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Donor-Derived Natural Killer Cell Infusion after Human Leukocyte Antigen–Haploidentical Hematopoietic Cell Transplantation in Patients with Refractory Acute Leukemia

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The optimum method of donor natural killer cell infusion (DNKI) after allogeneic hematopoietic cell transplantation (HCT) remains unclear. Fifty-one patients (age range, 19 years to 67 years) with refractory acute leukemia underwent HLA-haploidentical HCT and underwent DNKI on days 6, 9, 13, and 20 of HCT. Median DNKI doses were .5, .5, 1.0, and 2.0×10^8/kg cells, respectively. During DNKI, 33 of the 45 evaluated patients (73%) developed fever (>38.3°C) along with weight gain (median, 13%; range, 2% to 31%) and/or hyperbilirubinemia (median, 6.2 mg/dL; range, 1.0 mg/dL to 35.1 mg/dL); the toxicity was reversible in 90% of patients. After transplantation, we observed cumulative incidences of neutrophil engraftment (≥500/μL), grade 2 to 4 acute graft-versus-host disease (GVHD), chronic GVHD, and nonrelapse mortality of 84%, 28%, 30%, and 16%, respectively. The leukemia complete remission rate was 57% at 1 month after HCT and 3-year cumulative incidence of leukemia progression was 75%. When analyzed together with our historical cohort of 40 patients with refractory acute leukemia who underwent haploidentical HCT and DNKI on days 14 and 21 only, higher expression of NKp30 (>90%) on donor NK cells was an independent predictor of higher complete remission (hazard ratio, 5.59) and less leukemia progression (hazard ratio, .57). Additional DNKI on days 6 and 9 was not associated with less leukemia progression (75% versus 55%).

INTRODUCTION

Acute leukemia is a group of heterogeneous malignant disorders, characterized by uncontrolled clonal expansion of blasts in the bone marrow, peripheral blood, and extramedullary tissue. Patients with acute leukemia refractory to primary induction chemotherapy or salvage chemotherapy after recurrence have a dismal prognosis, with a median survival of several months and virtually no chance of long-term survival [1,2]. Allogeneic hematopoietic cell transplantation (HCT) can be considered in these patients; however, they should be younger and have adequate performance status [3,4]. Allogeneic HCT from a related donor with a fully mismatched HLA haplotype is feasible using reduced-intensity conditioning [5-10]. In haploidentical HCT, donor availability is rarely a problem, additional cell donation is feasible, and when using reduced-intensity conditioning, HCT can be performed in older patients. However, recurrence of underlying leukemia after HCT remains the most frequent cause of treatment failure [7,8].

Natural killer (NK) cells are a unique subset of lymphocytes that express CD56 but not CD3 [11,12]. NK cell activation is enhanced by the decreased expression of MHC class I antigen on tumor cells because of a failure to engage inhibitory receptors, notably killer immunoglobulin-like receptors (KIR) on NK cells [13-15]. Lack of HLA ligand for donor-inhibitory KIR was correlated with decreased recurrence of myeloid leukemia after HLA-matched sibling [16] or

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unrelated donor [17,18] HCT. In MHC-matched [19] and -mismatched [20] murine HCT models, post-transplantation infusion of donor-type NK cells exhibits antileukemia effects without graft-versus-host disease (GVHD). Activation of NK cell–activating receptors, such as NKG2D and natural cytotoxicity receptors, by ligands expressed by leukemic cells but not by normal tissue is a basis of such beneficial effects [21-23].

Adoptive transfer of NK cells from allogeneic donors to patients with malignancies has been performed after HCT [24-29] and without prior HCT [30-35]. In most studies, donor NK cells were obtained from lymphapheresis products using a magnetic-activated cell-sorting system, generating approximately 1×10^7 NK cells/kg to 3×10^7 NK cells/kg of patient body weight from a single-day lymphapheresis [24,25,27,30-33]. Alternatively, NK cells can be generated by ex vivo culture using cytokines [26,28,34,36,37]. For example, using IL-15 and IL-21, we generated NK cells from $CD34^+$ progenitor cells from a mobilized leukapheresis product [26], with a median yield of $.9 \times 10^7$ cells/kg after a 6-week culture. These cells were well tolerated when infused 6 weeks after haploidentical HCT. Furthermore, when we generated NK cells from the $CD3^+$ cell-depleted portion (as opposed to $CD34^+$ cells) of the mobilized leukapheresis product, more cells were obtained (median yield, 2.0×10^8 cells/kg) after a shorter culture period. These cells were infused 2 to 3 weeks after haploidentical HCT without acute infusion-associated toxicity and without increased acute and chronic GVHD or nonrelapse mortality (NRM) when compared with a historical cohort of patients who underwent haploidentical HCT without high-dose donor NK cell infusion (DNKI) [28]. Although decreased post-transplantation leukemia progression was suggested in patients with refractory acute myelogenous leukemia (AML) compared with the historical cohort, recurrence (46%) remained the major cause of treatment failure.

Currently, much remains undetermined regarding optimum methods for antimalignancy NK cell therapy; particularly, NK cell generation methods (separation versus ex vivo expansion), dose, and mode of administration (with versus without prior allogeneic HCT). When administered after HCT, the timing in relation to HCT needs to be determined. In our previous study [28], 7 of 40 patients with refractory acute leukemia failed to clear their leukemia cells after HCT and showed early progression within 1 month, which suggested that earlier administration of DNKI may be necessary to achieve better post-HCT leukemia control. Therefore, we investigated the effect of DNKI administered sooner after haploidentical HCT (6 to 9 days)—along with DNKI administered over 2 to 3 weeks, as in our previous study [28]— in patients with refractory acute leukemia.

PATIENTS AND METHODS

Patients, HLA-Haploidentical HCT, and DNKI

This protocol for HLA-haploidentical HCT with subsequent DNKI (Figure 1A) began in February 2013; 56 consecutive eligible patients had enrolled by May 2015. Of these, 2 patients died during conditioning therapy without receiving HCT and 3 died early after HCT without receiving DNKI. Thus, the remaining 51 patients who received at least 1 DNKI after HCT were assessed. All enrolled patients had *refractory acute leukemia* defined as failure to achieve complete remission (CR) after 2 cycles of induction chemotherapy, CR duration <6 months, failure to achieve CR after salvage chemotherapy, or second recurrence. They also met the following inclusion criteria: a Karnofsky performance scale index ≥70 and adequate organ function (total bilirubin <3.0 mg/dL, aspartate aminotransferase <250 U/L, creatinine <2.0 mg/dL, and no clinically evident cardiac dysfunction). The protocol was approved by the Asan Medical Center institutional review board and Korean Food and Drug Administration and is registered at clinicaltrials.gov as #NCT01795378. All patients and donors provided written informed consent.

Patient and donor HLA-A, -B, -C, and -DRB1 typing and donor KIR genotyping were performed by PCR-based methods. The haploidentical HCT methodology used has been described previously [7,28,38]. Briefly, the conditioning regimen included intravenous busulfan 3.2 mg/kg/day on days –7 and –6 of HCT (day 0 being the first day of donor cell infusion), intravenous fludarabine 30 mg/m^2/day on days –7 to –2, and intravenous thymoglobulin (Genzyme Transplant, Cambridge, MA) 3 mg/kg/day on days –3 to –1. Granulocyte colony–stimulating factor (G-CSF) 450 μg was intravenously infused daily starting on day 5 until absolute neutrophil counts (ANC) recovered to 3000/μL. Although there was no specific goal of leukemia burden before HCT, to control a rapidly increasing white blood cell count before HCT, 18 patients in the study received intravenous cytarabine 200 mg/m^2/day to 500 mg/m^2/day for 2 to 5 days within the 2 weeks before the conditioning. For GVHD prophylaxis, all patients received cyclosporine.

Cell donors underwent 2 sessions of cell collection (Figure 1A). For the first session, the donors received G-CSF 10 μg/kg subcutaneously daily for 3 days beginning on day –10 and underwent large volume leukapheresis (Amicus, Fenwal, Inc, Lake Zurich, IL) on the fourth day (day –7). For the second session of cell collection, beginning on day –3, the donors received the same dose of G-CSF subcutaneously daily for 5 to 6 days and underwent daily leukapheresis for 2 to 4 days. The cells collected during the first 1 to 3 days of the second session were administered to patients through central venous catheters on the same day for the purpose of HCT. Cells collected in the first session and on the last day of the second session were transported to a Korean Food and Drug Administration–inspected laboratory, located in the hospital, for NK cell generation.

Patients received donor-derived NK cells 4 times: 6, 9, 13, and 20 days after HCT. A DNKI delay of up to 2 days was allowed, depending on the patient condition, NK cell generation delay, or holidays. In the initial stage of the study, per the protocol design, 2 3-patient cohorts each received $.2 \times 10^8$ and $.5 \times 10^8$ donor NK cells on days 6 and 9. In 5 of these 6 patients, we observed fever, weight gain, and hyperbilirubinemia (described below). Therefore, in the subsequent patients, the DNKI doses for days 6 and 9 were fixed at $.5 \times 10^8$ cells/kg without further increases. The cell doses for DNKI administered on days 13 and 20 were based on the quantity of cells available [28]. That is, for day 13, 1×10^8 donor NK cells/kg or about one-half of the available cell culture products were administered. For day 20, the remaining cell culture products were administered. Donor NK cells were infused over 1 hour through a central venous catheter, with pheniramine 45.5 mg administered by brief intravenous infusion 30 minutes earlier. No cytokines, such as IL-2 or IL-15, were administered along with DNKI.

Patient Evaluation

Criteria for neutrophil and platelet engraftment [7] and acute and chronic GVHD [39,40] have been described. Bone marrow was biopsied on the day before conditioning therapy and again 4 weeks after HCT. *CR* of acute leukemia was defined as the recovery of a peripheral blood ANC to >1000/μL and evidence of donor cell regeneration in a bone marrow aspirate with ≤5% blasts. In patients with documented CR, *leukemia progression* was defined as >5% leukemia blasts in the bone marrow or leukemia in extramedullary sites. In patients with persistent leukemia after HCT, the *day of progression* was defined as the day on which leukemia blasts reappeared in the peripheral blood. Serum levels of IFN-γ, TNF-α, IL-2, and IL-15 were measured by ELISA on days –8, 6, 13, and 20. Serum thymoglobulin levels were measured on days 6, 13, and 20 by an indirect method that detects rabbit serum on allogeneic lymphocytes stained with goat antirabbit fluorescein isothiocyanate [41]. Hematopoietic chimerism was assessed 30, 90, and 180 days after HCT by a PCR-based procedure using short tandem DNA repeats.

Donor NK Cell Generation and Evaluation

The methodologies for NK cell production and quality control have been described [26,28]. Briefly, $CD3^+$ cells were depleted from the leukapheresis product by the RosetteSep (StemCell Technologies Inc., Vancouver, BC, Canada) system. CD3-depleted cells were then cultured in media supplemented with human IL-15, IL-21, and hydrocortisone. After 2 to 3 weeks of cultivation, cells were harvested, suspended in human albumin solution (5%), and infused into patients. NK cell products were immunophenotyped by flow cytometry after fluorescence labeling. Cell viability was determined by labeling differentiated cells with propidium iodide and analyzing them by flow cytometry. The ability of donor NK cells to kill target cells was measured using Raj and K652 cells and a calcein-release assay. Donor NK cell IFN-γ secretion was determined in culture supernatant by ELISA.

Statistical Analysis

Any untoward reaction associated with DNKI was assessed. Post-transplantation events, such as engraftment, GVHD, and NRM, were recorded. Times to engraftment, GVHD, NRM, and leukemia progression were