Biol Blood Marrow Transplant 22 (2016) 705–709

Biology of Blood and
Marrow Transplantation

journal homepage: www.bbmt.org

Clinical Research: Alternative Donors

Phase II Study of Haploidentical Natural Killer Cell Infusion for Treatment of Relapsed or Persistent Myeloid Malignancies Following Allogeneic Hematopoietic Cell Transplantation

Brian C. Shaffer [1,2], Jean-Benoit Le Luduec [3], Christopher Forlenza [2,4], Ann A. Jakubowski [1,2], Miguel-Angel Perales [1,2], James W. Young [1,2,3], Katharine C. Hsu [1,2,3,*]

[1] Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York
[2] Weill Cornell Medical College, New York, New York
[3] Immunology Program, Sloan Kettering Institute for Cancer Research, New York, New York
[4] Bone Marrow Transplant Service, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York

Article history:
Received 29 September 2015
Accepted 31 December 2015

Key Words:
Natural killer cell
Allogeneic transplantation
AML
MDS

ABSTRACT

We conducted a phase 2 study to determine the efficacy of HLA-haploidentical related donor natural killer (NK) cells after cyclophosphamide-based lymphodepletion in patients with relapsed or progressive acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) following allogeneic hematopoietic cell transplantation (HCT). Eight patients (2 with MDS and 6 with AML) were treated with cyclophosphamide 50 mg/kg on day −3 and day −2 before infusion of NK cells isolated from a haploidentical related donor. One patient also received fludarabine 25 mg/m^2/day for 4 days. Six doses of 1 million units of interleukin-2 (IL-2) were administered on alternating days beginning on day −1. The median number of NK cells infused was 10.6×10^6/kg (range, 4.3 to 22.4×10^6/kg), and the median number of CD3 cells infused was 2.1×10^3/kg (range, 1.9 to 40×10^3/kg). NK infusions were well tolerated, with a median time to neutrophil recovery of 19 days (range, 7 days to not achieved) and no incidence of graft-versus-host disease after NK infusion. One patient with AML and 1 patient with MDS achieved a complete response, but relapsed at 1.7 and 1.8 months, respectively. One patient with MDS experienced resolution of dysplastic features but persistence of clonal karyotype abnormalities; this patient was stable at 65 months after NK cell therapy. The median duration of survival was 12.9 months (range, 0.8 to 65.3 months). Chimerism analysis of CD3$^-$/CD56$^+$ peripheral blood cells did not detect any circulating haploidentical NK cells after infusion. NK phenotyping was performed in 7 patients during and after IL-2 infusion. We found a slight trend toward greater expression of KIR2DL2/2DL3/2DS2 (5% versus 28%; $P = .03$) at 14 days in patients who survived longer than 6 months from NK cell infusion (n = 4) compared with those who died within 6 months of NK cell therapy (n = 3). In summary, our data support the safety of haploidentical NK cell infusion after allogeneic HCT.

© 2016 American Society for Blood and Marrow Transplantation.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) can result in durable remission of malignancies that arise from myeloid progenitor cells, such as myelodysplastic syndrome (MDS), acute myelogenous leukemia (AML), or chronic myelogenous leukemia (CML). This is due to an increasingly recognized graft-versus-leukemia (GVL) effect mediated by alloreactive donor lymphocytes, including T cells and natural killer (NK) cells [1,2]. Unfortunately, relapse occurs in 40% of patients undergoing HCT for myeloid malignancies, and there are limited treatment options for these individuals [3,4]. Chemotherapy may result in subsequent remission, but is poorly tolerated in HCT survivors, who typically have tenuous immune function and poor performance status.

A second strategy for addressing relapse is donor lymphocyte infusion (DLI) to increase the potential for GVL. DLI results in remission of CML in approximately 70% to 90% cases; however, response is much less frequent in patients with MDS and occurs only rarely in patients with AML [4-6]. DLI also may induce serious graft-versus-host disease (GVHD), and thus should be used with caution.

Thus, available therapies to treat relapsed myeloid malignancy, particularly MDS and AML, have low efficacy and

Financial disclosure: See Acknowledgments on page 709.

* Correspondence and reprint requests: Katharine C. Hsu, MD, PhD, Adult Blood and Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY.
E-mail address: hsuk@mskcc.org (K.C. Hsu).

http://dx.doi.org/10.1016/j.bbmt.2015.12.028
1083-8791/© 2016 American Society for Blood and Marrow Transplantation.

can incur serious adverse effects. Consequently, there are few long-terms survivors of relapse after HCT.

Natural killer (NK) cells play a key role in mediating the GVL effect against myeloid malignancies, particularly AML [7-11]. An array of activating and inhibitory cell surface receptors control NK effector function against target cells, including NKG2A, NKG2D, natural cytotoxicity receptors, and the killer Ig-like receptors (KIRs) [12,13]. Among these, the latter have gained considerable attention after the publication of reports associating donor KIR genotypes with NK alloreactivity and leukemia control in murine and human HLA-mismatched allogeneic HCT [10,14-16]. NK cells stochastically express inhibitory KIR that interact with specific epitopes in class 1 HLA on target cells. KIR2DL2/3 recognize HLA-C characterized by Lys80 (HLA-C1 allotypes); KIR2DL1 recognizes HLA-C characterized by Asn80 (HLA-C2 allotypes); and KIR3DL1 recognizes HLA-B and HLA-A allotypes with the Bw4 motif [17,18]. In addition, the activating KIR2DS1 recognizes HLA-C2 [19]. Malignant cells lacking HLA capable of binding inhibitory KIR will induce a net activating signal in NK cells, leading to improved effector function in the latter. This effect is pronounced when the inhibitory KIR ligand is expressed in the transplant donor, a phenomenon referred to as activation of licensed NK cells recognizing "missing self" HLA determinants in the host [14]. Thus, NK effector function may be enhanced by the intentional use of HLA-mismatched donors.

In the present study, we tested whether HLA-haploidentical purified NK cells can exert a GVL effect without inducing GVHD in patients with relapsed myeloid malignancy after HLA-matched HCT. Adoptive transfer of purified NK cells offers the advantage of leukemic cell toxicity without the risk of GVHD, a frequent complication of unmodified DLI. Moreover, administration of a purified NK product enables the use of highly HLA-mismatched NK cell donors, increasing the likelihood of a greater antitumor effect.

The primary study endpoint was to determine the feasibility and safety of adoptive transfer of haploidentical NK cells for patients with a myeloid malignancy who relapsed after undergoing HCT from an HLA-matched sibling or unrelated donor. Adoptive NK cell transfer after allo HCT is well tolerated and in the lymphodepleted post-transplant recipient may induce GVL without serious toxicity.

METHODS

Subject Eligibility, Response Assessment, and Treatment Plan

Patients of any age who had relapsed or persistent AML, MDS, or blastic CML following allogeneic HCT and were determined ineligible for second HCT were eligible for this study. The study was approved by the Institutional Review Board and regulatory authorities of Memorial Sloan Kettering Cancer Center. All patients gave informed consent. The study was registered at ClinicalTrial.gov (identifier NCT00526292).

Patients were required to have ≥5% bone marrow involvement, determined by morphology, karyotype, or fluorescence in situ hybridization. Patients with measurable extramedullary disease were excluded. Patients who received other treatments for relapsed disease were not excluded, as long as they met the criteria for bone marrow before NK infusion. Patients with GVHD were not excluded provided that they did not receive systemic immunosuppression for 2 weeks before enrollment. Toxicities were graded according to the National Cancer Institutes Common Terminology Criteria for Adverse Events, version 3.0. Clinical responses were assessed using established criteria [20,21]. Response was evaluated by bone marrow aspiration/biopsy at days +30, +100, +200, and +365. Neutrophil engraftment was defined as the first day of a blood neutrophil count of >500 for 3 or more consecutive days.

Cytoreductive Therapy, IL-2, and Supportive Care

Cytoreductive chemotherapy included cyclophosphamide 50 mg/kg/day i.v. on days −3 and −2. One patient received cyclophosphamide on days −6 and −5 in addition to fludarabine 25 mg/m^2/day on days −6 through −2. To promote in vivo expansion of donor NK cells, patients received IL-2 at a dose of 1 million units per m^2 every 48 hours for 6 doses, beginning on day −1. NK cells were infused on day 0. Patients were treated with prophylactic antimicrobials for *Pneumocystis jiroveci*, Herpesviridae, and *Candida* species during therapy according to institutional guidelines.

Donor Selection, Leukapheresis, and Immunomagnetic Isolation of Donor-Derived NK Cells for Adoptive Transfer

Eligible donors were HLA-haploidentical family members who met standard criteria for cell donation based on Foundation for the Accreditation of Cellular Therapy/National Marrow Donor Program guidelines and who provided informed consent. All donors underwent KIR typing as described below. The selected donor underwent a standard 10-L apheresis the day before the anticipated cell infusion. Isolated peripheral blood mononuclear cells (PBMCs) were used to enrich NK cells on a CliniMACS clinical cell selection device (Miltenyi Biotech, Gladbach, Germany) following a 2-step procedure. In the first step, PBMCs were first depleted of CD3$^+$ cells (CD3 Reagent; Miltenyi Biotec). The CD3$^-$ cell product was collected, washed once and underwent a positive selection for CD56$^+$ cells (CD56 Reagent; Miltenyi Biotec). Viability was assessed, and phenotyping was performed to evaluate the absolute numbers of CD45$^+$, CD3$^+$, and CD56$^+$ cells. Cytokines were not used during in vitro NK cell processing. NK cells were considered acceptable for administration if the total number of CD3$^+$ cells did not exceed 2×10^5/kg, the CD56 enrichment resulted in ≥90% CD3$^-$/CD56$^+$ pure cell product, the viability was ≥70%, endotoxin was ≤5 EU/mL, and mycoplasma, Gram stain, and bacterial/fungal cultures were negative. Sterility samples from the final infused product were obtained and monitored in culture for 14 days.

Immunophenotyping and Cell Culture Conditions

PBMCs were isolated by Ficoll density gradient centrifugation. Effector cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% sodium pyruvate, and 1% 2-mercaptoethanol, supplemented with human IL-2 (Proleukin; Prometheus Laboratories, San Diego, CA) at 200 U/mL, and incubated at 37°C with 5% CO_2 for 12 to 16 hours before assays of function. CD107 mobilization and intracellular IFN-γ production served as indicators of effector cell activation [22-24]. PBMCs (5×10^5/well) or NK cells (1×10^5/well) were incubated with the human erythroleukemia cell line K562 (American Type Culture Collection, Manassas, VA) serving as target cells, at ratios of 1:5 and 1:1, respectively, for 4 hours in 96-well U-bottom plates with 200 μL of medium, described above, per well. APCH7-conjugated anti-CD107a (clone H4A3, BD Biosciences; Franklin Lakes, NJ) was added to each well before incubation. Cells were stained with anti-CD3-BV650 (clone SK7; BD Biosciences), anti−CD56-ECD (clone N901; Beckman Coulter; Brea, CA); anti−KIR2DL1/2DS1-PEcy5.5 (clone EB6B; Beckman Coulter), anti−KIR2DL2/2DL3/2DS2-FITC (clone CH-L; BD Biosciences), anti−KIR3DL1/S1-APC (clone Z27; Beckman Coulter), anti−KIR3DL1-Alexa Fluor 700 (clone DX9; Biolegend, San Diego, CA), anti−NKG2A-PEcy7 (clone Z199; Beckman Coulter), and anti−LIR-1-PE (clone HP-F1; Beckman Coulter). For IFN-γ evaluation, brefeldin-A (2 μg/mL; Sigma-Aldrich, St Louis, MO) and GolgiStop (1 μg/mL; BD Biosciences) were added to the mixture 1 hour after incubation, and FIX & PERM (Invitrogen, Carlsbad, CA) was used for staining. Cells were analyzed using multicolor flow cytometry on a FACS LSRFortessa instrument with FACS Diva software (BD Biosciences). Results were interpreted using FlowJo (FlowJo; Ashland, OR) and GraphPad Prism (GraphPad Software; La Jolla, CA) software.

RESULTS

Study Population

The study population is described in Table 1. The median age at treatment was 19.0 years (range, 1.9 to 55.9 years). Seven patients underwent transplantation from a HLA-matched sibling, and 1 patient did so ofrom a HLA-matched unrelated donor. The median time to relapse after HCT was 3.5 months (range, 1 to 94 months). The median time from HCT to NK infusion was 6.8 months (range, 3.9 to 152 months). Five of 8 patients had cytoreductive chemotherapy before NK infusion. One patient with extramedullary AML underwent surgical cytoreduction but had residual disease at the time of study enrollment. One patient was treated with azacytidine followed by DLI without response

Download English Version:

https://daneshyari.com/en/article/2101351

Download Persian Version:

https://daneshyari.com/article/2101351

Daneshyari.com