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Single and Multiple Dose MultiStem (Multipotent Adult Progenitor Cell) Therapy Prophylaxis of Acute Graft-versus-Host Disease in Myeloablative Allogeneic Hematopoietic Cell Transplantation: A Phase 1 Trial

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A B S T R A C T

We conducted a multicenter, phase 1 dose escalation study evaluating the safety of the allogeneic multipotent adult progenitor cell (MAPC, MultiStem, Athersys, Inc., Cleveland, OH) stromal product administered as an adjunct therapy to 36 patients after myeloablative allogeneic hematopoietic cell transplantation (HCT). Patients received increasing doses of MAPC (1, 5, or 10 million cells per kilogram recipient weight) as a single i.v. dose on day +2 after HCT (n = 18), or once weekly for up to 5 doses (1 or 5 million cells per kilogram; n = 18). Infusional and regimen-related toxicities were assessed for 30 days after the last MAPC dose. Of 36 allogeneic HCT donors (17 related and 19 unrelated), 35 were 6/6 HLA matched. MAPC infusions were well tolerated without associated infusional toxicity, graft failure, or increased incidence of infection. Median times to neutrophil (n = 36) and platelet (n = 31) engraftment were 15 (range, 11 to 25) and 16 (range, 11 to 41) days, respectively. The overall cumulative incidences of grades II to IV and III and IV acute graft-versus-host disease (GVHD) at day 100 were 37% and 14%, respectively (n = 36). In the group that received the highest single MAPC dose (10 million cells/kg), day 100 incidence of grade II to IV GVHD was 11.1% (1 of 9) with no observed cases of grade III and IV GVHD. We found no evidence for MHC class II allogeneic antibody induction, although some patients showed an increase in serum anticlass I titers compared with baseline. MAPC contribution to blood chimerism was negligible. These phase I data support the safety of stromal stem cell therapy and suggest that MAPC should be tested prospectively as a novel therapeutic option for GVHD prophylaxis after HCT.

INTRODUCTION

Because of diverse and novel properties, stromal stem cell therapies have been under active investigation as a novel adjunct treatment in conjunction with hematopoietic cell transplantation (HCT). The biologic and immune-privileged traits of mesenchymal stromal cells (MSC) fueled interest in their clinical development as cellular therapeutics in the field of HCT [1,2]. Lazarus et al. were the first to report the feasibility and safety of using ex vivo–expanded autologous and donor allogeneic MSC for infusion into patients undergoing myeloablative autologous and allogeneic HCT [1,3-5]. Although several groups originally reported a positive impact of MSC infusion on engraftment after HCT [3,4,6], greater focus has been for treatment of acute graft-versus-host disease (GVHD), taking advantage of the MSC immunomodulatory capacities. Le Blanc et al. [6] were the first to report

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that infusion of haploidentical MSC into a patient successfully controlled life-threatening acute GVHD in the gastrointestinal tract and liver. Since that time, subsequent reports have demonstrated that MSC were successful in directing sustained suppression of GVHD in additional patients experiencing severe, steroid-refractory acute GVHD [7-9]; however, preliminary reports of phase 3 trials of MSC for treatment of newly diagnosed or steroid-refractory GVHD failed to achieve sustained remission endpoints [10,11].

Multipotent adult progenitor cells (MAPC) are a class of adult stem cells with commonality to MSC but with higher proliferation capability and broader differentiation potential [12]. MAPC are an adult adherent bone marrow (BM)–derived progenitor cell population that meet criteria applied to MSC, including expression of particular surface antigens as well as the capacity to differentiate along mesenchymal lineages. MAPC differ from MSC as they are isolated and grown in hypoxic conditions with media, supplemented with growth factors, and grown at some confluent culture densities. These conditions allow for maintaining activity of the telomerase enzyme, which, as a consequence, contributes to an increase in expansion capacity before senescence [13]. MAPC also possess active immunomodulatory and anti-inflammatory properties [14-16], and comparison studies with MSC in side-by-side cultures have been favorable [13]. The MultiStem (Athersys, Inc., Cleveland, OH) product is a clinical-grade expanded MAPC population that appears nonimmunogenic, can suppress activated T cell proliferation, and has anti-inflammatory and angiogenic properties in both in vitro and in vivo rodent models [15,17]. The isolation conditions employed to produce the Multistem clinical product allow expansion to 60 population doublings or greater, compared with expansion limits of about 30 population doublings under MSC conditions. The expansion potential allows creation of a master cell bank as a manufacturing intermediate with repeated production from the master cell bank, such that all products used in the described clinical study are derived from the same donor, are expanded to the same population doublings, and also would allow potential future phase 2 and phase 3 trials. GVHD studies, arising from this first study, would use product derived from the same donor. Preclinical studies have shown the safety of i.v. infusion of MAPC as well as a survival benefit in a haploidentical acute GVHD rodent model when administered in a prophylactic manner [18-20].

We designed a clinical study to address the safety and efficacy of MAPC product administration during the first month after allogeneic HCT—the critical period for engraftment and early onset of acute GVHD—in patients with hematologic malignancy. Additional preclinical studies were undertaken to support the phase 1 observations and provide insights on the dose and schedule of MAPC that could be necessary for optimal clinical application. Herein, we present these first-in-human phase 1 and preclinical findings using escalating single dose and multiple dose MAPC therapy after allogeneic HCT.

PATIENTS AND METHODS

This open-label, phase 1, multicenter, dose-escalation trial evaluating single and repeated administration of allogeneic MAPC (MultiStem) was undertaken in patients with acute myeloid (AML) or lymphoid leukemia (ALL), chronic myeloid leukemia, or myelodysplasia. The study was approved by the institutional review board at each center and each patient provided written, informed consent after the study had been explained in detail by the treating physician and staff.

Study Population

Eligibility was specified for patients 18 through 65 years of age who were eligible to undergo an allogeneic BM or mobilized peripheral blood HCT from a matched related (MRD) or matched unrelated donor (MUD) at HLA-A, -B, -DR (6/6 allelic match or 5/6 single allelic mismatch, with provision that the DRB1 was molecularly matched). Diagnosis eligibility was specified for AML or ALL in second or subsequent remission, induction failure with <20% BM blasts, or first remission AML with high cytogenetic risk (including del(5q)/-5, del(7q)/-7, abn(3q), (9q), (11q), (20q), (21q), (17p), del(9q), t(6;9), t(9;22), and complex karyotypes [3 or more]), or with identified mutations within the FLT3 gene, or high-risk ALL in first remission (including t(9;22)(q34;11), t(4;11)(q21;q23), t(8;14)(q24.1;q32), low hypodiploidy or near triploidy, complex karyotype with more than 4 abnormalities). Chronic myeloid leukemia patients intolerant of tyrosine kinase inhibitor therapy or whose disease was resistant to therapy (accelerated phase, first, or second chronic phase), or myelodysplasia (intermediate/high or high risk by International Prognostic Scoring System) of lower risk by International Prognostic Scoring System with patient having progressed after prior therapy also were deemed eligible. *Complete remission* was defined as adequate blood counts and the absence of blasts in the peripheral circulation at the time of enrollment and <5% blasts in the BM within 28 days of enrollment.

Four myeloablative conditioning regimens were allowed, based on the patient's disease and status, including: (1) cyclophosphamide (Cy) and total body irradiation (TBI) with a TBI dose of at least 1200 cGy of fractionated TBI; (2) etoposide and TBI, with a TBI dose of at least 1200 cGy of fractionated TBI; (3) busulfan (Bu) and Cy, with at least 14 mg/kg Bu orally, 12.8 mg/kg intravenously, or targeted dose Bu; and (4) fludarabine (Flu) and melphalan (Mel) with a total dose of at least 120 mg/m^2 of Flu administered in divided doses in no less than 3 days and 100 mg/m^2/day but no greater than 140 mg/m^2/day of Mel administered on transplantation day −1. GVHD prophylaxis regimens permitted included methotrexate (MTX) at 15 mg/m^2 i.v. on day +1 and 10 mg/m^2 i.v. on days +3, +6, and +11 in combination with either tacrolimus, administered i.v. or orally to maintain blood levels between 5 and 15 ng/mL, or cyclosporine (CSA), administered to maintain blood levels between 150 and 300 ng/mL. Patients were excluded from the study if their calculated creatinine clearance <50 mL/minute, Karnofsky score <60%, serology was positive for human immunodeficiency virus, they had uncontrolled infections, or they had previously undergone hematopoietic cell or solid organ transplantation. For each patient, data were collected through 100 days after transplantation to encompass endpoints of this phase 1 study or until study withdrawal.

Study Product

The MultiStem product is an expanded population of MAPC originating from adherent adult stem cells harvested from the BM of an unrelated volunteer, healthy donor [13,15,17,21]. For this study, stem cells isolated from a single qualified donor were expanded ex vivo in cell factories to create a cryopreserved master cell bank under current good manufacturing practice. Subsequent expansions were used to manufacture the current good manufacturing practice–grade MultiStem products. The clinical donor and master cell bank were negative for presence of infectious agents, including human immunodeficiency virus 1, human immunodeficiency virus 2, hepatitis B and C, West Nile virus, syphilis, Human T-Cell Lymphotropic Virus-1 and -2 (HTLV-1 and HTLV-2), cytomegalovirus (CMV), and Epstein-Barr virus. Transmissible spongiform encephalopathy and Creutzfeldt-Jakob disease risks were ruled out via health and questionnaire assessments. During expansion for dose production from the master cell bank, in-process testing was performed for bacterial and fungal contamination and for chromosomal stability. The clinical MultiStem product is produced using fetal calf serum, but before cryopreservation, the cells are washed in human serum albumin so that if remnants of fetal bovine serum were present, they were below detectable level. After expansion, the clinical cryopreserved products contain DMSO, which after dilution before patient infusion has a concentration of 5%.

Assigned cryopreserved batches were stored at each clinical site in liquid nitrogen vapor phase until use for infusion. For infusion, clinical grade MAPC product was thawed, diluted in an equal volume of Plasmalyte A (Baxter, Deerfield, IL) and sampled for measurement of the viable cell count and retested for bacterial and fungal contamination. The dose of MAPC was diluted based on the actual body weight of the recipient and, after reconstitution, cells were transferred into an infusion bag. The reconstituted MAPC product was infused intravenously within 6 hours after thaw via the patient's central venous catheter at a rate of approximately 5 to 10 mL per minute by gravity. Vital signs were recorded before dosing, every 15 ± 5 minutes for 2 hours, and then every 4 ± 1 hour for 48 hours from the start of MAPC infusion during hospitalization. If dosing was performed on outpatient basis, vital signs were collected before dosing and then every 15 ± 5 minutes for 2 hours, then at 6 ± 1 hours after infusion. Premedication before