Biol Blood Marrow Transplant xxx (2016) 1–9

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Haploidentical Natural Killer Cells Infused before Allogeneic Stem Cell Transplantation for Myeloid Malignancies: A Phase I Trial

Dean A. Lee [1,*], Cecele J. Denman [1], Gabriela Rondon [2], Glenda Woodworth [2], Julianne Chen [2], Tobi Fisher [2], Indreshpal Kaur [2], Marcelo Fernandez-Vina [3], Kai Cao [4], Stefan Ciurea [2], Elizabeth J. Shpall [2], Richard E. Champlin [2]

[1] Division of Pediatrics, Cell Therapy Section, University of Texas MD Anderson Cancer Center, Houston, Texas
[2] Division of Cancer Medicine, Department of Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, Texas
[3] Histocompatibility, Immunogenetics, and Disease Profiling Laboratory, Stanford University Medical School Blood Center, Palo Alto, California
[4] Division of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston, Texas

Article history:
Received 5 December 2015
Accepted 11 April 2016

Key Words:
Stem cell transplant
Natural killer (NK) cells
Adoptive immunotherapy
Acute myeloid leukemia
Chronic myelogenous leukemia
Myelodysplastic syndrome

A B S T R A C T

Allogeneic stem cell transplantation is an effective treatment for high-risk myeloid malignancies, but relapse remains the major post-transplantation cause of treatment failure. Alloreactive natural killer (NK) cells mediate a potent antileukemic effect and may also enhance engraftment and reduce graft-versus-host disease (GVHD). Haploidentical transplantations provide a setting in which NK cell alloreactivity can be manipulated, but they are associated with high rates of GVHD. We performed a phase I study infusing escalating doses of NK cells from an HLA haploidentical–related donor—selected for alloreactivity when possible—as a component of the preparative regimen for allotransplantation from a separate HLA-identical donor. The goal of infusing third-party alloreactive NK cells was to augment the antileukemic effect of the transplantation without worsening GVHD and, thus, improve the overall outcome of hematopoietic transplantation. Twenty-one patients with high-risk acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), or chronic myelogenous leukemia refractory or beyond first remission received a preparative regimen with busulfan and fludarabine followed by infusion of apheresis-derived, antibody-selected, and IL-2–activated NK cells. Doses were initially based on total nucleated cell (TNC) content and later based on $CD56^+$ cells to reduce variability. $CD56^+$ content ranged from .02 to 8.32 $\times$ 10^6/kg. IL-2, .5 $\times$ 10^6 units/m^2 subcutaneously was administered daily for 5 days in the final cohort (n = 10). $CD3^+$ cells in the NK cell product were required to be $< 10^5$/kg. Median relapse-free, overall, and GVHD-free/relapse-free survival for all patients enrolled was 102, 233, and 89 days, respectively. Five patients are alive, 5 patients died of transplantation-related causes, and 11 patients died of relapse. Despite the small sample size, survival was highly associated with $CD56^+$ cells delivered (P = .022) and development of $\geq$ grade 3 GVHD (P = .006). There were nonsignificant trends toward higher survival rates in those receiving NK cells from KIR ligand–mismatched donors and KIR-B haplotype donors. There was no association with disease type, remission at time of transplantation, or KIR content. GVHD was not associated with TNC, $CD56^+$, or $CD3^+$ cells infused in the NK cell product or the stem cell product. This trial demonstrates a lack of major toxicity attributable to third-party NK cell infusions delivered in combination with an HLA-compatible allogeneic transplantation. The infusion of haploidentical alloreactive NK cells was well tolerated and did not interfere with engraftment or increase the rate of GVHD after allogeneic hematopoietic transplantation. Durable complete remissions occurred in 5 patients at high risk for disease recurrence. This approach is being further developed in a phase I/II trial with ex vivo–expanded NK cells to increase the NK cell dose with the objective of reducing relapse and improving the outcome of allogeneic hematopoietic transplantation for AML/MDS.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is effective for myeloid malignancies supporting administration of high-dose chemotherapy and inducing an immunologic

Financial disclosure: See Acknowledgments on page 8.

* Correspondence and reprint requests: Dean A. Lee, MD, PhD, Division of Pediatrics, Unit 853, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.
E-mail address: dalee@mdanderson.org (D.A. Lee).

http://dx.doi.org/10.1016/j.bbmt.2016.04.009
1083-8791/

graft-versus-leukemia (GVL) effect. However, relapse remains the major post-transplantation cause of treatment failure [1].

Natural killer (NK) cells have been appreciated as contributing to the GVL effect without directly causing graft-versus-host disease (GVHD) [2]. NK cell number, as measured by the dose in the stem cell graft or recovery after transplantation, has been associated with a decreased relapse rate [3,4]. NK cells are governed by activating and inhibitory receptors. NK cells can be selected for increased alloreactivity by mismatch of licensed inhibitory receptors in a setting of missing HLA ligands (KIR receptor:ligand mismatch); these cells may have more potent GVL activity and may also enhance engraftment and reduce GVHD [5] by eliminating host T cells and antigen-presenting cells required for priming a GVHD response [6]. When GVHD is established, however, NK cells may cooperate with the adaptive immune response and exacerbate GVHD [7]. In addition to the release of inhibition caused by missing self, NK cells respond to activating signals to trigger lysis of tumor targets. Activating ligands of NKG2D (MIC and ULBP family members) are upregulated by virus-infected and malignant cells as a consequence of stress [8] and may be further upregulated through genotoxic stress caused by radiation or chemotherapy, sensitizing tumors to NK cell lysis [9].

Haploidentical donors may be selected for the presence of KIR-ligand mismatch, thereby establishing a setting in which the donor NK cells are reactive against recipient tumor cells because of a missing KIR ligand. Haploidentical stem cell transplantation has historically been complicated by excessive GVHD, infection, and treatment-related mortality [10]. We hypothesized that haploidentical third-party NK cells could be added to an HLA-identical hematopoietic transplantation to increase GVL effects without exacerbating GVHD. We designed a phase I clinical trial to determine whether haploidentical NK cells could be safely administered after high-dose chemotherapy and before an HLA-matched allogeneic HSCT, a time of maximum stress sensitization and minimum disease burden.

MATERIALS AND METHODS

Patient Population

Twenty-one patients with high-risk myeloid malignancies were enrolled on protocol 2005-0508 (NCT00402558, phase I dose escalation) or 2010-0099 (NCT01390402, phase II expansion) to receive a 10/10 HLA–matched allograft between October 2006 and June 2013. *High-risk disease* was defined as acute myeloid leukemia (AML) past first remission or primary induction failure, myelodysplastic syndrome (MDS) with intermediate or high-risk international prognostic scoring system score, or chronic myelogenous leukemia (CML) that had failed control with tyrosine kinase inhibitor or in accelerated or blast phase. Patients were required to meet standard institutional transplantation criteria for cardiac, liver, renal, and pulmonary organ function, and they were initially required to be $\leq$ 60 years of age. The age requirement was later extended to age $\leq$ 70 years.

Hematopoietic progenitor cells were obtained from granulocyte colony–stimulating factor–mobilized peripheral blood collected by apheresis. The protocol required collecting peripheral blood stem cells of at least 6×10^6 CD34$^+$ cells/kg recipient weight (goal 8×10^6 CD34$^+$ cells/kg). On day 0, 4×10^6 CD34$^+$ cells/kg were infused, retaining at least 2×10^6 CD34$^+$ cells/kg as a backup in case of graft failure. Donors were related (n = 13) or unrelated (n = 8). Hematopoietic stem cells procured from unrelated donors were obtained through the National Marrow Donor Program.

For the purpose of selecting haploidentical NK cell donors from available family members, NK cell alloreactivity was initially determined using the KIR ligand:ligand mismatch model, defined as the presence of a KIR-ligand (HLA group C1, C2, or Bw4) in the donor that was missing in the recipient. KIR genotyping for the selected donor was then obtained to confirm KIR mismatch by establishing the presence of the KIR-receptor gene relevant to the mismatched KIR ligand. Donors were required to have mismatch for the phase I dose-escalation study. Because many studies report that KIR:KIR ligand mismatch is not required for a response to treatment with NK cells, the phase II expansion study preferred—but did not require—a mismatched donor when possible.

The protocols were approved by the institutional review board of MD Anderson Cancer Center and conducted under Investigational New Drug applications from the Food and Drug Administration. All patients and NK cell donors provided written informed consent to the protocol.

Transplantation Regimen

All patients received fludarabine 40 mg/m^2/day and busulfan 130 mg/m^2/day (adjusted for ideal body weight) for 4 days on days −13 through −10. NK cells were infused on day −8. Thymoglobulin 1.5 mg/kg/day was given on days −3 to −1 to all patients for GVHD prophylaxis and to prevent the NK cells from hindering engraftment. Tacrolimus was started on day −2 and discontinued after 3 months if there was no evidence of GVHD. Methotrexate 5 mg/m^2 was given on days 1, 2, 6, and 11. All patients received granulocyte colony–stimulating factor 5 mcg/kg/day starting on day 7 and until absolute neutrophil counts were $> 500 \times 10^9$/L for 3 consecutive days. Standard antimicrobial prophylaxis was provided with voriconazole, pentamidine or trimethoprim-sulfamethoxazole, and acyclovir or valacyclovir for fungal, pneumocystis jiroveci, and herpes simplex, respectively.

NK cell product

The NK cell product was produced from a steady state apheresis product of up to 2×10^{10} peripheral blood mononuclear cells by first depleting T cells using the CliniMACS device and magnetic-activated cell sorting colloidal super-paramagnetic anti-CD3 monoclonal antibody (Miltenyi Biotec, Auburn, CA). A second-step CD56-positive selection was performed for the first 3 patients but discontinued thereafter to improve cell yield. The NK cell product was then cultured overnight (~16 hours) in complete media containing 1000 IU/mL recombinant human IL-2 (Proleukin; Chiron, Emeryville, CA) and washed twice with normal saline using a COBE 2991 cell processor (COBE BCT, Lakewood, CO) before intravenous infusion.

Patients were treated in 4 dose levels of the NK cell–enriched product based on total nucleated cell (TNC) content, as follows: (1) 10^6 cells/kg, (2) 5×10^6/kg, (3) 3×10^7/kg, and (4) 3×10^7/kg (or all cells collected) followed by systemic IL-2 .5 million units/m^2 subcutaneously daily for 5 days. A subsequent phase II study in CML patients used a fixed dose of 5×10^6 CD56$^+$/kg, which was the maximum dose that could be routinely obtained from a steady-state apheresis from normal donors. The median number of NK cells infused at dose levels 3 and 4 was 5×10^6/kg (range, .97 to 8.32). CD3$^+$ cells in the NK cell product were required to be $< 10^5$/kg (median infused, 0×10^5/kg; range, 0 to 1.7).

Trial Design

The primary objective of 2008-0508 was to assess the safety of infusing alloreactive NK cells from a haploidentical relative and to determine the maximum tolerated dose (MTD) of these cells given in combination with busulfan, fludarabine, Thymoglobulin, and allogeneic transplantation from a separate HLA-identical related donor for treatment of AML/MDS. The secondary objectives were to determine if infusion of alloreactive haploidentical NK cells with busulfan and fludarabine/antithymocyte globulin will improve progression-free survival after allogeneic stem cell transplantation from an HLA-compatible donor compared with historical controls, and to determine the rate of engraftment, GVHD, immune reconstitution, and survival after infusion of alloreactive haploidentical NK cells.

The trial was designed as a phase I dose-escalation study followed by a cohort expansion at the identified MTD, or maximum feasible dose if no MTD were reached. The continual reassessment method was used for dose finding, with a target probability for transplantation-related mortality of .30, the baseline rate seen historically in this patient population for this regimen without NK cells.

HLA and KIR Typing and Determination of KIR Content/Matching

Patients and stem cell and NK cell donors were HLA typed at the intermediate-resolution level for alleles at HLA-A, -B, -C, -DRB1, and -DQB1 loci by PCR amplification and oligonucleotide hybridization using commercial kits from Invitrogen (Carlsbad, CA), ELPHA, and/or One Lambda (Canoga Park, CA). The patients and selected donors were typed for the same loci by high-resolution methods using PCR amplification and nucleotide sequencing (Abbott, Abbott Park, IL, or Protrans, Hockenheim, Germany).

KIR genotyping was performed for the selected NK cell donors with reverse sequence-specific oligonucleotide methodology using fluorescently labeled beads conjugated to oligonucleotide probes (One Lambda). KIR typing was performed in 17 of the 21 donors. The revised typing kit allowing discrimination of functional (Func) versus deletion (Del) variants of KIR2DL4 was used for 15 donors. KIR typing was not performed for stem cell donors.

KIR-ligand:ligand mismatch was predicted using the KIR Ligand Calculator maintained by the European Bioinformatics Institute of the European