International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-2, February 2016 Pages 113-116

DCAG in Combination with HLA-mismatched Stem-Cell Microtransplantation for the Treatment of Refractory or Relapsed AML in Elderly Patients

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***Abstract*— No reference salvage regimen has been established in elderly patients with refractory or relapsed acute myeloid leukemia (AML). In our study, six patients older than 60 diagnosed with refractory or relapsed AML received programmed infusions of granulocyte colony-stimulating factor (G-CSF)–mobilized HLA-mismatched donor peripheral-blood stem cells after the DCAG regimen of chemotherapy treatment. The complete remission (CR) rate was 33.33% after the first therapy cycle. The median recovery times of neutrophils and platelets were 12 days and 14.5 days, respectively. No acute graft-versus-host disease (GVHD) or chronic GVHD was observed in any of the patients. The median overall survival (OS) time was 6.2 months (range, 0.9-26.2), and one out of six patients (16.7%) achieved persistent CR. In Conclusion, DCAG regimenin in combination with microtransplantation was well tolerated and showed a promising clinic efficacy in elderly patients with refractory or relapsed AML.**

***Index Terms*— DCAG regimen; microtransplantation; elderly; Refractory or relapsed; Acute myeloid leukemia.**

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I. INTRODUCTION

Acute myeloid leukemia (AML) is most often encountered in the patients older than 60 years of age [1]. The effect of the intensive chemotherapy in elderly patients with AML, because of their biologic characteristics and poor tolerance, is not as well as younger patients [2]-[4]. However, most elderly patients who achieve a complete remission (CR) eventually relapse and die from AML [5]. There has been little progress in the treatment of refractory or relapsed AML in elderly patients [6]-[8], and the management of these patients remains a major challenge.

In our previous study, 85 evaluable elderly patients with newly diagnosed AML were treated with decitabine in combination with granulocyte colony-stimulating factor, low-dose cytarabine and aclarubicin (DCAG) regimen which showed a high CR rate (64.7%) and safety [9]. Recently, Guo [10],[11] first reported human leukocyte antigen (HLA)-mismatched stem-cell microtransplantation combined with conventional chemotherapy can improve the outcome and avoid graft-versus-host disease (GVHD), which provide a safe and effective therapy, especially for elderly patients with AML. Kong et. al also found that microtransplantation maintenance therapy following consolidation therapy is feasible in elderly patients with AML [12]. It has been reported that decitabine induces CD80 expression on cancer cells and stimulates tumor-specific cytotoxic T-lymphocyte responses [13].

On the basis of these observations, we investigated the effect and safety of decitabine-based chemotherapy regimen (DCAG) in combination with microtransplantation (DCAG-M) on outcomes of refractory or relapsed AML in elderly patients.

II. MATERIALS AND METHODS

A. Patients

Six patients who lacked HLA-matched sibling donors or refused to receive HLA-haploidentical stem cell transplantation were enrolled in this study between November 2013 and October 2015. The characteristics of those 6 patients were summarized in Table Ⅰ. Those patients consisted of 3 males and 3 females with a median age of 63.5 years (range, 60 years to 87 years). Two patients were aged over 70 years. All patients were diagnosed with morphological examination, cytochemical, cytogenetic and immunophenotypic analyses according to World Health

Organization classification [14]. The definition of refractory AML referred to not achieving CR after two cycles of a standard regimen. Relapse was defined as over 5% of blast cells by bone marrow puncture. The criteria used to describe a cytogenetic clone and karyotype followed the recommendations of the International System for Human Cytogenetic Nomenclature [15]. Molecular markers such as FLT3-ITD, C-kit, CEBPα, and NPM1 were analyzed.

Table1 .Clinical Characteristics of elderly Patients With refractory or relapsed AML

No	Sex/ Age	FAB type	status	ECOG	Molecular markers(F -LT3,C-K -it,NPM1, CEBPα)	chromosome	HLA matched loci	cycles	OS	Outcome
1	F/60	M2	relapsed	1	C-Kit(+)	45,X,-X,t(8;21)(q22;q22)[7]/46,XX[3]	H5/10	5	26.2	survival(CR)
2	F/66	M2	relapsed	1	Not done	46,XX[10]	H5/10	4	9.9	death
3	F/76	M2	refractory	2	negative	47,XX,+8[2]/47,idem,t(1;12)(p33;q24)[8]	H5/10	1	0.9	death
4	M/87	M6	relapsed	1	negative	84-88,XXYY, del(1)(q22)X2,-3,-3,del(5)(q11)X2,del(6)(q14)X2,-8,der(9)X2,-12,-13,-13,-14,-14,-17,-20,-21[5cp]	H5/10	2	3.3	death
5	M/60	M2	refractory	1	negative	46,XY,t(7;11)(p15;p15)[4]	H0/10	2	2.2	death
6	M/61	M2	relapsed	1	negative	47,XY,+8[5]	H0/10	4	9	death

The protocol was approved by the Human Ethics Committees of the Jiangsu province hospital, Nanjing. All patients and donors gave written informed consent before enrollment onto the study.

B. Treatment Design

All of the 6 patients received DCAG regimen [9] induction chemotherapy before microtransplantation: 15 mg/m^2 intravenously over 4h for 5 consecutive days (day 1-5) and G-CSF of 300 μg/day (day 0-9) for priming combined with cytarabine of 10 mg/m2 q12h for 7 days (day 3-9), aclarubicin of 10 mg/day for 4 days (day 3-6). The G-CSF priming was discontinued if white blood count (WBC) was >20×10^9/L. Each infusion of the cells was performed at 36 hours post-chemotherapy.

Before transplantation, donor and recipient HLA-A, -B, -C, -DRB1, and -DQB1 alleles were typed. Of the 6 patient/donor pairs, 4 were mismatched at 5/10 of HLA loci and 2 were at 0/10.

C. Mobilization and Apheresis of donor peripheral mononuclear cells

Apheresis of donor peripheral mononuclear cells was collected with a CS-3000S cell separator at the fifth and the sixth day after the donors were subcutaneously injected with 5-10 ug/kg granulocyte colony-stimulating factor (G-CSF) twice a day. Donor cells were aliquoted and cryopreserved in liquid nitrogen, but fresh collected cells were used as the first cycle [10]-[11]. The median numbers of mononuclear (MNC), CD34$^+$, CD3$^+$, natural killer (NK) cells, NK-T cells infused per cycle were 3.33×10^8/kg (range, 2.69×10^8 /kg - 4.97×10^8 /kg), 2.94×10^6 /kg (range, 0.6×10^6/kg - 5.09×10^6/kg), 0.91×10^8/kg (range, 0.76×10^8/kg - 3.33×10^8/kg), 0.2×10^8/kg (range,0.1×10^8/kg - 0.29×10^8/kg), and 0.07×10^8/kg (range,0.04×10^8/kg -0.21×10^8/kg), respectively (see Table Ⅱ).

Table2 Cells content in products for microtransplantation

NO	MNC(10^8/kg)	CD34$^+$(10^6/kg)	CD3$^+$(10^8/kg)	NK(10^8/kg)	NK-T(10^8/kg)
1	3.22	3	0.96	0.2	0.16
2	3.25	3.12	0.91	0.21	0.04
3	3.41	2.88	0.9	0.2	0.07
4	4.33	5.09	3.33	0.29	0.21
5	4.97	0.6	0.76	0.15	0.07
6	2.69	1.53	0.76	0.1	0.06

D. Supportive care

The blood routine, lactate dehydrogenase, liver and renal function were dynamically performed during the period of therapy. Empirical antibiotics and anti-fungal agents were administered when the body temperature was over 38.5℃. At the same time, etiologic examinations including hemoculture and secretion culture were monitored. Irradiated blood components were infused if hemoglobin was under 70 g/L or platelet under 20×10^9 /L, respectively. Bone marrow aspiration was taken at the time of recovery of peripheral hemogram, or 4 weeks later after microtransplantation.

E. Treatment responses and evaluation of outcomes

Treatment responses were defined according to the modified 2003 IWG criteria [14]. Morphologic CR included normalization of bone marrow blasts (≤5% blasts) and peripheral blood counts (absolute neutrophil count ≥1.0×10^9/L and platelet >100 ×10^9/L). Time to hematopoietic recovery was measured from the first day of 3 consecutive days on which the neutrophil count was >0.5×10^9/L and platelet count was more than 20×10^9/L, respectively. Early death (ED) was defined as mortality within the first 4 weeks after induction chemotherapy. GVHD was defined according

to the published criteria [15]. OS was calculated from day 1 of treatment with DCAG-G regimen, until death from any cause or last follow-up.

III. RESULTS

A. Response to treatment

Six patients had a CR rate of 33.3% after the first cycle. After the second cycle, only one patient with CR acquired a second CR. Upon final analysis (December 31, 2015), only one patient had a consecutive CR. For all patients, the median OS of all patients was 6.2 months (range, 0.9-26.2).

B. Treatment Toxicity

ED occurred in one patient (16.7%) because of severe infection and poor performance (ECOG PS of 2). The most frequently seen adverse effect was myelosuppression. The median time to neutrophil and platelet recovery was 12 days (range, 0 to 38), 14.5 days (range, 5 to 39) during the treatment period, respectively. The incidence of non-hematological toxicities was low. One patient developed slight skin rash after the first cycle of the therapy, which disappeared after antiallergic treatment. Febrile neutropenia, septicemia, pulmonary infection, oral infection and intestinal infection occurred in 72.2%, 16.7%, 27.8%, 11.1%, and 5.6% of patients during the 18 cycles of therapy, respectively. Five of 6 patients died and reasons for patient death included non-remission disease (20%), severe infections (60%), and cardiac dysfunction (20%). Neither acute GVHD nor chronic GVHD was observed in any of the patients during the entire treatment and follow-up period.

IV. DISCUSSION

The treatment of patients with AML in elderly has improved [9],[10],[17], whereas prognosis of those patients with refractory or relapsed AML following intensive chemotherapy is dismal. Tamjeed [7] evaluated high dose cytarabine, mitoxantrone and L-asparaginase regimen salvage for relapsed or refractory AML in the elderly. Results showed that CR/CRi (all marrow criteria of CR but without either complete platelet or neutrophil recovery) was achieved in 33% and median OS was 5.2 months of patients ≥60 years old. A recent study of azacitidine chemotherapy on 130 older patients with relapsed and refractory AML obtained a CR/CRi rate of 17%, and a median OS of 8.4 months in all patients [8]. Our study showed that the DCAG-M regimen achieved a higher CR rate of 33.3% compared with chemotherapy treatment [7],[8]. In consistent with the previous reports [10],[11], many more NK cells (0.1×10^8/kg-0.29×10^8/kg) and $CD3^+$ cells (0.76×10^8/kg-3.33×10^8/kg) were infused. The size of alloreactive donor purified NK cells repertoire is correlated with reduced relapse rate after NK cell immunotherapy in elderly patients with AML[18]. In particular, the number of circulating T regulatory cells after NK cell infusion and the post-chemotherapy serum concentrations of some cytokines, such as interleukin(IL)-15 and IL-35, critically influences the capacity of infused NK cells to expand and to kill AML cells [19],[20]. Moreover, microtransplantation was reported to have significantly high OS because of a high dose of $CD3^+$ cells (0.4×10^8/kg-2.4×10^8/kg) infused [11]. Thus, the favorable result was due to the remarkable decitabine in combination with microtransplantation immunotherapy.

Relapsed or refractory AML is associated with an extremely poor prognosis, especially in the elderly. Adverse cytogenetics, poor performance status (ECOG PS of 3), as already reported [9], were identified as the independent adverse prognostic factors in elderly AML. In our research, adverse factors that had a significant influence on the short OS (＜4 months) for the three patients included secondary AML (100%), poor karyotype (66.7%), and age older than 70 years (66.7%), whereas one patient with good performance status and favorable karyotype of t (8; 21) achieved persistent CR. These effects suggest the need to carefully evaluate the eligibility to DCAG-M treatment, which should be proposed only to therapy-fit patients.

Our results revealed the median recovery time for neutrophils and platelets was 12 days and 14.5 days, respectively, similar to the published researches [10],[11], and it indicated that microtransplantation can promote hematopoietic recovery. The primary toxicity of our treatment was infection associated with neutropenia. Infection occurred in 61.1% of 6 patients during the whole 18 cycles of therapy. However, no patients suffered from acute GVHD or chronic GVHD during any cycles and follow-up period. It has been suggested that DCAG-M regimen was much safer than the allogeneic hematopoietic stem-cell transplantation [21],[22].

In conclusion, salvage DCAG-M regimen appeared to be a feasible, safe and effective for elderly refractory or raplased AML patients, including more than 70-year-old patients. Further studies on large numbers of patients are needed.

ACKNOWLEDGMENT

This study was supported by grants from National Natural Science Foundation of China (81270614, 81300379, 81570134, 81570141, 81522001). National Public Health Grand Research Foundation (No. 201202017), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institute (No. JX10231801), Program for Development of Innovative Research Teams in the First Affiliated Hospital of Nanjing Medical University, Project of National Key Clinical Specialty, National Science & Technology Pillar Program (No. 2014BAI09B12), and Project Funded by Jiangsu Provincial Special Program of Medical Science (No. BL2014086).

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