Ultra-Low-Dose Decitabine Combined With Autologous Cytokine-Induced Killer Cells for Elderly Patients With Acute Myeloid Leukemia Transformed From Myelodysplastic Syndrome

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ABSTRACT

Purpose: Elderly acute myelocytic leukemia (AML) patients have limited treatment options because they poorly tolerate standard-dose chemotherapy. The present article describes our experience with ultra-low-dose decitabine combined with infusion of autologous cytokine-induced killer (CIK) cells for 2 elderly patients with myelodysplastic syndrome–transformed AML.

Methods: Decitabine (10 mg) was given on days 1 to 5, and CIK cells on day 14 with 2 to 8 $\times$ 10^9 cells per infusion.

Findings: The therapeutic regimen resulted in marked hematologic recovery and was associated with better than expected survival in both cases.

Implications: Our experience suggests that the combination therapy is safe and effective for elderly patients with myelodysplastic syndrome–transformed AML. (*Clin Ther*. 2014;36:1104–1111)

Key words: acute myelocytic leukemia, AML, cytokine-induced killer cells, CIK cells, decitabine, elderly, myelodysplastic syndrome, MDS.

INTRODUCTION

Myelodysplastic syndrome (MDS) is a group of clonal bone marrow disorders with increased risk for leukemic transformation.[1,2] The presence of an antecedent MDS is an established prognostic factor for acute myelocytic leukemia (AML), which is one of the most common hematologic malignancies involving elderly persons.[3] The overall incidence of AML is 3.4 cases per 100,000 persons in the United States, with a median age at diagnosis of 67 years.[4]

Currently, allogeneic stem cell transplantation remains the only curative option, but, unfortunately, this is not viable for the majority of MDS patients due to their age and comorbidity. Most elderly patients also cannot tolerate standard- dose chemotherapy, and even with the best supportive care and therapeutic regimens available, the prognosis of elderly AML patients remains dismal, with the 5-year survival at 5% to 15%.[5,6]

Decitabine is a DNA-demethylating agent and has recently emerged as a potent therapeutic agent for MDS and leukemia.[5–7] Recent findings indicate that

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decitabine is safe and effective for MDS and MDS-transformed AML; however, its use in the elderly is limited because they poorly tolerate the bone marrow toxicities of the drug. Currently, it remains unknown whether there is an optimal dose of the drug that achieves the best therapeutic efficacy with the least bone marrow suppression.

Our earlier studies found that infusion of autologous cytokine-induced killer (CIK) cells partially induced hematologic improvement or partial remission in elderly AML patients.[8–10] Considering that low-dose decitabine causes a sustained antitumor response even after the drug is discontinued and after specific killing of leukemia cells by CIK cells,[11,12] we speculated that ultra-low-dose decitabine combined with infusion of autologous CIK cells could achieve a more desirable outcome with a more benign safety profile in elderly patients with AML. The present article describes our experience with the use of the combination therapy for 2 elderly patients with MDS-transformed AML that resulted in marked hematologic improvement and better- than-expected survival.

PATIENTS AND METHODS

Large-Scale Expansion of CIK Cells

A total of 54 mL of venous blood was collected from the median cubital vein of the patient into evacuated heparinized tubes. Human peripheral blood mononuclear cells (PBMCs) were isolated by using Ficoll-Hypaque density-gradient centrifugation and then washed 3 times. After adjustment to a final concentration of 2×10^6 cells/mL with CIK medium (Takara Bio Inc, Japan), PBMCs were cultured in 75-cm^2 culture flasks pretreated with 8 mL of phosphate-buffered saline containing 5 μg/mL of anti-human CD3 monoclonal antibody (Takara Bio Inc, Japan) at 4°C overnight. After overnight incubation (day 0), 1000 U/mL of recombinant human interferon gamma (PeproTech, Inc, Rocky Hill, New Jersey) and 1000 U/mL of recombinant human interleukin-2 (rhIL-2; PeproTech, Inc, Rocky Hill, New Jersey) were added to the culture medium (day 1). The cells were then cultured in a humidified 5% CO_2 incubator at 37°C. After 6 hours, fresh CIK medium with 1000 U/mL of rhIL-2 and 1000 U/mL of interferon gamma were added to remove nonadherent cells. After 4 days, the cells were transferred from the coated flasks to fresh (uncoated) flasks. Fresh CIK medium and 1000 U/mL of rhIL-2 were added every 3 days thereafter. The overall composition and purity of CIKs harvested after 14-day incubation periods were assessed by using flow cytometry (FACS-420, USA; Cellfit software, USA).

Quality Control Measures

CIK cells were washed 3 times with normal saline and resuspended in 100 mL of normal saline before administration. The CIK cells were phenotyped with appropriate monoclonal antibodies including CD4-fluorescein isothiocyanate, CD8-phycoerythrin, CD3-chlorophyll protein complex, and CD56-allophycocyanin antibodies. All CIK cells met the following "CIK gate" criteria: $\geq$70% $CD3^+$ cells, $\geq$40% $CD8^+$ cells, and $\geq$15% $CD3^+CD56^+$ cells. The final cell products were assessed for viability by using the trypan blue dye exclusion test and checked twice for possible contamination by bacteria, fungi, and endotoxins. The viable cells accounted for $\geq$95% of all cells.

Flow Cytometry

The following antihuman antibodies were used to stain cell surface markers to determine CIK phenotype: CD4-fluorescein isothiocyanate, CD8-phycoerythrin, CD3-chlorophyll protein complex, and CD56-allophycocyanin antibodies. The antibodies and isotype-matched monoclonal antibodies were purchased from BD Biosciences (San Jose, California). Data acquisition was performed by using a FACSCalibur flow cytometer (BD Biosciences).

CASE REPORTS

Case 1

An 83-yearold male patient was admitted in April 2006 because of fatigue and night sweating for >3 months. The patient had a history of alveolar carcinoma of the right lung, coronary heart disease, and chronic kidney insufficiency (Table I). The family history was insignificant, and physical examination showed no remarkable findings. Routine complete blood count upon admission revealed white blood cells (WBCs) at 3.1×10^9/L (normal, 3.5–4 $\times 10^9$/L), neutrophils at 34% (normal, 50%–70%), lymphocytes at 56% (normal, 20%–40%), monocytes at 5% (normal, 3%–8%), hemoglobin at 143 g/L (normal, 110–160 g/L), and platelets at 149×10^9/L (normal, 100–300 $\times 10^9$/L). The erythrocyte sedimentation rate, serum folic acid, and vitamin B_{12} contents were normal. Bone marrow aspiration revealed abnormal