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Stem cell transplantation in acute myeloid leukemia;history, drivers and challenges

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ABSTRACT

Stem cell therapy for Acute Myeloid Leukemia (AML) involves the transfusion of mutlipotent cells derived from bone marrow, peripheral or cord blood. Cells are harvested from the same individual (autologous) before leukemic cells have been destroyed using chemotherapy or radiation therapy, and returned post marrow ablation; alternatively replacement post ablation can be from an HLA-matched donor (allogeneic). One disadvantage in allogeneic transplants is the development of a potentially lethal immune response, graft versus host disease (GVHD). An advantage of allogeneic grafts is the potential for a graft-versus leukema (GVL) response, where the renewing immune system destroys residual malignant cells; this can be an important contribution to achieving remission. Because the autologous option makes use of stem cells from the patient, the possibility of malignant contamination remains, and there is no GVL response; the advantage however is that autologous grafts do not cause GVHD. A major obstacle to HSC therapy is immunological rejection. Technological advances in genomics and proteomics, gene therapy as well as improved stem cell banking technology have the potential to improve the clinical utilization of hematopoietic stem cells for AML therapy.

1.Introduction:

An understanding of blood stem cell biology advanced with the development of radio-biological research [1-3]. Blood cells have short lifespan and need to be replenished continuously. Their production depends directly on the presence of hematopoietic stem cells (HSCs) (Figure 1). When HSCs are destroyed by radiation the entire blood lineage fails. Knowledge in the field of HSC homeostasis and differentiation has had far reaching affects beyond the understanding of acute myeloid leukemia and can be generalized to other areas of stem cell research. The major advances have included [4]:

· Characterizing cell hierarchy - essential for making sense of impact of genetic and molecular aberrations.

· Isolation of cell subsets, and development of in vitro colony-forming assays - supporting molecular research in both normal and malignant hematopoiesis, and transplant medicine.

· Understanding of the different properties of HSCs dependent on location (e.g., foetal liver, bone marrow, or placenta), and organism age - thorough study of cell biological environments is fundamental to delineating stem cell regulation.

· Realization that a 'classical' hierarchy (Figure I) with orderly linear development of progenitors is over-simplistic - HSCs have varying and 'plastic' developmental potential, controlled by coupled and competing transcription factor regulation. Cellular programming is not simply linear.

· Recognition of links between chromosomal translocations, hematopoietic transcription factors and malignancy - disturbances of transcription lie at the heart of oncogenesis.

· Observation that although animal model mechanisms differ from that in humans, certain critical signaling and transcriptional pathways are in common - parallel study using different species-models takes advantage of the strengths of each.

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2.History, drivers and challenges.

The developmental milestones in stem cell therapy (SCT) from the 1950s preclinical trials to the successful application in human transplantation in the late 1970s are shown in Table 1. These laid the foundation for many areas of stem cell research, as well as current hematopoietic stem cell transplantation (HSCT) practices largely driven by stem cells potentials as a renewable source of adult cells [5] . This self regenerative benefit of stem cells is being overshadowed by recent findings that leukemia is also maintained by leukemia stem cells (LSCs) that are capable of self-renewal [6-9].The similarities between LSC and normal stem cells(SCs) and the many useful targets in AML that requires simultaneous targeting have hampered the development of targeted therapies that are selective against LSCs [10-13]. Four sources of HSCT are available and each has its pros and cons (Table 2). The vast majority of clinical trials have been in allogeneic and autologous stem cell transplants. Despite our learning of how best to use these therapies the challenges remain better techniques for identification and purification ,cryopreservation, and understanding the influence of single nucleotide polymorphisms(SNPs), non-HLA genetics, and cytokine genes [14-16].

2.1.Identifying and purifying HSCs

The ability of HSCs to repopulate all of the hematopoietic lineages in vivo and sustain the production of these cells for the life span of the individual is critical to sustaining life [17,18]. Aside from reliable cell surface markers ,HSCs are identified and enumerated based on functional long-term, multilineage, in vivo repopulation assays[19].The extremely low frequency of HSCs in any tissue and the absence of a specific HSC phenotype have made their purification and characterization a highly challenging goal [20,21].HSC are characterized by in vitro and in vivo assays[22-32]. The in vivo assays including the Till & McCulloch colony-forming assay (CFU-S) modified to the competitive repopulating unit assay (CRU) that detects transplantable mouse hematopoietic stem cells with the capacity to regenerate all of the blood cell lineages for extended time periods in vivo[33].While the in vitro assays include the Long-Term Culture-Initializing Cell (LTC-IC) assay and Cobble-stone Area Forming Cell (CAFC) assay [34]. HSC are isolated by enriching for a rare cell population with a combination of monoclonal antibodies [35]. They are purified by ficoll-density fractionation, pre-sort enrichment, and cell sorting with cell-surface and metabolic markers such as CD34, which has become the most important cell-surface marker for positively selecting a rare cell population [36-38]. Within the CD34+ cell population, the differential expression of Thy-1, CD38, and AC133 have been used to fractionate HSCs and progenitors [39]. In order to sub fractionate CD34+ cells by these markers, the cells can be further purified by flow cytometry [29,40].

Cryopreservation of stem cells

Hematopoietic stem cell transplantation represents a critical approach for the treatment of Acute Myeloid leukemia[41].An essential pre-requisite to the clinical application of stem cell therapy are suitable cryopreservation protocols for long-term storage[42].It has long been known that the process of cryopreservation and thawing plays an important role in the decline of viability in cryopreserved cells. Factors that are suspected of causing such an occurrence are the type and concentration of the cryoprotectant and the cooling and warming rate used during cryopreservation and thawing and the use of straw[43-45]. Recently, numerous research groups have endeavoured to optimize the procedure of freezing and thawing and to develop new cryopreservative media and instruments in order to minimize the damage caused by the freezing - thawing cycle (FTC) and to improve cell survival [46-49]. The improvement in conventional slow cooling protocols and the novel container systems being developed are likely to provide systems that are compatible with the requirements of GMP, regulation, automation, and scale-up[50-53].

2.2.Influence of SNPs, Non-HLA genetics and cytokine genes on stem cell therapy.

After allogeneic stem cell transplantation, the establishment of the donor's immune system in an antigenically distinct recipient confers a therapeutic graft-versus-malignancy effect, but also causes graft-versus-host disease (GVHD) and protracted immune dysfunction due to genetic heterogeneity caused by host-donor antigen disparity influenced by functional polymorphisms in the transplanted antigens[54-56].Identifying these clinically relevant transplant antigens is critical for improved donor selection[57].

A number of cytokine gene polymorphisms have been studied in the context of HSCT, including polymorphisms in IL10, TNF, IFNG, IL6, IL1, and TGFB1 [58-60]. Typically, studies have focused on SNPs in regulatory regions of these genes because these regions may influence the amount of cytokine produced at a steady state or after stimulation[55,61]. Most of these studies have been performed at single institutions in patients who received stem cells from HLA-matched siblings after myeloablative conditioning[55]. The majority have attempted to correlate SNP genotype with risk of acute GVHD[62-64]. In the future, evaluation before transplantation is likely to comprise a more detailed genetic analysis of patient and donor with the ultimate goal of individualized patient treatment based on predictive risk scoring [55,65].

3.Conclusion

Acute myeloid leukemia (AML) arises as a consequence of cytogenetic and somatic gene mutations that result in abnormal HSC and myeloid precursor cell. Many gene mutations are associated with specific abnormalities of transcription factor function.Stem cell biology have provided insight into the genetic and molecular mechanisms of HSC and progenitor cell self-homeostasis, transcription programming, and phenotype expression at different stages of cell maturation as well as mechanisms of cancer cell replication and proliferation in general. Similarly such knowledge has been used in developing techniques for propagating and maturing cell lines from bone marrow and peripheral blood in HSC transplant therapy and in predicting outcomes. Though stem cell transplantation is in it developmental stage, the results achieved so far has raised great expectations in the field of regenerative medicine.

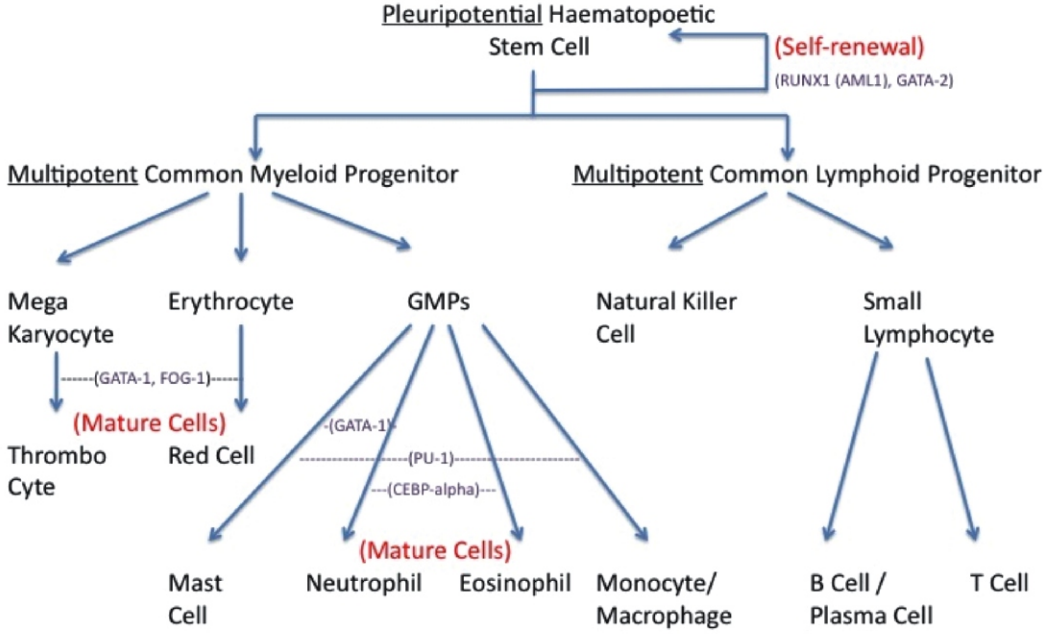
Table 1: Developmental Milestones in HSCT

Year	Development of Hematopoietic Stem Cell Transplantation	Challenges
1949	Spleen shielding experiment of Jacobson.	Limited knowledge of radiation in immune-suppression
1957	First human twin transplants for leukemia.	Relapse
1962	Successful allogeneic transplants in dogs	Understanding of human histo-compatibility
1968	First successful allogeneic transplants in humans.	Graft-vs.-Host Disease (GVHD), limited understanding of details of human histocompatibility, lack of experience with the use of immunosuppressive drugs, and shortcomings in supportive care techniques
1977	Successful application of autologous marrow transplantation	Lack of genetic markers, poor cryopreservation technology
1979	Encouraging results in patients with acute myeloid leukemia transplanted in first remission	GVHD and complications, Relapse, toxicity, and limited donor compatibility and availability.

Table 2. Pros, Cons and Challenges of the different types of HSCT

Type of sct	pros	cons	challenges
Allogeneic SCT	GVL effect represents one of the most powerful anti-leukemia treatments	GVHD, Treatment-related mortality (TRM)	Improving current sources of transplantation and incorporating novel therapies to mitigate TRM
Autologous SCT	Immunologic compatibility between infused hematopoietic stem cell.	Absence of GVL which is crucial to achieving good outcome in SCT, shorter DFS	Contaminated sample, elucidating autologous stem cell transplantation in conjunction with gene therapy
Umbilical Cord Blood	Greater availability, increase in eligible donors and decreased incidence of GVHD	Decreased numbers of stem cells, increase graft failure and mortality	Overcoming cell dose limitation.
Induced pluri-potent stem cells	Prospects to generate SC uncontaminated for autograft without ethical complications	Genomic instability, tumor formation, and the lengthy time requirements needed to obtain these cells via retrovirus development	Locating pluri-potent stem cell sources without the need for reprogramming protein integration

Figure I. Diagram showing the hematopoietic cell lineage from pluri-potent, self-regenerating stem cell, to mature blood cells, with examples of associated transcription factors.



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4.References

[1] Kraft A. Atomic Medicine: the Cold War Origins of Biological Research. History Today. 2009;59(11).

[2] Bizzozero O, Johnson K, Ciocco A. Radiation-related leukemia in Hiroshima and Nagasaki, 1946-1964. I. Distribution, incidence and appearance time. N Engl J Med. 1966; 274 (20): 1095-101.

[3] Yoshinaga S, Mabuchi K, Sigurdson A, Doody M, Ron E. Cancer risks among radiologists and radiologic technologists: review of epidemiologic studies. Radiology. 2004; 233 (2): 313-21.

[4] Orkin HS, Zon LI. Hematopoiesis: An evolving paradigm for stem cell biology. Cell. 2008; 132(4): 631-43.

[5] Ahn S M , Goode R J A, Simpson R J .Stem cell markers: Insights from membrane proteomics? Proteomics. 2008; Volume 8 : Issue 23-24; Pages: 4946-4957.

[6] Passegué E, Catriona HMJ, Laurie EA, Irving LW. Normal and leukemic hematopoiesis: Are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? PNAS. 2003; vol.100,Suppl 1: 11842-11849.

[7] Huntly BJP, Gary D. Gilliland leukemia stem cells and evolution of cancer stem cell research. Nat Rev Cancer. 2005; 5:311–321.

[8] Derrick J. R, Catriona H.M. J, Irving L. W. Stems Cells and the Pathways to Aging and Cancer, Cell.2008;132, 681–696.

[9] J.A Martinez-Climent , Lorena F, Randy D. G, Reiner S ,Felipe P. Lymphoma stem cells: enough evidence to support their existence? haematol.2010;vol. 95(2):293-302.

[10] Craig T J, Monica L G. Mechanisms controlling pathogenesis and survival of leukemic stem cells, Oncogene.2004;23, 7178–7187.

[11] Mary ES, Janet SS, Hua Z, Kenneth LL. Differential Destruction of Stem Cells: Implications for Targeted Cancer Stem Cell Therapy, Cancer Res.2009; 69:24

[12] Thomas X. Targeting leukemia stem cells: The new goal of therapy in adult acute myeloid leukemia, World J Stem Cells. 2009;1(1): 49–54.

[13] Roboz GJ. Novel. Approaches to the Treatment of Acute Myeloid Leukemia. Hematology. December 1, 2011; 2011(1): 43 - 50.

[14] Brenner MK. The contribution of marker gene studies to hematopoietic stem cell therapies. Stem Cells. 1995;13(5):453-61.

[15] Jacob H, Hube A. Preservation of stem cells. Organogenesis. 2009; 5(3):134-7.

[16] Riggs JR, Wanta SM, Lekic N, Craig MJ, Gallicano GI. Current and prospectivetherapies to treat leukaemia. Journal of Hematological Malignancies. 2011;1(1):24-34.

[17] Muller-Sieburg C E, Cho R H, Karlsson L, Huang J F, Sieburg H B. Myeloid-biased hematopoietic stem cells have extensive self-renewal capacity but generate diminished lymphoid progeny with impaired IL-7 responsiveness . Blood June 1, 2004; vol. 103(11): 4111-4118.

[18] Reya T. Regulation of Hematopoietic Stem Cell Self-Renewal. Endocrine Reviews January 1, 2003; vol. 58(1):283-295.

[19] Bhatia M. Hematopoietic Development from Human Embryonic Stem Cells. ASH Education Book January 1, 2007; vol. 2007(1):11-16.

[20] Wognum AW, Eaves AC, Thomas TE. Identification and isolation of hematopoietic stem cells. Arch Med Res. Nov-Dec 2003; 34(6):461-75.

[21] Grant A C, Nathan B, Karen K L, Margaret A G. Mouse Hematopoietic Stem Cell Identification And Analysis. Cytometry A. January 2009; 75(1): 14–24.

[22] Zanjani ED, Silva MR, Flake AW. Retention and multi lineage expression of human hematopoietic stem cells in human sheep chimeras. Blood Cells. 1994; 20:331-338.

[23] Pettengell R, Luft T, Henschler R, et al. Direct comparison by limiting dilution analysis of long-term culture-initiating cells in human bone marrow, umbilical cord blood, and blood stem cells. Blood. 1994; 84:3653-3659.

[24] Broxmeyer HE, Hangoc G, Cooper S, et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. Proc Natl Acad Sci U S A. 1992;89:4109-4113.

[25] Mayani H, Dragowska W, Lansdorp PM. Characterization of functionally distinct subpopulations of CD34+ cord blood cells in serum-free long-term cultures supplemented with hematopoietic cytokines. Blood. 1993;82:2664-2672

[26] Vormoor J, Lapidot T, Pflumio F, et al. Immature human cord blood progenitors engraft and proliferate to high levels in severe combined immunodeficient mice. Blood. 1994;83:2489-2497.

[27] DiGiusto DL, Lee R, Moon J, et al. Hematopoietic potential of cryopreserved and ex vivo manipulated umbilical cord blood progenitor cells evaluated in vitro and in vivo. Blood. 1996;87:1261-1271.

[28] Larochelle A, Vormoor J, Hanenberg H, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. Nat Med. 1996;2:1329-1337.

[29] Bhatia M, Wang JCY, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci U S A. 1997;94:5320-5325.

[30] Piacibello W, Sanavio F, Severino A, et al. Engraftment in nonobese diabetic severe combined immunodeficient mice of human Cd34(+) cord blood cells after ex vivo expansion: evidence for the amplification and self-renewal of repopulating stem cells. Blood. 1999;93:3736-3749

[31] Holyoake TL, Nicolini FE, Eaves CJ. Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. Exp Hematol. 1999;27:1418-1427.

[32] Rosler E S, Brandt J E, Chute J, Hoffman R. An in vivo competitive repopulation assay for various sources of human hematopoietic stem cells. Blood November 15, 2000 vol. 96 (10): 3414-3421.

[33] Williams DE, Lu L, Broxmeyer HE. Characterization of mouse hematopoietic stem and progenitor cells. Immunol Res. 1987; 6(4): 294-304.

[34] Van Os RP, Dethmers-Ausema B, de Haan G. In Vitro Assays for Cobblestone Area-Forming Cells, LTC-IC, and CFU-C. Methods Mol Biol. 2008; 430: 143-57.

[35] Reitsma MJ, Lee BR, Uchida N. Method for purification of human hematopoietic stem cells by flow cytometry. Methods Mol Med. 2002;63:59-77.

[36] Robert W. Storms,Aliana P. Trujillo, James B. Springer, Lisa Shah,* O. Michael Colvin,Susan M. Ludeman, Clay Smith. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. Proc Natl Acad Sci U S A. August 3,1999; 96(16): 9118–9123

[37] Scharenberg C W, Harkey M A, and Torok-Storb B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors . Blood January 15, 2002; vol. 99(2): 507-512

[38] Miltenyi S. CD34+ Selection: The Basic Component for Graft Engineering. The Oncologist December 1997; vol. 2(6); 410-413

[39] Danet G H, Luongo J L, Butler G,Lu M M,Tenner A J,Simon M C,Bonnet D A. C1qRp defines a new human stem cell population with hematopoietic and hepatic potential. Proc Natl Acad Sci U S A. August 6,2002; 99(16): 10441–10445

[40] Reitsma MJ, Lee BR, Uchida N. Method for Purification of Human Hematopoietic Stem Cells by Flow Cytometry. Methods Mol Med. 2002; 63: 59-77.

[41] Berz D,* McCormack M E, Winer E S, Colvin G A, and Quesenberry P J. Cryopreservation of Hematopoietic Stem Cells. Am J Hematol. 2007 June; 82(6): 463–472.

[42] Hunt C J. Cryopreservation of Human Stem Cells for Clinical Application: A Review. Transfus Med Hemother. April,2011; 38(2): 107–123.

[43] He X, Park E Y H, Fowler A , Yarmush M L, Toner M. Vitrification by Ultra-fast Cooling at a Low Concentration of Cryoprotectants in a Quartz Microcapillary: A Study Using Murine Embryonic Stem Cells. Cryobiology. June,2008; 56(3): 223–232.

[44] Xu F, Moon S, Zhang X, Shao L, Song Y S, and Demirci U. Multi-scale heat and mass transfer modelling of cell and tissue cryopreservation. Philos Transact A Math Phys Eng Sci.February 13,2010; 368(1912): 561–583.

[45] Zhang X,Catalano P N,Gurkan U A,Khimji I,and Demirci U. Emerging technologies in medical applications of minimum volume vitrification. Nanomedicine (Lond).August,2011; 6(6): 1115–1129

[46] Lermen D, Blomeke B, Rowne R, Clarke A, Dyce PW, Fixemer T, Fuhr G, Holt WV, Ewgenow KJ, Lloyd RE, Lotters S, Paulus M, Reid GMc, Rapoport DH, Rawson D, Ringleb J, Ryder OA, Sporl G, Schmitt T, Veith M, Muller P. Cryobanking of viable biomaterials: implementation of new strategies for conservation purposes. Mol Ecol 2009, 18:1030-1033.

[47] De Rosa A, De Francesco F, Tirino V, Ferraro GA, Desiderio V, Paino F, Pirozzi G, D'Andrea F, Papaccio G. A new method for cryopreserving adipose-derived stem cells: An attractive and suitable large-scale and long-term cell banking technology. Tissue Eng, Part C s. 2009;15:659-667.

[48] Xu X, Cowley S, Flaim CJ, James W, Seymour L, Cui Z. The roles of apoptotic pathways in the low recovery rate after cryopreservation of dissociated human embryonic stem cells. Biotechnol Prog 2010, in press.

[49] Afrimzon E, Zurgil N, Shafran Y, Ehrhart F, Namer Y, Moshkov S, Sobolev M, Deutsch A, Howitz S, Greuner M, Thaele M, Meiser I, Zimmermann H and Deutsch M. The individual-cell-based cryo-chip for the cryopreservation, manipulation and observation of spatially identifiable cells. II: Functional activity of cryopreserved cells. BMC Cell Biology. 2010; 11:83.

[50] Thirumala S, Goebel W S, Woods E J. Clinical grade adult stem cell banking. Organogenesis. Jul-Sep,2009; 5(3): 143–154.

[51] Coopman, K. Large-scale compatible methods for the preservation of human embryonic stem cells: Current perspectives. Biotechnol Progress.2011;27: 1511–1521

[52] Amps KJ, Jones M, Baker D, Moore HD. In situ cryopreservation of human embryonic stem cells in gas-permeable membrane culture cassettes for high post-thaw yield and good manufacturing practice. Cryobiology. June,2010; 60(3):344-50

[53] Ausubel LJ, Lopez PM, Couture LA. GMP scale-up and banking of pluripotent stem cells for cellular therapy applications. Methods Mol Biol. 2011;767:147-59.

[54] Barrett J A, Rezvani K, Solomon S, Dickinson A M, Wang X N, Stark G, Cullup H, Jarvis M, Middleton P G , Chao N. New Developments in Allotransplant Immunology. ASH Education Book January 1, 2003; vol. 2003(1): 350-371.

[55] Mullally A ,Ritz J. Beyond HLA. the significance of genomic variation for allogeneic hematopoietic stem cell transplantation. Blood February15,2007;vol. 109(4):1355-1362.

[56] Pfistershammer K, Lawitschka A , Klauser C , Leitner J,Weigl R,Heemskerk M H M,Pickl W F, Majdic O,Böhmig G A,Fischer G F, Greinix H T,Steinberger P. Allogeneic disparities in immunoglobulin-like transcript 5 induce potent antibody responses in hematopoietic stem cell transplant recipients Blood September 10, 2009; vol. 114(11): 2323-2332.

[57] Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K, Ishikawa Y, Kato S, Sao H, Sakamaki H, Kawa K, Hamajima N, Shigetaka A,Kodera Y .The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood June 1, 2002; vol. 99(11): 4200-4206.

[58] Goussetis E, Varela I, Peristeri I, Kitra V, Spanou K, Moraloglou O, Paisiou A, Karatasaki S, Soldatou A, Constantinidou N, Graphakos S. Cytokine gene polymorphisms and graft-versus-host disease in children after matched sibling hematopoietic stem cell transplantation: a single-center experience. Cell Mol Immunol. 2011 May;8(3):276-80

[59] Laguila Visentainer JE, Lieber SR, Lopes Persoli LB, Dutra Marques SB, Vigorito AC, Penteado Aranha FJ, de Brito Eid KA, Oliveira GB, Martins Miranda EC, Bragotto L, de Souza CA. Relationship between cytokine gene polymorphisms and graft-versus-host disease after allogeneic stem cell transplantation in a Brazilian population. Cytokine. Nov 3,2005;32(3-4):171-7

[60] Viel DO, Tsuneto LT, Sossai CR, Lieber SR, Marques SB, Vigorito AC, Aranha FJ, de Brito Eid KA, Oliveira GB, Miranda EC, de Souza CA, Visentainer JE. IL2 and TNFA gene polymorphisms and the risk of graft-versus-host disease after allogeneic haematopoietic stem cell transplantation. Scand J Immunol. Dec 2007;66(6):703-10.

[61] Girnita D M, Burckart G, Zeevi A. effect of cytokine and pharmacogenomic genetic polymorphisms in transplantation. Curr Opin Immunol. October,2008; 20(5): 614–625.

[62] Chien J W, Zhang X C,Fan W, Wang H, Zhao L P, Martin P J , Storer B E, Boeckh M , Warren E H ,Hansen J A.Evaluation of published single nucleotide polymorphisms associated with acute GVHD. Blood .2012; vol. 119(22): 5311-5319.

[63] Hansen JA, Chien J W, Warren E H, Zhao L P, Martin P J. Defining Genetic Risk for GVHD and Mortality Following Allogeneic Hematopoietic Stem Cell Transplantation. Curr Opin Hematol.November,2010; 17(6): 483–492.

[64] Tseng LH,Storer B,Petersdorf E, Lin M T, Chien J W, Grogan B M,Malkki M, Chen P J, Zhao L P, Martin P J, Hansen J A. IL10 and IL10 receptor gene variation and outcomes after unrelated and related hematopoietic cell transplantation. Transplantation. March 15,2009; 87(5): 704–710.

[65] Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E; Eurobank members. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol. Dec,2004;127(5):479-90.