saving, and has the potential to open up exciting new avenues for cell, drug, and gene therapy.

194

Assessment of T-Cell Reconstitution After Two Step Haploidentical Stem Cell Transplants by Measurement of T-Cell Receptor Excision Circles (TREC)

John L. Wagner [1], Irina Kakhniashvili [2], Bijoyesh Mookerjee [3], Joanne Filicko [4], Dolores Grosso [1], Neal Flomenberg [1].
[1] Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; [2] Medical Oncology, Kimmel Cancer Center Thomas Jefferson University; [3] Blood and Marrow Transplantation, Thomas Jefferson Medical College, Philadelphia, PA; [4] Thomas Jefferson University Hospital, Philadelphia, PA

Hematopoietic stem cell transplantation (HSCT) can associated with T cell immunodeficiency which can contribute to a high incidence of infections in patients. In the thymus, hematopoietic progenitor cells undergo rapid proliferation and differentiation to become mature T cells. Factors which can affect the function of the thymus include: age, high dose chemotherapy and radiotherapy, GVHD, relapse and the occurrence of some opportunistic infections. T cell rearrangement continues even in the elderly, and because the TRECs are non-replicating episomal DNA and their number can be decreased only by cell death, dilution through cell division or both, the measurement of the number of TRECs can be a measure of immunity. The purpose of this study was to measure T cell reconstitution after hematopoietic stem cell transplantation in adult patients with hematological malignancies by measuring TREC at various time points after transplant.

To measure thymic output after HSCT, TREC levels were measured in sixteen patients ages 23-68 (median age 53). Two patients had CLL, one had follicular lymphoma and the rest had acute leukemia (ten AML and three ALL). Ten patients received myeloablative transplants and six received reduced intensity transplants. All patients achieved full donor chimerism at the time of analysis and all survived a minimum of 6 months after transplant (all but one survived at least 500 days post-transplant). For the myeloablative transplants, patients received 12 Gy of TBI followed by a donor lymphocyte infusion of 2 x 108 CD3 cells/kg followed two days later by cyclophosphamide at 60mg/kg/day for two days followed one day later by a CD34 selected stem cell product. For the non-myeloablative transplants, patients received fludarabine 30mg/m2 for four doses and cytarabine 2gm/m2 for four doses followed by 2 Gy of TBI then as described for the myeloablative transplants. All patients received tacrolimus and mycophenolate mofetil for GVHD prophylaxis. The CD34 dose ranged from 1.40 - 5.89 x 106 cells/kg (median dose 3.34 x 106 cells/kg).

Peripheral blood samples were purified using the AUTOMACS magnetic cell sorter (Miltenyi Biotec). To determine the purity of the CD4+ and CD8+ cells, two color cell staining using fluorochrome-conjugated antibodies against CD3+, CD4+, CD8+ and CD56+ and flow cytometry was performed. TRECs were quantified by real-time polymerase chain reaction (PCR) analysis using the 5' nuclease (Taqman) assay. A standard curve was plotted using samples with known amounts of TREC, and TREC values for each sample was calculated by the ABI7700 software. Samples were run and analyzed in triplicate.

Our analysis of the data showed that in general there were more TREC's per 50,000 CD8 cells then CD4 cells. Recipients with donors that had higher TRECs had more TRECs after transplant and that with one exception the number of TRECs in the recipient did not exceed that of the donor.

195

The Effect of Sirolimus Based Regimen On Immune Reconstitution After Allogeneic Stem Cell Transplantation

Jong-Ho Won [1], Seong Kyu Park Sr. [2], Se Hyung Kim Jr. [3], Jina Yun Jr. [4]. [1] Soon Chun Hyang University Hospital, Seoul, South Korea; [2] Hematology, Soonchunhyang University Hospital, Bucheon, South Korea; [3] Hematology, Soonchunhyang Universit Hospital, Bucheon, South Korea; [4] Hematology/Oncology, Soonchunhyang Universit Hospital, Bucheon, South Korea

Background: Reconstitution of the immune system following allogeneic stem-cell transplantation (allo-SCT) is a complex process that requires successful engraftment of the hematopoietic stem cell, as well as adequate thymic function. Although GVHD control, improvements in antibiotic spectra, and circumspection in the use of immunosuppressants have helped, too many patients still die of infections because of insufficient immunologic recovery. Sirolimus has been used alone and in combination with calcineurin inhibitors for prevention of allograft rejection after solid organ transplantation. In the field of hematopoietic stem cell transplantation, the combination of sirolimus and tacrolimus has also resulted in a low incidence of acute GVHD and reduced transplant-related toxicity.
Methods: We evaluated the immune recovery status of 24 patients who received the combination of sirolimus and tacrolimus as a GvHD prophylaxis compared to a historical control (n=21) using tacrolimus and methotrexate (MTX). They were conditioned with myeloablative regimens.
Results: The incidence of acute GVHD in patients with sirolimus based regimen was lower. And the incidence of CMV or EBV reactivation in the same group was higher. The recovery of $CD4^+$ T cells and natural killer (NK) cells seemed to be more delayed in recipients with sirolimus based regimen compared to those in patients with tacrolimus and MTX at 1 month after transplantation (8.8 ± 1.6 vs 14.9 ± 5.8 for $CD4^+$ T cells, 35.7 ± 6.4 vs 53.4 ± 19.6 for NK cells). However, there was no significant difference between in the recovery of $CD4^+$T cells and NK cells at 3 months after transplantation. In the aspect of humoral immunity, there was a trend to be lower in immunoglobulin-A and Ig-M levels during 1 month and 3 months after transplantation in patients with sirolimus based regimen. This difference was overcome around post-transplant 6 months. And regulatory T cells (CD4+CD25+Foxp3+) seemed to be higher in patients with sirolimus based regimen.
Conclusion: The sirolimus based regimen is associated with well-controlled acute GVHD. Although the correlation between the increased risk of infection and delayed immune reconstitution was not confirmed, the regimen might be cause of increasing the risk of opportunistic infection. Therefore we need an effort of early tapering of immunosuppressants and a careful monitoring for opportunistic infections in patients received sirolimus based regimen.