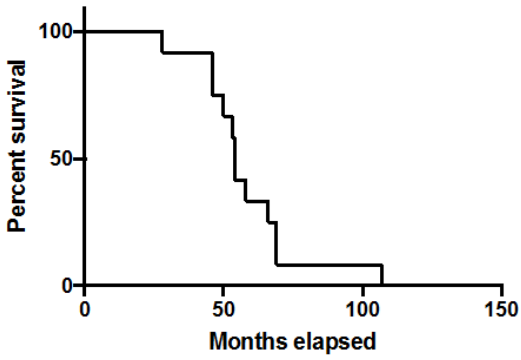

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Figure 2: Endpoint analysis of the responses to cell therapy. a) Progression-free survival (PFS) by Kaplan–Meier curve. PFS was defined from the date of definitive treatment to the date of first documented disease progression or death or date of the cutoff date, whichever came first. The Kaplan–Meier estimate of median PFS was 43 months; b) Overall survival by Kaplan–Meier curve. Overall survival was defined as the time from study day one until the date of death due to any cause or to the cutoff date, including data from long-term follow-up. The Kaplan–Meier estimate of median OS was 54 months.

Before and after each infusion day 1 and 6, the peripheral T cell subsets were collected and assessed for phenotypes by flow cytometry. Fluorescent-labeled monoclonal antibodies (mAbs) against CD3, CD4, CD8, CD16, CD19, CD56, HLA DR (Beckman Coulter) were used to stain the cells per the manufacturer's recommendations. The flow cytometry was performed by an FC500 flow cytometer (Beckman Coulter). A total of 50,000 events were collected and analyzed using CXP software (Beckman Coulter).

Physical examinations were performed before and after treatment. Also, patient's symptoms and complaints were recorded and analyzed. During the treatment, the ultrasonography for abdominal and superficial lymph nodes was performed once every three months, while chest and abdominal MRI, whole body PET-CT scans, and gastroscopy were performed once every six months. A primary clinical assessment was conducted by radiographic images (CT/PETCT), whereas subsequent clinical effect evaluation was involved in the analysis of T-cell subsets, biochemistry, hemogram, bone marrow morphology test and clinical symptoms as well. Side effects, mortality rate, and complications were included in the safety evaluation. After immunotherapy, the patients were monitored every three months in the first year and every six months after that, with a median follow-up time of 54 months (ranging 5-63 months). The disease status was assessed based on physical examination and imaging (CT, PET scan, etc.). If metastases or recurrence occurred during the follow-up periods, the following treatments were recommended including surgery, chemotherapy or radiation. The status and correlating treatments of the patients have updated accordingly in the database.

Statistical evaluation

Statistical analysis was done by GraphPad Prism version 7.0 (GraphPad Software, Inc., San Diego, CA). The therapeutic efficacy was determined by Cheson Revised Response Assessment Criteria [26]. The response criteria were: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The overall response rate (ORR) was calculated as the proportion of patients with CR and PR. A corresponding two-sided 95% exact binomial confidence interval (CI) was constructed using Wilson/Brown method and repeated with individual CR, PR, SD, and PD. The follow-ups were regularly conducted through in-patient visits or telephone consultations for each patient. Progression-free survival (PFS) was described from the date of the treatment to the date of first documented disease progression or death or date of the cut-off date, whichever came first. Patients who died were considered censored at their date of death. Overall survival (OS) was stated as the time from study day one until the date of death because of any cause or to the cut-off date, including data from long-term follow-up. Kaplan and Meier's method was used to estimate both OS and PFS, and to compare both with the log-rank test. The results were expressed in Kaplan–Meier plots. In the analysis of $CD4^+$ and $CD8^+$ cells from the patients one day after each infusion, the complete response (n=6) *vs.* the stable and progression disease (n=4) cases were separately regrouped and assessed using no matching, regular two-way ANOVA. Also, the non-parametric Mann-Whitney test was used to analyze the difference of the medians of $CD3^+CD4^+HLADR^+$ and $CD3^+CD8^+HLADR^+$ subsets one day after each infusion from CR patients (n=5) *vs.* SD or PD patients (n=4) in the cell therapy group. The difference between two groups was statistically significant at $p<0.05$.

Results

Clinical efficacy evaluation

By analysis of response rates within a median follow-up of 54 months (ranging 5-63 months), the overall response rate was 66.7% (95% CI: 39.1, 86.2), with 6 cases achieving a complete response and 2 cases a partial response by immunotherapy (Table 2). The disease events (stable, progression, or death) were observed in 4 out of 12 cases (33.3%). The duration of response (DOR) ranged from 18-68 months by cell therapy, as compared to those same patients treated with the chemotherapies previously, which reached up to 30 months (patient #1) at best (Table 3).

Subject	Prior therapy	Duration of response to prior therapy (Mo)	Duration of response to cell therapy (Mo)
CR			
1	R-CHOP x6	30 (CR)	68 (response ongoing)
	R-DHAP x1	- (PD)	
3	R-CHOP x6	6.5 (PR)	47 (response ongoing)
	DHAP x11	7 (PR)	
4	R-CHOP x1	5 (PR)	47 (relapsed and died)
5	R-CHOP x6	5 (CR)	61 (response ongoing)
6	R-CHOP x1	3 (CR)	44 (response ongoing)
8	R-CHOP x6	- (SD)	18 (response ongoing)
PR			
7	ABVD x6	3 (PR)	42 (response ongoing)
11	ABVD x4	4 (PR)	38 (response ongoing)
SD			
9	ABVD x2	- (SD)	-
	BEACOPP x4		
10	R-CHOP x4	18 (PR)	-
12	R-CHOP x6	- (SD)	-
PD			
2	CHOP x3	-(SD)	-(died)
	DHAX x4	13 (PR)	

Table 3: Clinical outcomes evaluated for efficacy in the patients receiving cell therapy (CR: Complete Response; DHAP: Cytarabine, Cisplatin, and Dexamethasone; DHAX: Cytarabine, Dexamethasone, and Oxaliplatin; PD: Progressive Disease; PR: Partial Response; R-CHOP: Cyclophosphamide, Doxorubicin, Prednisone, Rituximab, and Vincristine sulfate; R-DHAP: Rituximab, Cytarabine, Cisplatin, and Dexamethasone; SD: Stable Disease).

The median progression-free survival (PFS) was 43 months for those with the immunotherapy (Figure 2a), whereas the median overall survival (OS) was 54 months (Figure 2b). At 50 months of the treatment, OS was 75 percent of patients treated. Here we also note that by the analysis cut-off date, there had been two patients (#2 and #4) who experienced the disease relapse and died of the disease at 28 and 54 months separately after the start of the first immunotherapy treatment.

Adverse effects

No noticeable adverse events (infection, allergy, pulmonary, renal symptoms, hepatic function failure, or autoimmune disorder) were observed among the patients who had the immunotherapy after multiple infusions, though some patients developed mild fatigue and low-grade fever at the initial infusion, which resolved after treatment. No patients withdrew from the treatment. At the end of follow-up period, no patients showed liver and kidney dysfunction, change in hemogram or other symptoms.

Impact of potential factors on the efficacy of immunotherapy

In the multivariate analysis of this study, we sought to identify a couple of factors including specific cell subsets, which could contribute to the likely clinical outcomes. The peripheral blood from all 12 patients in the immunotherapy group was collected and assessed before the cell preparation for expansion and after one day of the infusion each time. Although the numbers of infused cells showed no significant impact on the clinical outcomes (Data not shown), the phenotyping analysis by flow cytometry suggested specific subsets might play such a role in clinical outcomes. Here we characterized the lymphocyte subsets as percentages of $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD19^+$, $CD3^+/CD56^+$, $CD4^+$/HLA DR^+ and $CD8^+$/HLA DR^+. We

separately pooled together the percentages of CD4 and CD8 cells collected after one day of each infusion cycle and compared the $CD4^+$ and $CD8^+$ subsets between 6 CR patients and 3SD/1PD patients, as shown in Figure 3a. Interestingly, the comparison revealed that the percent of $CD4^+$ T cells in the blood one day after infusion was highly correlated with achieving CR. That was because all CR patients had significantly higher percentages of $CD4^+$ cells (a mean of 17.7%) than those (a mean of 7.3%) in the stable disease (SD)/progression of disease (PD) patients (p=0.0246).

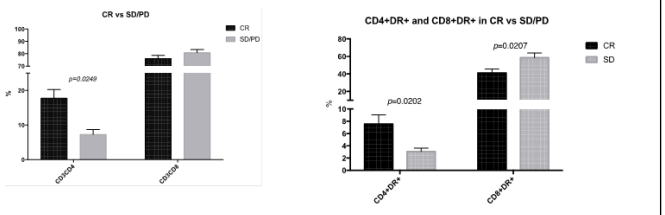

Figure 3: Impact of cellular composition on clinical efficacy. a) Comparison of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells post day one of infusion from CR patients (n=6) *vs.* SD or PD patients (n=4) in the cell therapy group. Those cells were collected and analyzed from above patients after the first day of the infusion. Two groups were assessed using no matching, regular two-way ANOVA. The results were considered significant at $p<0.05$. b) Comparison of percentages of $CD3^+CD4^+HLADR^+$ and $CD3^+CD8^+HLADR^+$ cells post day one of infusion from CR patients (n=5) *vs.* SD or PD patients (n=4) in the cell therapy group. Those subsets pooled from above patients each after the first day of the infusion were analyzed by flow cytometry. The Mann–Whitney test was used to compare medians of two groups. The results were considered significant at $p<0.05$.

Further, in Figure 3b, $CD4^+$ cells in all CR patients were shown to express a higher frequency of HLA DR (a mean of 7.6%) than those (a mean of 3.1%) of 3SD/1PD patients (p=0.0202). However, $CD8^+$ cells in all CR patients demonstrated having a lower expression of HLA DR (a mean of 41.2%) than those (a mean of 58.6%) of 3SD/1PD patients (p=0.0207).

Discussion

In general, chemotherapy-refractory lymphoma is difficult to treat and needs innovative approaches to treatment. High-dose chemotherapy and autologous stem cell transplantation (ASCT) are considered the standard of care for chemo sensitive relapsed Hodgkin and non-Hodgkin lymphomas, leading to increased disease-free survival [27,28]. However, disease relapse or progression is still the primary cause of treatment failure following ASCT for non-Hodgkin lymphomas (NHL). Also, the efficacy, feasibility, and affordability limit its extensive application [29,30]. Therefore, appropriate and effective strategies are being developed to contend in this field. Recently adoptive immunotherapy has made significant advances, as a variety of treatment modalities have been explored thus far, including new T-cell products that are genetically engineered with chimeric antigen receptors (CARs) targeting CD19. CD19 CAR T cells have proven to be a great success and have broad application for treatment of relapse and refractory lymphomas or other B cell malignancies [31,32].

CIK therapy represents a mature, promising, and potent cytotoxic, but a less profound tool in the treatment of certain cancers. In our pilot study, we determined the anti-tumor efficacy, tolerability, and safety of anti-CD3 expanded T cells in the patients with refractory lymphomas. Their straightforward ex-vivo expansion, along with the MHC-unrestricted tumor killing capability may overcome some crucial problems that have limited the outreaching and clinical translation in other forms of immunotherapy [17,33]. In contrast, the treatment of genetically modified T cells has been associated with several potential safety concerns, such as cytokine release storm (CRS) and neurologic toxicity, both of which have been linked to a marked increase in serum proinflammatory cytokine levels [34,35].

Our preliminary study indicates that the cell immunotherapy has potential to provide substantial clinical benefit in heavily pre-treated patients with malignant lymphoma, especially in the case of lack of adequate alternatives. Also, overall survival has often been regarded as the most reliable endpoint in evaluating an experimental strategy for cancer treatment. Thus, its improvement is an essential benchmark for efficacy and regulatory approval of a new therapy. On the other hand, we found the commonly used endpoints, PFS, OS, and response rate were not sufficient to assess and describe the value of clinical efficacy when compared with different therapeutic modalities. In our cases, the response rate did not adequately capture the potential outcomes with our immunotherapy, and additional metrics are needed, especially when the new agents or therapies have mechanisms of action that may translate into different clinical effects.

In consideration of our clinical outcomes, the HLA-DR was up regulated during $CD4^+$ T cell activation and proliferation following T cell receptor engagement, which suggests their activation and presentation of antigenic information in anti-tumor immune response [36,37]. However, the expression of HLA DR by $CD8^+$ cells was inversely correlated to the clinical outcomes in this case. They could be considered as regulatory $CD8^+$ T cell subsets that played a suppressive role in tumor patients [38] and might shorten the progression-free survival in our patients. Interestingly our observations suggest that higher levels of HLA-DR expressed by $CD4^+$ and $CD8^+$ T cells reflect different roles in tumor-bearing patients. Therefore, the results demonstrated that the higher percentages of $CD4^+$ T cells, $CD4^+$HLA DR^+ T cells and lower percent of $CD8^+$HLA DR^+ in T cell-mediated immunotherapy could be useful biomarkers to predict a favorable prognosis for the patients with refractory lymphoma. Thus, the therapeutic strategies that aim to overcome $CD8^+$ Treg activity as a means of enhancing anti-tumor immune responses must consider this unique subset circulating in the patients' blood.

In conclusion, our study provides an informative reference for subsequent clinical trials using anti-CD3 expanded PBMCs and demonstrates that the method of the immunotherapy preparation is an effective alternative in the treatment of patients with refractory and relapsed malignant lymphomas when compared with the complicated preparation methods of other immunotherapies mediated by TCR T and CAR T cells.

Acknowledgement

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval and Ethical Standards

The study protocol and informed-consent forms were reviewed and approved by the affiliated hospital of Guiyang Medical University ethical committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All individual participants included in the study must give informed consent.

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References

1. Lester JF, Dojcinov SD, Attanoos RL, O'Brien CJ, Maughan TS, et al. (2003) The clinical impact of expert pathological review on lymphoma management: a regional experience. Br J Haematol 123: 463-468.
2. Friedberg JW (2011) Relapsed/refractory diffuse large B-cell lymphoma. Hematology Am Soc Hematol Educ Program: 498-505.
3. Chao MP (2013) Treatment challenges in the management of relapsed or refractory non-Hodgkin's lymphoma-novel and emerging therapies. Cancer Manag Res 5: 251-269.
4. Balducci L, Yates J (2000) General guidelines for the management of older patients with cancer. Oncology 14: 221-227.
5. Zagonel V, Monfardini S, Tirelli U, Carbone A, Pinto A (2001) Management of hematologic malignancies in the elderly: 15-year experience at the Aviano Cancer Center, Italy. Crit Rev Oncol Hematol 39: 289-305.
6. Pinto A, Zagonel V, Ferrara F (2001) Acute myeloid leukemia in the elderly: biology and therapeutic strategies. Crit Rev Oncol Hematol 39: 275-287.
7. Tirelli U, Carbone A, Monfardini S, Zagonel V (2001) A 20-year experience on malignant lymphomas in patients aged 70 and older at a single institute. Crit Rev Oncol Hematol 37: 153-158.
8. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, et al. (2002) Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298: 850-854.
9. Zhen YH, Liu XH, Yang Y, Li B, Tang JL, et al. (2015) Phase I/II study of adjuvant immunotherapy with sentinel lymph node T lymphocytes in patients with colorectal cancer. Cancer Immunol Immunother 64: 1083-1093.
10. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, et al. (2013) CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 5: 177ra38.
11. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, et al. (2011) Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 29: 917-924.
12. Brockelmann PJ, Borchmann P, Engert A (2016) Current and future immunotherapeutic approaches in Hodgkin lymphoma. Leuk Lymphoma 57: 2014-2024.
13. Bachanova V, Burns LJ, McKenna DH, Curtsinger J, Panoskaltsis-Mortari A, et al. (2010) Allogeneic natural killer cells for refractory lymphoma. Cancer Immunol Immunother 59: 1739-1744.
14. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, et al. (1985) Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 313: 1485-1492.
15. Rosenberg SA, Spiess P, Lafreniere R (1986) A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 233: 1318-1321.
16. Schmidt-Wolf IG, Negrin RS, Kiem HP, Blume KG, Weissman IL (1991) Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. J Exp Med 174: 139-149.
17. Olioso P, Giancola R, Di Riti M, Contento A, Accorsi P, et al. (2009) Immunotherapy with cytokine induced killer cells in solid and hematopoietic tumours: a pilot clinical trial. Hematol Oncol 27: 130-139.
18. Hontscha C, Borck Y, Zhou H, Messmer D, Schmidt-Wolf IG. Clinical trials on CIK cells: first report of the international registry on CIK cells (IRCC). J Cancer Res Clin Oncol 137: 305-310.
19. Jakel CE, Schmidt-Wolf IG (2014) An update on new adoptive immunotherapy strategies for solid tumors with cytokine-induced killer cells. Expert Opin Biol Ther 14: 905-916.
20. Jakel CE, Vogt A, Gonzalez-Carmona MA, Schmidt-Wolf IG (2014) Clinical studies applying cytokine-induced killer cells for the treatment of gastrointestinal tumors. J Immunol Res 2014: 897214.
21. Chen JL, Lao XM, Lin XJ, Xu L, Cui BK, et al. (2016) Adjuvant Cytokine-Induced Killer Cell Therapy Improves Disease-Free and Overall Survival in Solitary and Nonmicrovascular Invasive Hepatocellular Carcinoma After Curative Resection. Medicine 95: e2665.
22. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, et al. (1989) Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol 7: 1630-1636.
23. Weng DS, Zhou J, Zhou QM, Zhao M, Wang QJ, et al. (2008) Minimally invasive treatment combined with cytokine-induced killer cells therapy lower the short-term recurrence rates of hepatocellular carcinomas. J Immunother 31: 63-71.
24. Li JJ, Gu MF, Pan K, Liu LZ, Zhang H, et al. (2012) Autologous cytokine-induced killer cell transfusion in combination with gemcitabine plus cisplatin regimen chemotherapy for metastatic nasopharyngeal carcinoma. J Immunother 35: 189-195.
25. Bonanno G, Iudicone P, Mariotti A, Procoli A, Pandolfi A, et al. (2010) Thymoglobulin, interferon-gamma and interleukin-2 efficiently expand cytokine-induced killer (CIK) cells in clinical-grade cultures. J Transl Med 8:129.
26. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, et al. (2007) Revised response criteria for malignant lymphoma. J Clin Oncol 25: 579-586.
27. Schmitz N, Pfistner B, Sextro M, Sieber M, Carella AM, et al. (2002) Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. Lancet 359: 2065-2071.
28. Dahi PB, Tamari R, Devlin SM, Maloy M, Bhatt V, et al. Favorable outcomes in elderly patients undergoing high-dose therapy and autologous stem cell transplantation for non-Hodgkin lymphoma. Biol Blood Marrow Transplant 20: 2004-2009.
29. Rivera-Rodriguez N, Cabanillas F (2013) Recent advances in the management of mantle cell lymphoma. Curr Opin Oncol 25: 716-721.
30. Nagle SJ, Woo K, Schuster SJ, Nasta SD, Stadtmauer E, et al. (2013) Outcomes of patients with relapsed/refractory diffuse large B-cell lymphoma with progression of lymphoma after autologous stem cell transplantation in the rituximab era. Am J Hematol 88: 890-894.

31. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, et al. (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 368: 1509-1518.
32. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 33: 540-549.
33. Leemhuis T, Wells S, Scheffold C, Edinger M, Negrin RS (2005) A phase I trial of autologous cytokine-induced killer cells for the treatment of relapsed Hodgkin disease and non-Hodgkin lymphoma. Biol Blood Marrow Transplant 11: 181-187.
34. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, et al. (2011) T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 3: 95ra73.
35. Davila ML, Riviere I, Wang X, Bartido S, Park J, et al. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 6: 224ra25.
36. Lamb JR, Fledmann M (1982) A human suppressor T cell clone which recognizes an autologous helper T cell clone. Nature 300: 456-458.
37. LaSalle JM, Ota K, Hafler DA (1991) Presentation of autoantigen by human T cells. J Immunol 147: 774-780.
38. Arruvito L, Payaslian F, Baz P, Podhorzer A, Billordo A, et al. (2014) Identification and clinical relevance of naturally occurring human CD8+HLA-DR+ regulatory T cells. J Immunol 193: 4469-4476.