CHIMERISM
2015, VOL. 6, NO. 3, 47–49
http://dx.doi.org/10.1080/19381956.2015.1128624

Taylor & Francis
Taylor & Francis Group

ARTICLE ADDENDUM

Deep-sequencing of the T-cell receptor repertoire in patients with haplo-cord and matched-donor transplants

Houda Alachkar[a] and Yusuke Nakamura[b]

[a]School of Pharmacy, University of Southern California, Los Angeles, CA, USA; [b]Section of Hematology/Oncology, Department of Medicine, The University of Chicago, Chicago, IL, USA

ABSTRACT

Haplo-cord transplant has emerged as a feasible and reliable approach for haematopoietic stem cell transplant in patients who are unable to find matched-donor. This approach provides fast myeloid recovery, low incidence of graft vs host disease (GVHD) and favorable graft versus leukemia (GVL) effects. T cell recovery plays an important role in preventing infectious complications; it also mediates the GVHD and the GVL effects. Here, we utilized a novel RNA-based sequencing approach to quantitatively characterize the T cell receptor (TCRs) repertoire in patients underwent haplo-cord transplant in comparison with those underwent matched-donor transplant. Our study shows that higher percentage of cord cells early post transplant were associated with significantly higher TCR diversity. TCR diversity was significantly lower in patients with GVHD and in relapsed patients. A larger cohort study is needed to validate these data and to provide useful information on the specific TCR clones correlated with clinical outcome.

ARTICLE HISTORY
Received 17 November 2015
Accepted 26 November 2015

KEYWORDS
chimerism; GVHD; GVL; Haplo-cord; relapse; stem cell transplant; T cell receptors; T cell repertoire

The use of umbilical cord blood (UCB) as an alternative stem cell source for patients who have no matched related- or unrelated- donor has the advantage of being feasible and readily available, and provides minimal donor-related risks.[1,2] Furthermore, studies suggest lower risk of graft vs. host disease (GVHD), and possibly lower risk of disease relapse associated with this approach.[2-4] However, delays in immune reconstitution characterized by prolonged neutropenia remains a major challenge facing the use of UCB in haematopoietic stem cell transplant (HSCT).[4-6] One method to evade this delayed immune recovery is supplementing UCB with $CD34^+$ selected cells from an adult donor (haploidentical).[7-9] This approach has proven feasibility and resulted in earlier neutrophils and platelets recovery.[9,10] In our recent publication,[11] we applied RNA-based next-generation sequencing approach of the T cell receptors (TCRs) to characterize the T cell repertoire in patients with acute myeloid leukemia (AML) underwent haplo-cord transplant. Our study found that the TCR repertoires in these patients exhibit similar diversity to those in patients underwent the conventional matched donor transplant (MD). A novel and important finding in our study is the correlation between haplo-cord chimerism and TCR diversity. We found that patients with high percentage (above the median) of cord blood cells in their blood measured at day 30 after transplant had significantly higher TCR diversity assessed at day 100. This suggests that haplo-cord chimerism at an early time-point predicts later TCR diversity. This is in concordance with recent finding by another study showing that early post-transplant chimerism dynamics in PB and T cells predicts cord blood (CB) graft failure. On the other hand, early increase in percentages of CB T cells correlates with ultimate CB engraftment.[12]

The curative potential of HSCT is largely driven by the GVL effects, and challenged by a limiting toxicity and transplant-related mortality caused by the GVHD.[13] The link between GVHD and GVL has been recognized and supported by experimental and clinical lines of evidence. Although patients with GVHD, especially chronic GVHD, are likely to have lower risk of relapse, the increased non-relapse mortality

CONTACT Houda Alachkar, PharmD, PhD alachkar@usc.edu USC School of Pharmacy, 1985 Zonal Ave PSC 608, Los Angeles CA, USA 90089-9121.
Addendum to: Yew P, Alachkar H, Yamaguchi R, Kiyotani K, Fang H, Yap K, Liu H, Wickrema A, Artz A, Besien K, Imoto S, Miyano S, Bishop MR, Stock W, and Nakamura Y. Quantitative characterization of T cell repertoire in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplantation 2015; PMID:26052909; dx.doi.org/10.1038/bmt.2015.133

remains a cause for the relatively poor overall survival.[13] Research efforts to fine-tune the balance between the GVL and GVHD include manipulation of post-transplantation immune suppression, source of stem cells, and cytokine use.[14] These approaches aim to enhance the GVL effect after the transplantation in order to reduce the relapse rates and improve clinical outcome.

The role of activated donor-derived cytotoxic T cells in the pathophysiology of GVHD and GVL has been well established.[15,16] Therefore, identifying TCR clones responsible for the GVL effect and those responsible for the GVHD will be the tenet for segregating the GVL effect from the GVHD. Characterization of the T cell repertoires should enable us to identify T cell clones responsible for both the GVL and GVHD. Although the number of patients analyzed in our recent study is not large enough to identify particular clones responsible for GVHD or GVL, we observed an expansion of TRAV38-2/DV8-TRAJ30 and TRBV15-1-TRBJ2-1 in 2 acute GVHD patients who shared all of 3 major HLA class I alleles (*HLA-A*02, HLA-B*07* and *HLA-C*07*) and 2 of the 3 major class II loci (*HLA-DR*04, HLA-DQ*03* and *HLA-DQ*06*). More importantly, our comprehensive approach enabled us to obtain important insights into the correlation between the TCR diversity, and each of the relapse status and the occurrence of acute GVHD. Our study showed higher expansion of particular clones in patients with GVHD in comparison with patients without GVHD. This was consistent with decrease in the TCR repertoire diversity in patients with GVHD. On the other hand, among non-GVHD subset, the relapse-free patients have significantly higher diversity than relapsed patients. Although this finding might seem counterintuitive, it is plausible to think that higher diversity at early days after transplant is essential for a later expansion of a particular clone(s) and the development of the GVL effect. Furthermore, the expansion of certain T-cell populations in the relapsed patients may reflect an increased levels of regulatory T-cells that may function as suppressive of the GVL and GVHD effects.[17] Consistent with this argument, an opposite correlation were found in patients with GVHD, where relapsed patients exhibit higher TCR repertoire diversity than non-relapsed patients. This further supports the strong link between the GVL and GVHD effects and the need for further analysis to separate these 2 effects.

In addition to the patients population analyzed in our study which included high-risk patients with AML that underwent haplo-cord transplant, the unique approach for characterizing the TCR repertoire added a great value to this study. Most studies reporting the TCR repertoire after HSCT have focused on analyzing the TCRB only.[18,19] Our study reports detailed analysis of TCRA and TCRB, these information are needed for further functional analysis of expanded T cell clones. Additionally, we applied 5′RACE PCR to amplify all TCRA and TCRB transcripts using a single primer set, this method minimizes the amplification bias of a TCR-specific multiplex PCR using exon-specific multiplex primers.[20,21] More importantly, our technique enables the discovery of novel exons. Indeed, we found a transcript generated by recombination between TRDV1 (annotated as a V segment of TCR delta) and various J segments of TCRA in our samples, indicating this TRDV1 may function as one of V segments in the TCR α gene.

In summary, our study has implied that although haplo-cord transplant has similar T cell recovery to that of matched donor transplant, the percentages of the engrafted cord cells few weeks after transplant may determine the T cell repertoire diversity and recovery. Although the significance of the clonally expanded T cells in the induction of acute GVHD remains non-conclusive, our study showed that significant expansion of certain T cell clones is likely to be associated with GVHD and/or relapse of the disease. Future studies to evaluate a large cohort of patients with shared common HLA alleles, may facilitate the identification of the TCR clones responsible for GVHD and/or GVL.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, Ciocci G, Carrier C, Stevens CE, Rubinstein P. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 1996; 335:157-66; PMID:8657213; http://dx.doi.org/10.1056/NEJM199607183350303

[2] Gluckman E. Milestones in umbilical cord blood transplantation. Blood Rev 2011; 25:255-9; PMID:21764191; http://dx.doi.org/10.1016/j.blre.2011.06.003

[3] Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W, Sirvent A, Champlin RE, Chao N, Gee AP, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol 2010; 11:653-60; PMID:20558104; http://dx.doi.org/10.1016/S1470-2045(10)70127-3

[4] Ballen KK, Koreth J, Chen YB, Dey BR, Spitzer TR. Selection of optimal alternative graft source: mismatched unrelated donor, umbilical cord blood, or haploidentical transplant. Blood 2012; 119:1972-80; PMID:22210876; http://dx.doi.org/10.1182/blood-2011-11-354563

[5] Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 2004; 351:2265-75; PMID:15564543; http://dx.doi.org/10.1056/NEJMoa041276

[6] Barker JN, Hough RE, van Burik JA, DeFor TE, MacMillan ML, O'Brien MR, Wagner JE. Serious infections after unrelated donor transplantation in 136 children: impact of stem cell source. Biol Blood Marrow Transplant 2005; 11:362-70; PMID:15846290; http://dx.doi.org/10.1016/j.bbmt.2005.02.004

[7] Fernandez MN, Regidor C, Cabrera R, García-Marco J, Briz M, Forés R, Sanjuán I, McWhinnie A, Querol S, García J, et al. Cord blood transplants: early recovery of neutrophils from co-transplanted sibling haploidentical progenitor cells and lack of engraftment of cultured cord blood cells, as ascertained by analysis of DNA polymorphisms. Bone Marrow Transplant 2001; 28:355-63; PMID:11571507; http://dx.doi.org/10.1038/sj.bmt.1703143

[8] Kwon M, Balsalobre P, Serrano D, Pérez Corral A, Buño I, Anguita J, Gayoso J, Diez-Martin JL. Single cord blood combined with HLA-mismatched third party donor cells: comparable results to matched unrelated donor transplantation in high-risk patients with hematologic disorders. Biol Blood Marrow Transplant 2013; 19:143-9; PMID:22960386; http://dx.doi.org/10.1016/j.bbmt.2012.08.019

[9] Jain N, Liu H, Artz AS, Anastasi J, Odenike O, Godley LA, Joseph L, Marino S, Kline J, Nguyen V, et al. Immune reconstitution after combined haploidentical and umbilical cord blood transplant. Leuk Lymphoma 2013; 54:1242-9; PMID:23088744; http://dx.doi.org/10.3109/10428194.2012.739688

[10] Lindemans CA, van Besien K. Topping it up: methods to improve cord blood transplantation outcomes by increasing the number of CD34+ cells. Cytotherapy 2015; 17:723-9; PMID:25791069; http://dx.doi.org/10.1016/j.jcyt.2015.02.005

[11] Yew PY, Alachkar H, Yamaguchi R, Kiyotani K, Fang H, Yap KL, Liu HT, Wickrema A, Artz A, van Besien K, et al. Quantitative characterization of T-cell repertoire in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2015; 50:1227-34; PMID:26052909; http://dx.doi.org/10.1038/bmt.2015.133

[12] Kwon M, Martínez-Laperche C, Balsalobre P, Serrano D, Anguita J, Gayoso J, Díez-Martín JL, Buño I. Early peripheral blood and T-cell chimerism dynamics after umbilical cord blood transplantation supported with haploidentical cells. Bone Marrow Transplant 2014; 49:212-8; PMID:24212562; http://dx.doi.org/10.1038/bmt.2013.177

[13] van Besien K. Allogeneic transplantation for AML and MDS: GVL versus GVHD and disease recurrence. Hematology Am Soc Hematol Educ Program 2013; 2013: 56-62; PMID:24319163; http://dx.doi.org/10.1182/asheducation-2013.1.56

[14] Li JM, Giver CR, Lu Y, Hossain MS, Akhtari M, Waller EK. Separating graft-versus-leukemia from graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. Immunotherapy 2009; 1:599-621; PMID:20191089

[15] Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. Nat Rev Cancer 2004; 4:371-80; PMID:15122208; http://dx.doi.org/10.1038/nrc1365

[16] Schmaltz C, Alpdogan O, Muriglan SJ, Kappel BJ, Rotolo JA, Ricchetti ET, Greenberg AS, Willis LM, Murphy GF, Crawford JM, et al. Donor T cell-derived TNF is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. Blood 2003; 101:2440-5; PMID:12424195; http://dx.doi.org/10.1182/blood-2002-07-2109

[17] Barrett AJ, Battiwalla M. Relapse after allogeneic stem cell transplantation. Expert Rev Hematol 2010; 3:429-41; PMID:21083034; http://dx.doi.org/10.1586/ehm.10.32

[18] Chapman M, Warren EH, 3rd, Wu CJ. Applications of next-generation sequencing to blood and marrow transplantation. Biol Blood Marrow Transplant 2012; 18:S151-60; PMID:22226099; http://dx.doi.org/10.1016/j.bbmt.2011.11.011

[19] van Heijst JW, Ceberio I, Lipuma LB, Samilo DW, Wasilewski GD, Gonzales AM, Nieves JL, van den Brink MR, Perales MA, Pamer EG. Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. Nat Med 2013; 19:372-7; PMID:23435170; http://dx.doi.org/10.1038/nm.3100

[20] Woodsworth DJ, Castellarin M, Holt RA. Sequence analysis of T-cell repertoires in health and disease. Genome Med 2013; 5:98; PMID:24172704; http://dx.doi.org/10.1186/gm502

[21] Meier J, Roberts C, Avent K, Hazlett A, Berrie J, Payne K, Hamm D, Desmarais C, Sanders C, Hogan KT, et al. Fractal organization of the human T cell repertoire in health and after stem cell transplantation. Biol Blood Marrow Transplant 2013; 19:366-77; PMID:23313705; http://dx.doi.org/10.1016/j.bbmt.2012.12.004