Biol Blood Marrow Transplant 21 (2015) 1237–1245

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Dynamical System Modeling of Immune Reconstitution after Allogeneic Stem Cell Transplantation Identifies Patients at Risk for Adverse Outcomes

Amir A. Toor [1,*], Roy T. Sabo [2], Catherine H. Roberts [1], Bonny L. Moore [1], Salman R. Salman [1], Allison F. Scalora [1], May T. Aziz [3], Ali S. Shubar Ali [1], Charles E. Hall [1], Jeremy Meier [1], Radhika M. Thorn [1], Elaine Wang [1], Shiyu Song [4], Kristin Miller [5], Kathryn Rizzo [6], William B. Clark [1], John M. McCarty [1], Harold M. Chung [1], Masoud H. Manjili [7], Michael C. Neale [8]

[1] *Bone Marrow Transplant Program, Massey Cancer Center, Department of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia*
[2] *Department of Biostatistics, Virginia Commonwealth University, Richmond, Virginia*
[3] *Department of Pharmacy and Therapeutics, Virginia Commonwealth University, Richmond, Virginia*
[4] *Department of Radiation Oncology, Virginia Commonwealth University, Richmond, Virginia*
[5] *Department of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia*
[6] *Department of Pathology, Virginia Commonwealth University, Richmond, Virginia*
[7] *Department of Microbiology & Immunology, Virginia Commonwealth University, Richmond, Virginia*
[8] *Departments of Psychiatry & Human Genetics, Virginia Commonwealth University, Richmond, Virginia*

Article history:
Received 19 December 2014
Accepted 14 March 2015

Key Words:
Stem cell transplant
Dynamical system
Immune reconstitution
T cells
Graft-versus-host disease

A B S T R A C T

Systems that evolve over time and follow mathematical laws as they evolve are called *dynamical systems*. Lymphocyte recovery and clinical outcomes in 41 allograft recipients conditioned using antithymocyte globulin (ATG) and 4.5-Gy total body irradiation were studied to determine if immune reconstitution could be described as a dynamical system. Survival, relapse, and graft-versus-host disease (GVHD) were not significantly different in 2 cohorts of patients receiving different doses of ATG. However, donor-derived $CD3^+$ cell reconstitution was superior in the lower ATG dose cohort, and there were fewer instances of donor lymphocyte infusion (DLI). Lymphoid recovery was plotted in each individual over time and demonstrated 1 of 3 sigmoid growth patterns: Pattern A (n = 15) had rapid growth with high lymphocyte counts, pattern B (n = 14) had slower growth with intermediate recovery, and pattern C (n = 10) had poor lymphocyte reconstitution. There was a significant association between lymphocyte recovery patterns and both the rate of change of donor-derived $CD3^+$ at day 30 after stem cell transplantation (SCT) and clinical outcomes. GVHD was observed more frequently with pattern A, relapse and DLI more so with pattern C, with a consequent survival advantage in patients with patterns A and B. We conclude that evaluating immune reconstitution after SCT as a dynamical system may differentiate patients at risk of adverse outcomes and allow early intervention to modulate that risk.

INTRODUCTION

Allogeneic stem cell transplantation (SCT) results in widely disparate outcomes in individual transplant recipients, regardless of uniformity of histocompatibility criteria applied in donor selection [1-3] and therapeutic interventions [4-6] used for pretransplant conditioning regimens and graft-versus-host disease (GVHD) prophylaxis. Standard methodology is to examine clinical outcomes using statistical analytic techniques based on probability theory [7,8]. These analytic techniques are useful in determining odds of clinical outcomes in populations of patients transplanted using uniform conditioning regimens but are inadequate for determining the course an individual might take after SCT. This is because of the underlying assumption that within the constraints of certain critical parameters, such as donor type or HLA matching, the probability distribution of these clinical outcomes is essentially random. As an example, the

Financial disclosure: See Acknowledgments on page 1245.
* Correspondence and reprint requests: Amir A. Toor, MD, Associate Professor of Medicine, Massey Cancer Center, Virginia Commonwealth University, 1300 E Marshall St, PO Box 980157, Richmond, VA 23298-0157.
E-mail address: atoor@mcvh-vcu.edu (A.A. Toor).

http://dx.doi.org/10.1016/j.bbmt.2015.03.011
1083-8791/

addition of antithymocyte globulin (ATG) to the conditioning regimen reduces the *likelihood* of developing severe acute GVHD [9], and higher levels of donor T cell chimerism at day 30 after reduced-intensity conditioning (RIC) results in a lower *probability* of relapse after SCT [10]. Such population-based estimates are used to guide clinical decision-making at various time points during the transplant process, but individual patients undergoing SCT remain at risk for competing causes of adverse outcomes.

To improve outcome predictions in individuals undergoing SCT in real time, a closer examination of the immunobiology of transplantation is necessary. In SCT treatment, failure is often attributable to either the development of immune-mediated GVHD or the lack of an immune graft-versus-malignancy (GVM) effect [11-16]. Donor-derived immune reconstitution is driven, in part, by the disparity in minor histocompatibility antigens encountered in each instance of SCT [17,18]. It has been demonstrated that HLA-matched donor–recipient pairs have extensive variation in their exomes [19]. When analyzed in silico, this translates to a massive array of minor histocompatibility antigens, which may be presented by the HLA in the recipients [20]. This minor histocompatibility antigen difference may be considered an alloreactivity potential between donors and recipient pairs and appears to mirror the complex T cell clonal frequency observed in the T cell repertoire in SCT donors and recipients [21]. This suggests that clinical outcomes related to T cell alloreactivity after SCT may not be primarily stochastic in nature but when taking a quantitative perspective may be better considered mathematically to account for the large alloreactivity potential between SCT donors and recipients.

Systems that follow mathematically defined laws are common in the natural world and are called *dynamical systems* [22-25]. A dynamical system is defined as any *iterating* physical system that *evolves* over time in a manner such that *future states* of the system are predicated on *all* the *preceding states* and the evolution of the system can be modeled using ordinary differential equations. Population growth constrained by environmental pressures is a nonlinear example of such a system. In the context of SCT, this implies that if considered as a system of interacting donor T cell clones and recipient antigens, immune reconstitution after SCT is a dynamic, evolving process that can be modeled mathematically and allow more precise determination of the odds of clinical outcomes, such as engraftment, GVHD, and survival, in an individual [26]. A feature of dynamical systems is that early events in the system set the trajectory of the series of events to follow and thus determine the eventual outcome. In nonlinear dynamical systems, this implies that small variations in the early state of the system can produce large measurable effects late in the evolution of this system. In SCT a large body of literature now supports the notion that both early interventions [27-29] and the magnitude of immune reconstitution early in the course of SCT impacts late clinical outcomes [13,30,31], supporting the validity of considering SCT as a nonlinear dynamical system.

In this article the results of a prospective, randomized, phase II clinical trial are reported and considered in the light of immune reconstitution kinetics modeled as a dynamical system. The trial investigated 2 doses of rabbit ATG (Thymoglobulin; Sanofi-Aventis, Quebec, Canada) in patients undergoing RIC. The trial was designed to determine the optimal dosing of ATG to be given in combination with reduced-intensity total body irradiation (TBI) to ensure adequate engraftment. The clinical outcomes from this trial are analyzed with an underlying assumption that each transplant represents an example of a dynamical system. Each donor–recipient pair is composed of a unique set of recipient alloantigens and a set of donor-derived immune effectors interacting over time after the transplant event, in this instance modulated by 2 different ATG doses. The focus of the work presented here is on total lymphoid and T cell reconstitution and the resulting clinical effect. We demonstrate that lymphoid reconstitution after transplantation follows the ubiquitous quantitative rules describing constrained growth, that is, it occurs as a logistic function of time.

Table 1
Patient Characteristics

	ATG 5.1	ATG 7.5
N	19	22
Male	12	14
Median age (range)	57 (44-69)	57 (40-68)
Donor		
Matched related donor	9	10
Unrelated donor	10	12
HLA Mismatch	2	2
Stem cell source		
Bone marrow	2	2
Peripheral blood	17	20
Diagnosis and prior therapy		
MM	5	4
NHL	7	8
AML	1	3
MDS	0	2
CLL	4	3
PLL	2	2
Median no. of prior regimens (range)	4 (2-10)	4 (1-15)
Prior autologous SCT	6	8
Median cell dose infused		
$CD3^+ \times 10^6$/kg (range)	2.3 (.1-5.7)	2.9 (.2-11.3)
$CD34^+ \times 10^8$/kg (range)	5.8 (1.7-10.4)	5.1 (1.6-7.5)

MM indicates multiple myeloma; NHL, non-Hodgkin lymphoma; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; PLL, prolymphocytic leukemia.

METHODS

Patients

Between 2009 and 2013, 41 patients were enrolled in a prospective, randomized, phase II clinical trial, approved by the institutional review board at Virginia Commonwealth University (ClinicalTrials.gov Identifier: NCT00709592). To be eligible, patients had to be ≤70 years old with recurrent or high-risk hematological malignancy. Patients were required to have a 7/8 or 8/8 locus matched related or unrelated donor, with high-resolution typing performed for HLA-A, -B, -C, and -DRB1. Two different doses of ATG were tested in the 2 arms of this trial, and patients were stratified for donor type (matched related or unrelated donor) and disease status (first complete remission or higher) at the time of randomization. Patient characteristics are given in Table 1. Patient follow-up was updated as of October 2014.

Rabbit ATG and Low-Dose TBI Conditioning Regimen

Patients were randomized between 2 different doses of rabbit ATG, either 2.5 or 1.7 mg/kg, adjusted ideal body weight per day given intravenously from day −9 to −7 (ATG 7.5 or ATG 5.1 cohorts), followed by TBI to a total dose of 4.5 Gy, delivered in 3 1.5-Gy fractions, administered twice on day −1, with the final dose on day 0 (see Supplemental Figure 1 and Supplemental Material). Tacrolimus given orally for GVHD prophylaxis from day −2, with taper commencing around 12 weeks post-transplant, in an initial cohort of patients (n = 24) and according to donor-derived T cell counts in the remaining patients. Mycophenolate mofetil (MMF) was given orally at a dose of 15 mg/kg twice daily from days 0 to 30. Escalating-dose donor lymphocyte infusion (DLI) was permitted beyond 8 weeks post-SCT