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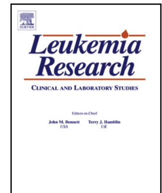

Research paper

# Allogeneic hematopoietic stem cell transplantation following donor CIK cell infusion: A phase I study in patients with relapsed/refractory hematologic malignancies

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ABSTRACT

Patients with relapsed/refractory hematologic malignancies after allogeneic stem cell transplantation have a poor prognosis due to the high rate of relapse. Techniques capable of decreasing post-transplantation relapse rates are urgently sought. This study aimed to explore the feasibility and safety of allogeneic hematopoietic stem cell transplantation (HSCT) following infusion of donor cytokine-induced killer (CIK) cells. CIK cells were generated in vitro from donor peripheral blood mononuclear cells, and were phenotyped using flow cytometric analysis. CIK cells were administered to 15 high-risk relapsed/refractory hematologic malignancy patients who were not in complete remission in multiple infusions. These patients also received allogeneic HSCT. The side effects and outcomes were recorded. All patients achieved engraftment and complete remission. After CIK cell infusion, two patients developed graft-versus-host disease (GvHD), which was controlled by additional immunosuppressant drugs. At the last follow-up, nine patients had died and six patients were surviving at a median follow-up of 1513 days (range, 771–1655 days). In conclusion, allogeneic HSCT combined with sequential infusion of donor CIK cells is well tolerated in salvage relapsed/refractory hematologic malignancy patients.

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## 1. Introduction

Cases of relapsed or refractory hematologic malignancy have a very poor prognosis. The only effective method for curing such diseases is allogeneic hematopoietic stem cell transplantation (HSCT) [1]. HSCT is largely dependent on graft versus leukemia performance, which is exerted by immunologically active cells transferred with the graft [2]. The traditional therapeutic approach to relapsed hematologic malignancy following HSCT involves the use of multi-agent re-induction chemotherapy to achieve a second complete remission as a transition to subsequent efforts at salvage and cure. However, the therapeutic efficacy of this approach is limited by tumor cell resistance, and in the vast majority of patients, these re-induction approaches are unsuccessful due to refractory disease or early relapse [3]. Patients who relapse shortly after a previous transplantation, especially when relapse occurs particularly early after autologous transplantation, often do not respond to salvage chemotherapy and have a very poor prognosis, with a 2-year OS rate of below 20% and almost no patients surviving past 5 years. Relapse remains the leading cause of death following HSCT, as tumor cell resistance continues to present an unsolved problem in high-risk hematological malignancies [4,5].

Due to the high failure rate of re-induction therapy, the adoption of conventional salvage chemotherapy or immediate transplantation for relapsed patients is controversial. Among patients who experience relapse after allogeneic transplant, re-induction chemotherapy is also frequently unsuccessful, with long-term survival reportedly as low as 5% [6]. In these high-risk cases, immunosuppression is often withdrawal. Donor lymphocyte infusion (DLI) is the most common approach, and a second allogeneic HSCT may also be an effective treatment option [7], but the cost

*Abbreviations:* ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; ATG, anti thymus globulin; CIK, cytokine-induced killer; CML, chronic myelogenous leukemia; CML-BP, chronic myeloid leukemia; DLI, Donor lymphocyte infusion; F-Bu-Cy, fludarabine busulfan and cyclophosphamide regimen; FITC, fluorescein isothiocyanate; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MM, multiple myeloma; MMF, mycophenolate mofetil; NHL, non-Hodgkin lymphoma; NK, natural killer; PE, phycoerythrin; PMNCs, peripheral blood mononuclear cells; s-AML, second acute myeloid leukemia.

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of this therapy restricts its application to a minority of patients in China [8,9]. There is a great need for a method of decreasing relapse after allogeneic HSCT.

The adoptive transfer of donor cytokine-induced killer (CIK) cells may serve as an alternative approach to donor lymphocyte infusions. CIK cells are activated and expanded in vitro from peripheral blood mononuclear cells (PMNCs) by addition of cytokines. These expanded CIK cells comprise a heterogeneous population of $CD3^+CD56^-$ T cells and $CD3^+CD56^+$ natural killer (NK) cells. CIK cells can eradicate a variety of hematological diseases in a non-major histocompatibility complex (MHC)-restricted manner without exerting substantial alloreactive complications [10,11]. In vitro analysis suggests that conventional CIK cells exhibit no substantial alloreactive potential, but provide stronger anti-tumor efficacy than isolated CD3/CD56+ CIK cells [12]. Nishimura et al. previously evaluated the trafficking and survival of luciferase-expressing CIK cells in an allogeneic bone marrow transplant model, and found that CIK cells have the potential to separate graft-versus-tumor effects from GVHD [13].

To further examine this technique, in this study we reviewed the outcome of allogenic CIK administration followed by allogeneic HSCT in 15 patients with high-risk relapsed/refractory hematologic malignancy patients who were not in complete remission, and reviewed the patient outcomes.

## 2. Material and methods

### 2.1. Patients

This prospective phase I clinical trial enrolled patients from the Second Affiliated Hospital of Chongqing Medical University between June 2010 and December 2012. Inclusion criteria were: (1) hematologic malignancy relapse within six months of prior autologous stem cell transplantation; (2) chronic myelogenous leukemia (CML) in the blast phase that was unresponsive after TKI or two cycles of chemotherapy; (3) acute myeloid leukemia (AML) that did not achieve partial remission with standard chemotherapy of two cycles. Exclusion criteria were: (1) aged less than 15 years or more than 70 years; (2) Eastern Cooperative Oncology Group scores over 1; (3) severe liver, kidney, or heart conditions, or active infections. All study participants provided written informed consent, and the procedure was approved by the Institutional Review Board of The Second Affiliated Hospital of Chongqing Medical University (Clinical trial number) (ChiCTR-TNC-11001676).

### 2.2. Conditioning regimen

Of the 15 enrolled patients, 11 received treatment with the fludarabine, busulfan, and cyclophosphamide regimen (F-Bu-Cy) prior to transplantation. Cyclophosphamide (50 mg/kg/day; Haizheng, China) was administered on the third and second days before transplantation. Busulfan (4 mg/kg/day, Schering Pharmaceutical Ltd, Germany) was administered orally on the sixth to fourth days before transplantation. Fludarabine (30 $mg/m^2$/day) before transplantation (GlaxoSmithKline, United Kingdom) was administered by intravenous drip on the ninth to sixth days before transplantation. One patient received the same regimen excluding busulfan. The ALL and NHL patients additionally received 200 $mg/m^2$/day of lomustine (Nanjing Pharmaceutical Ltd, China) by intravenous drip two days prior to transplantation. Two patients with MM also received 140 $mg/m^2$/day of melphalan (GlaxoSmithKline, United Kingdom) by intravenous drip on days three and two prior to transplantation and 25 $mg/m^2$/day fludarabine (GlaxoSmithKline, United Kingdom) by intravenous drip on days 8–4 prior to transplantation.

### 2.3. GvHD prophylaxis

All patients received 2.5 mg/kg/day of anti-thymus globulin (ATG, Gene, France) by intravenous drip for 2 days before transplantation. On the day before transplantation 2.5 mg/kg/day of cyclosporine (CsA, Novartis, Switzerland) was administered intravenously until symptoms of nausea, vomiting and hiarrhea subsided, and was then administered orally (5 mg/kg for 3–6 months).

To prevent GvHD after transplantation, we also administered 15 $mg/m^2$ of methotrexate intravenously on day one after transplantation, and 10 $mg/m^2$ of methotrexate intravenously on the third day after transplantation. HLA mismatch patients received 0.5 g mycophenolate mofetil (MMF, Cellcept, Shanghai Roche Pharmaceuticals Ltd) orally every 12 h starting on day 7 before transplantation, and continuing until engraftment after transplantation, in addition to 20 mg of basiliximab (Novartis, Switzerland) intravenously on the day of transplantation. The whole-blood CsA concentration was monitored weekly using a fluorescence polarization immunoassay (Aechitect i1000, Abbott ltd, USA).

### 2.4. CIK cell culture

CIK cells were generated from donor PMNCs. Briefly, 80 mL of mononuclear cells was collected from each donor (who donate stem cells) sample using a blood cell separator (Fresenius Kabi, Germany). Lymphocytes were separated using lymphocyte separation medium and either cryopreserved or cultured in RPMI1640 medium containing 10% autologous plasma (culture medium). After 4–6 h, the suspended cells were centrifuged and resuspended at $1 \times 10^6$/mL in culture medium containing IFN-γ (1000 U/mL) and incubated at 37 °C in 5% $CO_2$. After 24 h, the medium was replaced with CIK medium (culture medium containing 100 mg/mL of mouse anti human CD3 monoclonal antibody and 1000 U/mL IL-2). The medium was replaced by the fresh medium every 3 days, and after 10–14 days of in vitro stimulation the CIK cells were harvested and used for infusion, as previously described [14]. After the 10–14-days culture period, cells were expanded to $1 \times 10^8$–$10^9$/kg.

The cytotoxic capacities of the expanded CIK cells were verified by measuring killing of LDH-loaded target cell lines K562 and U266 (purchased from the Cell Bank of Shanghai Cancer Institute, Shanghai, China). Cultivated CIK cells were incubated with U266 or K562 cells at a CIK:target ratio of 5:1, 10:1, or 20:1 at 37 °C in 5% $CO_2$ for 4 h. The LDH content of supernatant was assessed by an automatic biochemistry analyzer (Siemens, Germany). Baseline release (in the absence of CIK cells) and maximal NP-40-induced release were recorded. The kill rate was calculated according to the following formula: kill rate (%) = (test total − target cell natural release total)/target cells (target cells release total − largest natural release total) × 100%. Cytotoxic populations capable of killing at least 20% of U266 and K562 in 4 h at a CIK:target ratio of 5:1 were considered sufficiently cytotoxic, as previously described [15].

### 2.5. Flow cytometric analysis

The CIK phenotype was established by staining with CD4-fluorescein isothiocyanate (FITC), CD8-phycoerythrin (PE), CD3-FITC, CD16-PE, CD-25-FITC, and CD56-allophycocyanin (all BD Biosciences, San Jose, CA, USA), and staining was detected using a FACS Calibur flow cytometer (BD Biosciences).

### 2.6. CIK cell infusion

At the first infusion, patients received $1 \times 10^6$ cells/kg and this number was adjusted in subsequent infusions according to the patient's status. Patients first received an infusion on days +30, +60, and +90. Thereafter, if disease did not recur or if GVHD did not occur, tha petients received CIK every 3 months. Hematopoietic reconstitution was considered achieved when the patient's blood contained $>0.5 \times 10^9$/L neutrophils, and $>20 \times 10^9$/L platelets.

### 2.7. Follow up

After discharge from hospital, patients underwent routine blood tests once every 2 weeks in the first 3 months, once every month in the second 3 months, then after 6 months once every 2 months, and after 1 year, once every 3 months. Routine blood tests measures liver and kidney function and chromosomal or molecular abnormalities, as previously described [16,17].

### 2.8. Statistical analysis

Continuous variables are presented as means ± standard deviations (SD) and were compared using independent samples *t*-tests. Categorical variables are presented as frequencies and percentages; chi-square tests were used for comparison. All analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA). $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Patient characteristics

The patients enrolled in this prospective trial were diagnosed with AML ($n = 3$), second acute myeloid leukemia (s-AML) ($n = 1$); non-Hodgkin lymphoma (NHL) ($n = 4$); acute lymphocytic leukemia (ALL) ($n = 2$); multiple myeloma (MM) ($n = 2$), and chronic myeloid leukemia (CML-BP) ($n = 3$) (Table 1).

### 3.2. Characterization of expanded CIK cells

CIK cells were generated from donor PMNCs by incubation with IFN-γ, human CD3 monoclonal antibody, and IL-2 for 10–14 days. Fluorescent staining confirmed that CIK were 88.8 ± 5.2% $CD3^+$, 12.0 ± 5.6% $CD3^+CD4^+$, 49.0 ± 7.4% $CD3^+CD8^+$, 38.3 ± 4.5% $CD3^+CD56^+$, 0.57 ± 0.2% $CD3^-CD19^+$, and 1.0 ± 0.3% $CD3^-CD56^+$ ($n = 10$). The cytotoxic capacities of the expanded CIK

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