Mediterranean Journal of Hematology and Infectious Diseases

Scientific Letter

Impact of Donor-Specific anti-HLA Antibodies and Donor KIR Characteristics in Haploidentical HSCT for Beta-Thalassemia

Keywords: Haploidentical HSCT, KIR receptors, anti-HLA antibodies.

Published: March 1, 2017 **Received: December 5, 2016** **Accepted: January 23, 2017**

Citation: Andreani M., Testi M., Sodani P., Troiano M., Di Luzio A., Testa G, Falco M., Poggi E., Gaziev J., Piazza A. Impact of donor-specific anti-HLA antibodies and donor KIR characteristics in haploidentical HSCT for beta-thalassemia. Mediterr J Hematol Infect Dis 2017, 9(1): e2017020, DOI: http://dx.doi.org/10.4084/MJHID.2017.020

Allogeneic hematopoietic stem cell transplantation (HSCT) still remains a potentially curative treatment for many children affected by life-threatening, onco-hematological or genetic non-malignant disorders. Unfortunately, the probability of finding a HLA-identical sibling donor is 25% and a suitable genotypically HLA-compatible unrelated donor can be promptly located for less than 70% of the remaining patients. Currently, for patients requiring transplantation but lacking a related or an unrelated HLA-matched donor the HLA-haploidentical HSCT (haplo-HSCT) represents a widely available approach. Over the last 20 years, haplo-HSCT outcomes have substantially improved due to the development of novel GVHD prophylaxis strategies, advances in supportive care and the increased usage of grafts with high stem cell and low T lymphocyte content, making this procedure an attractive potentially curative option.[1-4]As already reported, haplo-HSCT has been proposed as a curative approach also for the treatment of severe non-malignant disorders, such as Beta-Thalassemia.[5-7]Between 2004 and 2015 fifty-two pediatric patients affected by thalassemia major (n=43), drepanothalassemia (n=2), or sickle cell anemia (n=7) received haplo-HSCT at the IME Foundation-Mediterranean Institute of Hematology at the Policlinic of Tor Vergata in Rome. Details relative to the conditioning regimen used, the graft manipulation and the post-transplant immunosuppression are reported in reference.[6,7]Briefly, all patients received a pre-transplant conditioning regimen with oral or weight-based i.v. Busulfan, Thiotepa, Cyclophosphamide, and ATG preceded by cytoreduction/immunosuppression with hydroxyurea, azathioprine and fludarabine. Forty patients received $CD34^+$ selected grafts while12 patients alpha/beta T cells and $CD19^+$ B cells depleted grafts. In the present study we included 18 beta-thalassemic patients whose recipient serum and donor DNA samples were available to perform retrospective analyses of anti-HLA antibody and of KIR repertoire characteristics, respectively. Among this cohort, 8 patients showed a secondary graft failure, subsequent to a period of a period of full donor engraftment, while 10 had complete donor chimerism (CC). The aim of this study was to investigate the potential role of donor specific anti-HLA antibodies and/or of some donor KIR repertoire characteristics (i.e. NK alloreactivity and high B content value) on the high rate of graft rejection observed. In the last few years several papers have shown an association between donor-specific anti-human leukocyte antigen (HLA) antibodies (DSAs) with graft failure following haplo-HSCT, suggesting that anti-HLA sensitization should be routinely evaluated in HSCT with HLA mismatched donors.[8-10]In the present study, in a subgroup of 18 patients, we were able to retrospectively evaluate by Luminex single antigen beads (One Lambda, Canoga Park, CA) the presence in the recipients sera of anti-HLA antibodies, and in particular DSA. Anti-HLA antibodies where present in 6 of the 18 patients analyzed; 1 against class I antigens, 2 against class II and 3 against both, with a mean fluorescence intensity (MFI) varying from 1000

to 22000. When we analyzed their specificity, we found that in three cases, they could be classified according to donor HLA genotype as DSA with a percentage in our survey (17%) comparable to that reported in literature. Eight out of the 18 patients analyzed showed a secondary graft failure, while 10 reached a stable complete full engraftment. One out of 8 patients that rejected their grafts died, while 7 survived with thalassemia, returning to a transfusion dependent status. The analysis of correlation with graft rejection indicated that 5 of the 8 patients that lost their graft were positive for anti-HLA antibodies (62.5%), while only 1 out of 10 (10%) in the group of those with full donor engraftment revealed their presence, showing a statistically significant difference between the patients that rejected the graft and those showing a CC (p=0,042). Among the patients in which we detected anti-HLA antibodies, 2 were DSA positive for class I and 1 was DSA positive for both class I and II. These 3 DSA positive patients belonged to the group of the 8 patients that lost the graft, while none of 10 patients with CC, were found positive for DSA (p=0.068). On the other hand, the presence of anti-HLA non DSA was observed in 2 patients out of 8 that rejected the transplant and in 1 with CC. There are, in our opinion, two different reasons for precluding DSA to achieve a statistical significance: the first one is clearly due to the small sample size of our survey, the second one might be found in the fact that some anti HLA antibodies could be directed against HLA loci, such as DQA1, DPA1 or DRB3-4-5 that have not been analyzed in the recipient/donor couples and thus might have been under-estimated as DSA. Recent papers suggested a role of donor NK cells in the outcome of patients affected by malignant diseases receiving haplo-HSCT.[11-12]In this study we investigated if the absence of NK alloreactivity and/or a low B content value of donor KIR genotype may be correlated with graft failure. The molecular basis for NK alloreactivity is represented by NK receptors, namely killer immunoglobulin-like receptors (KIR), which are specific for allotypic determinants shared by different HLA-class I alleles (KIR-L). In an allogenic environment (such as in haplo-HSCT setting) molecular NK allorectivity is present when the donor is characterized by the presence of inhibitory KIR specific for KIR-L expressed in the donor and missing in the patient (i.e. when KIR/KIR-L mismatch in graft versus host direction occurs). Presence of NK alloreactivity in the donor has been correlated with a better patient outcome by producing a desired graft versus leukemia (GVL) effect (in onco-hematological patients) and contributing to the clearance of residual host dendritic cells and T lymphocytes, thus preventing GvHD and graft rejection. Moreover, the presence in the donor of peculiar KIR genotypes characterized by the presence of several activating KIR (collectively indicated as B/X and differing for B content value) was also correlated with a better patient outcome.[13-15]To investigate if the presence of NK alloreactivity in the donor could improve patient outcome mediating an allo-recognition of patient T lymphocytes and consequently limiting the cells mediating graft loss we analyzed donor KIR repertoire and donor/recipient HLA class I typing. Analyses of all KIR genes, as well as the common variants of KIR2DL5, KIR2DS4, and KIR3DP1genes were performed by a sequence-specific primer polymerase chain reaction (PCR-SSP) approach (Invitrogen - Brown Deer, WI, USA). Based on the obtained results, presence/absence of NK alloreactivity, donor KIR genotypes, and B content value were assigned. This analysis revealed that 7 donors were characterized by the presence of NK alloreactivity, 1 donor (~5,5%) had an A/A KIR genotype, while 17 (~94,5%) were typed with B/x KIR genotype. A B content value ≥2 was detected in 47% of the B/x donors. Our results showed no significant differences in the clinical outcome of the patients receiving the graft from a donor with NK alloreactivity or with a B content value ≥2. Although data reported in literature suggest that the donor KIR genotype might influence the outcome of transplant in HLA haplo-HSCT for thalassemic patients we did not detect any impact of the presence of alloreactive NK cells and/or B content value with graft failure. On the contrary, although a larger cohort of patients needs to be analyzed to draw any definitive conclusion, our data indicated that the presence of anti-HLA sensitization correlates with graft failure and it should be taken into account before approaching a HSCT from a HLA haplo-identical donor. Since allogeneic HSCT from haploidentical family members could provide donors for virtually all patients who need HSCT and often more than one donor is available for a single patient, optimal donor selection should include recipient anti-HLA

screening to avoid dangerous mismatches. In summary, in the present study we investigated the role of donor specific HLA antibodies (DSA) and donor KIR repertoire characteristics in a group of 18 patients affected by haemoglobinopathies who underwent haploidentical T cell depleted transplantation. Among these patients, 8 showed a secondary graft failure, subsequent to a period of full donor engraftment, while 10 had stable complete donor chimerism (CC). Five out of 8 patients (62.5%) who rejected the graft had anti-HLA antibodies in the sera collected before transplant, while only 1 patient out of 10 (10%) with CC showed their presence (p=0,042). Notably, of the 5 HLA antibodies positive patients who rejected the graft 3 had DSA (2 for class I and 1 for class I and II) while none of the patients with CC had DSA. Among the 8 patients that experienced graft failure 4 were transplanted with a donor characterized by the lack of NK alloreactivity and 5 with a donor with a B content value <2. Although we analyzed a small cohort of patients, our data indicated that the presence of anti-HLA antibodies in patient sera, but not donor KIR characteristics, correlates with graft failure thus suggesting that analysis of anti-HLA antibodies should be taken into account in haploidentical transplant setting.

Acknowledgement. With the contribution of Fondazione Berloni.

Marco Andreani[1], Manuela Testi[1], Pietro Sodani[2], Maria Troiano[1], Andrea Di Luzio[1], Giuseppe Testa[1],Michela Falco[3], Elvira Poggi[4], Javid Gaziev[2] and Antonina Piazza[4]

[1]Laboratory of Immunogenetics and Transplant Biology, IME Foundation at Polyclinic of Tor Vergata, Rome, Italy
[2]International Center for Transplantation in Thalassemia and Sickle Cell Anemia, IME Foundation at Polyclinic of Tor Vergata, Rome, Italy
[3]Laboratoryof Clinical and Experimental Immunology, Giannina Gaslini Institute, Genoa, Italy
[4]Laboratory of Tissue Typing and Transplant Immunology, National Council of Researches IFT Unit of Rome S. Camillo Hospital – Regional Transplant Center Lazio, Rome, Italy

Competing interests: The authors have declared that no competing interests exist.

Correspondence to: Marco Andreani. Laboratory of Immunogenetics and Transplant Biology, IME Foundation at Polyclinic of Tor Vergata, Rome, Italy. E-mail: m.andreani@fondazioneime.org

References:

1. Gaziev J, Marziali M, Isgrò A, Sodani P, Paciaroni K et al.Gallucci C, Andreani M, Testi M, De Angelis G, Alfieri C, Cardarelli L, Ribersani M, Armiento D, Lucarelli. Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach. Blood. 2013 Oct 10;122(15):2751-6. https://doi.org/10.1182/blood-2013-07-513473
2. Li Pira G, Malaspina D, Girolami E, Biagini S, Cicchetti E, Conflitti G, Broglia M, Ceccarelli S, Lazzaro S, Pagliara D, Meschini A, Bertaina A, Montanari M, Locatelli F. Selective Depletion of aß T Cells and B Cells for Human Leukocyte Antigen-Haploidentical Hematopoietic Stem Cell Transplantation. A Three-Year Follow-Up of Procedure Efficiency.Biol Blood Marrow Transplant. 2016 Nov;22(11):2056-2064. https://doi.org/10.1016/j.bbmt.2016.08.006
3. Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R, Pende D, Falco M, Handgretinger R, Moretta F, Lucarelli B, Brescia LP, Li Pira G, Testi M, Cancrini C, Kabbara N, Carsetti R, Finocchi A, Moretta A, Moretta L, Locatelli F. HLA-haploidentical stem cell transplantation after removal of alphabeta + T and B-cells in children with non-malignant disorders. Blood. 2014;124(5):822–6. https://doi.org/10.1182/blood-2014-03-563817 PMid:24869942
4. Bacigalupo A, Dominietto A, Ghiso A, Di Grazia C, Lamparelli T, Gualandi F, Bregante S, Van Lint MT, Geroldi S, Luchetti S, Grasso R, Pozzi S, Colombo N, Tedone E, Varaldo R, Raiola AM. Unmanipulatedhaploidentical bone marrow transplantation and post-transplant cyclophosphamide for hematologic malignanices following a myeloablative conditioning: an update. Bone Marrow Transplant. 2015 Jun;50Suppl 2:S37-9. https://doi.org/10.1038/bmt.2015.93
5. Locatelli F, Merli P, Strocchio L. Transplantation for thalassemia major: alternative donors. Curr Opin Hematol. 2016 Nov; 23(6):515-523. https://doi.org/10.1097/MOH.0000000000000280 PMid:27537474
6. Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring HarbPerspect Med. 2012 May;2(5):a011825. doi: 10.1101/cshperspect.a011825. Review. https://doi.org/10.1101/cshperspect.a011825
7. Sodani P, Isgrò A, Gaziev J, Paciaroni K, Marziali M, Simone MD, Roveda A, De Angelis G, Gallucci C, Torelli F, Isacchi G, Zinno F, Landi F, Adorno G, Lanti A, Testi M, Andreani M, Lucarelli G. T-cell depleted HLA-haploidentical stem cell transplantation in thalassemia young patients. Pediatr Rep. 2011 Jun 22;3Suppl 2:e13. doi: 10.4081/pr.2011.s2.e13 https://doi.org/10.4081/pr.2011.s2.e13
8. Chang YJ, Zhao XY, Xu LP, Zhang XH, Wang Y, Han W, Chen H, Wang FR, Mo XD, Zhang YY, Huo MR, Zhao XS, Y K, Liu KY, Huang XJ. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. J Hematol Oncol. 2015 Jul 10;8:84 https://doi.org/10.1186/s13045-015-0182-9 PMid:26156584 PMCid:PMC4496923
9. Morin-Zorman S, Loiseau P, Taupin JL, Caillat-Zucman S. Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol. 2016 Aug 12;7:307. doi: 10.3389/fimmu.2016.00307. Review. https://doi.org/10.3389/fimmu.2016.00307
10. La Nasa G, Vacca A, Littera G, Piras, Orru S, Greco M, Carcassi C, Caocci G. What unrelated hematopoietic stem cell transplantation in thalassemia taught us about transplant immunogenetics. Mediterr J Hematol Infect Dis 2016, 8(1): e2016048 https://doi.org/10.4084/mjhid.2016.048
11. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, Marsh SG, Geraghty D, Spellman S, Haagenson MD, Ladner M,

Trachtenberg E, Parham P, Miller JS. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood 2010 Oct 7;116(14):2411-9. doi: 10.1182/blood-2010-05-283051. Epub 2010 Jun 25. https://doi.org/10.1182/blood-2010-05-283051

12. Littera R, Orrù N, Vacca A, Bertaina A, Caocci G, Mulargia M, Giardini C, Piras E, Mastronuzzi A, Vinti L, Orrù S, Locatelli F, Carcassi C, La Nasa G. The role of killer immunoglobulin-like receptor haplotypes on the outcome of unrelated donor haematopoietic SCT for thalassaemia. Bone Marrow Transplant. 2010 Nov;45(11):1618-24. https://doi.org/10.1038/bmt.2010.24
13. Mancusi A, Ruggeri L, Urbani E, Pierini A, Massei MS, Carotti A, Terenzi A, Falzetti F, Tosti A, Topini F, Bozza S, Romani L, Tognellini R, Stern M, Aversa F, Martelli MF, Velardi A. Haploidentical hematopoietic transplantation from KIR ligand-mismatched donors with activating KIRs reduces non-relapse mortality. Blood. 2015;125(20):3173–82. https://doi.org/10.1182/blood-2014-09-599993 PMid:25769621
14. Moretta L, Locatelli F, Pende D, Sivori S, Falco M, Bottino C, Mingari MC, MorettaA.Human NK receptors: from the molecules to the therapy of high risk leukemias.FEBS Lett. 2011 Jun 6;585(11):1563-7. https://doi.org/10.1016/j.febslet.2011.04.061
15. Falco M, Moretta L, Moretta A, Bottino C. KIR and KIR ligand polymorphism: a new area for clinical applications? Tissue Antigens. 2013 Dec;82(6):363-73. Review. https://doi.org/10.1111/tan.12262