UDC 615.385.3:616.097

Pirozhkov I. A.[1, 2], Smolyaninov A. B.[1, 2], Chechetkin A. V.[3], Ivolgin D. A.[1, 2]

[1]*Pokrovsky stem cell bank, St. Petersburg, Russian Federation*
[2]*Research laboratory of cell technologies of the Northwest State Medical University I.I. Mechnikov, St. Petersburg, Russian Federation*
[3]*Russian Federal State Research Institute of Hematology and Transfusiology of Federal Medical-Biological Agency, St. Petersburg, Russian Federation*

e-mail: ipir@mail.ru

SCIENTIFIC-ORGANIZATIONAL ASPECTS FOR DEVELOPING AN INVENTORY OF THE DONORS OF UMBILICAL CORD BLOOD WITH *CCR5 DELTA32/DELTA32* GENOTYPE FOR *HIV* INFECTION TREATMENT

ABSTRACT

Projections of the using umbilical cord blood for HIV infection cure consist in the transplantation of umbilical cord blood hematopoietic stem cells from the donors of homozygous *CCR5 delta32* mutation carriers.

This work presents the results of screening evaluation of umbilical cord blood samples from the donors, included in the public registry of the Pokrovsky stem cells bank, for identification of the homozygous *CCR5 delta32* polymorphism carriers and their following *HLA* typing to see the perspectives for creating a public registry of *CCR5 delta32/delta32* donors of the umbilical cord blood for treatment of *HIV*-infected patients. Total 2860 umbilical cord blood samples were examined from which 29 samples with *CCR5 delta32/delta32* genotype were selected.

High frequency of the *HLA* alleles most prevalent in the North-West region of the Russian Federation has been found in the donors of umbilical cord blood with wild *CCR5* gene and among *CCR5 delta32/delta32* donors.

KEYWORDS: umbilical cord blood, HIV, *CCR5 delta32*, hematopoietic stem cells transplantation.

Chemokines and chemokine receptors play the key role in the regulation of targeted migration of leucocytes in the blood and tissues. They are involved in the pathogenesis of many diseases. Presently, special attention has been paid to the chemokine receptor *CCR5*. *CCR5* is ascribed to the superfamily of transmembrane receptors conjugated with G-protein. The *CCR5* protein is coded by *CCR5* gene located on short arm of chromosome 3 at position 21 (3p21) [1]. There exists *CCR5 delta32* polymorphism type representing deletion of 32 pairs of the nucleotides in the coding area of *CCR5* gene. As a result of mutant gene expression in the homozygous state, shortened and functionally inactive *CCR5* protein is translated [2, 3]. Homozygous carriers of *CCR5 delta32* make nearly 1% of the representatives of the European race and heterozygous on average 10-15 %. Among representatives of the Negroid and Mongoloid races no homozygous carriers of *CCR5 delta32* mutation have been found [4].

The prevalence of polymorphism under consideration decreases from north to the south of Europe. The highest prevalence (16 %) of deletion allele carriage has been found in the Finnish and Mordovian populations and the lowest (4 %) in Sardinia [5]. Also, high polymorphism prevalence was found in France (13.6 %) and Denmark (12.3 %). *Lucotte G.* and *G. Mercier* have reported decrease of polymorphism prevalence in southern countries: 5.6%in the Italians and 1.2%in the Corsicans [6]. The individuals of the Russian ethnic group of Pomors have the high rate of the *CCR5 delta32* polymorphism: 3.1%of homozygous and 30.2%of heterozygous. The prevalence of *CCR5 delta32* genotype is also high in the Gagausian ethnic group: 2.1%for homozygous and 19.9%for heterozygous; and in the West-Ukrainian ethnic group: 2.1%for homozygous and 20.8%for heterozygous [7].

As has been shown, the *CCR5 delta32* mutation carriers have lesser probability for development and more benign course of autoimmune diseases. Indeed, several authors reported favorable clinical course and better prognosis for patients with rheumatoid arthritis and multiple sclerosis in the presence of *CCR5 delta32* mutation, both in the homozygous

and heterozygous state [8, 9]. Lowering of *CCR5* protein expression on the membranes of malignant and stromal cells in the hematological patients with *CCR5 delta32* mutation brings about their reduced response on anti-inflammatory chemokines that may be the positive factor limiting tumor spreading at lymphoproliferative diseases [10].

In recent time *CCR5 delta32* polymorphism has been studied actively in connection with its influence on the pathogenesis of Human Immunodeficiency Virus (*HIV*) infection. Earlier studies reported that the virus finds membrane *CD4* receptor to be a suitable for its entry into cell [11, 12, 13]. However soon it became clear that for virus entry into cell it's binding with only one receptor is not sufficient. In 1996 five different research groups independently made a discovery that along with *CD4* the *HIV* virus uses chemokine receptor *CCR5* for penetration through cell membrane. Simultaneous expression of *CD4* and *CCR5* receptors occurs on *T*-lymphocytes, monocytes, macrophages and dendrite cells.

Presence of *CCR5 delta32* in homozygous state determines synthesis of defective *CCR5* receptor non-expressing itself on cell membrane. For this reason homozygous carriers of study polymorphism possess practically full resistance to *HIV* infecting [14, 15, 16, 17, 18]. One successful case of transplantation of peripheral blood hematopoietic stem cells (*HSC*) was performed in 2007 year in Germany to a HIV-infected patient with acute myeloid leucosis from the donor with *CCR5 delta32/delta32* genotype.

Following transplantation the highly active antiretroviral therapy was discontinued. Virus loading in blood plasma and bioptates of various organs including intestine, liver and lymph nodes remained at undetectable level till now [19, 20, 21]. However, despite a successful treatment this was the sole case and not a single *HSC* transplantation has been performed so far [22]. This circumstance is linked not only with rare incidence of *CCR5 delta32* polymorphism but also with a need of stringent donor-recipient *HLA* matching. For successful bone marrow HSC and peripheral blood transplantation minimum 7 of 8 alleles must coincide in four *HLA-A*, *-B*, *-C*, *-DRB1* loci during high-resolution typing [23, 24]. Such conditions create essential difficulties for selection of proper recipient in the inventory of donors of *HSC* and peripheral blood.

The hypothesis of the American research group headed by Prof. *L. D. Petz* states that HSC of umbilical cord blood with *CCR5 delta32/delta32* gene type could be used for transplantation to HIV-infected patients [25]. It is known that transplantation of umbilical cord blood HSC can be performed under less stringent conditions of histocompatibility: it is sufficient they are matched at 4 of 6 alleles in *HLA-A* and *B* loci at using low resolution typing and at *HLA-DRB1* loci at using high-resolution typing [26, 27].

At present the cord blood is thought to be equivalent source of *HSC* along bone marrow and peripheral blood. First successful transplantation of umbilical cord blood was performed in 1988 in France to a 5-year patient with Fanconi anemia [28]. By the year 2011 more than 20,000 umbilical-cord blood *HSC* transplantations had been performed at the transplantation centers worldwide [29]. Numerous comparative investigations on bone marrow [30, 31], peripheral blood [32, 33] and umbilical-cord blood HSC transplantations [34, 35, 36] demonstrate equivalent post-transplantation outcomes. Thus umbilical cord blood *HSC* transplantation could most probably find its clinical usage for cure of *HIV*-infected patients.

Hence the aim of this work was to evaluate the scientific-organizational possibilities for developing a public registry of umbilical cord blood with *CCR5 delta32/delta32* genotype for cure of *HIV*-infected patients.

MATERIALS AND METHODS

Total 2860 umbilical cord blood samples taken from an Registry of Pokrovsky bank of stem cells (St. Petersburg) were examined. These samples were screened for the presence of *CCR5 delta32* polymorphism and distribution of *HLA* alleles.

Assessment of CCR5 delta32 polymorphism

DNA was isolated from the cord blood samples frozen at -70°C using the *PROTRANS kit* (*Protrans*, Germany). Screening for *CCR5 delta32* alleles was performed by means of the polymerase chain reaction (PCR) in the amplificator *MyCycler, Ver. 1.065* (*BioRad*, USA). Polymorphism detection was done in 9%polyacrilamide gele using vertical electrophoresis. The length of PCR of fragments made 244 bp at wild type gene variant and 192 bp at homozygous *CCR5 delta32* polymorphism.

HLA typing

HLA typing of umbilical cord blood specimens was performed by sequence-specific priming (SSP) method. DNA was isolated from 0.5-0.7 ml of umbilical cord blood using *Protrans DNA Box 500* (*Protrans*, Germany). DNA concentration was estimated on spectrophotometer, mean value 70 μg/ml. Further we performed amplification by using Cyclerplate systems Protrans *HLA-A*,-B**, *-DRB1** (*Protrans*, Germany). Amplification was performed by using thermocycler *MyCycler* (*BioRad*, USA). Products of amplification placed in the gel wells and performed electrophoresis during 25 min at 170 V. In each of 96 wells there should appear control product for checking correct amplification. In some of the wells there should be the strip of specific product that defined the respective genotype by loci *HLA-A*, *HLA-B* and *HLA-DRB1*.

RESULTS AND DISCUSSION

For detection of *CCR5 delta32* polymorphism we examined 2860 umbilical cord blood samples from public registry of donors. As a result, 29 samples with *CCR5 delta32/delta32* genotype were selected that made 1.0 %. In 493 samples (17.2 %) the *CCR5 delta32* polymorphism was present in the heterozygous state **(Table 1)**.

Comparative analysis was carried out to see the distribution of *HLA* alleles among cord blood donors, selected at random, with the wild *CCR5* genotype and among cord blood donors with *CCR5 delta32/delta32* genotype **(Table 2)**.

High frequency of the most prevalent *HLA* alleles in the North-West of Russia was established among the donors of both groups **(Table 3)**.

Identification of the *CCR5 delta32* polymorphism opened new possibilities for HIV-infection cure. Of today, transplantation of HSCs with *CCR5 delta32/delta32* genotype has been the only method that allows eradicate HIV from the infected organism. However the use of bone marrow or peripheral blood samples from adult donors is practically inadmissible because of rare polymorphism occurrence in the population and observance of stringent compatibility conditions by *HLA* system in donor-recipient matching. At the same time cord blood HSCs could significantly more likely to be suitable for treating such patients.

The American investigators have defined that a Registry incorporating 300 units of mononuclear umbilical cord blood fraction with *CCR5 delta32/delta32* genotype will allow specimen selection for transplantation by *HLA* with 73.6%probability for children and 73.6%probability for adults

Table 1. *CCR5 delta32* allele prevalence in cord blood samples of donors from North-West Federal region of Russian Federation (Pokrovsky Stem Cell Bank data)

CCR5 GENOTYPE	CORD BLOOD SAMPLES	
	TOTAL NUMBER	%
WT/WT	2338	81,8
WT/CCR5 delta32	493	17,2
CCR5 delta32/delta32	29	1,0
All	**2860**	**100**

Note: *WT – wild type*

Table 2. *HLA* allele prevalence in donors with wild type genotype and genotype *CCR5 delta32*

LOCUS	THE MOST COMMON ALLELE	ALLELE FREQUENCY IN INDIVIDUALS WITH WILD TYPE GENOTYPE, %	ALLELE FREQUENCY IN INDIVIDUALS WITH GENOTYPE *CCR5 DELTA32/ DELTA32*, %
HLA-A	*02	37,5	25
	*03	5	17,5
	*01	10	7,5
HLA-B	*07	5	7,5
	*35	10	5
	*44	5	15
HLA-DRB1	*07	20	7,5
	*15	7,5	5
	*13	10	10

Table 3. The most common *HLA* alleles in Northwest region of Russian Federation

LOCUS	THE MOST COMMON ALLELE	FREQUENCY, %	THE AVERAGE FREQUENCY OF THIS ALLELE FOR CAUCASIANS [35]	FREQUENCY RANGE [35]
HLA-A	*02	28,3	25,01	7,2-39,6
	*03	15,8	6,87	1,6-25,6
	*01	13,6	14,07	5,3-28,1
HLA-B	*07	13,6	8,67	1,0-16,0
	*35	12,3	10,33	5,0-18,3
	*44	8,8	11,19	4,6-21,7
HLA-DRB1	*07	15,1	13,7	5,3-28,9
	*15	14,8	10,73	5,7-25,6
	*13	13,7	11,11	4,5-26,2

of the Europeoid race [25]. Moreover there are works demonstrating performance of successful transplantations of cord HSCs in combinations with haploidentical bone marrow transplantations [39, 40, 41]. In this case the probability of selection of appropriate cord blood donor for HSCs transplantation will reach 85.6%for children and 82.1%adult patients of the Europeoid race [25]. An additional proof of efficacious HSCs transplantation in HIV infection can be the clinical case where a double umbilical cord blood HSCs transplantation was performed in 34-year HIV-infected patient with acute myeloid leucosis. Retrospectively it was found that one of the cord blood donors was the homozygous carrier of *CCR5 delta32* polimorphism. Investigation of chimerism showed complete engraftment of *CCR5 delta32/delta32* cells. In vitro study carried out on day 123 following transplantation demonstrated that peripheral blood mononuclear cells of the patient were resistant to *HIV-1 BAL* (*CCR*-tropic strain) and *NL4-3* (*CXCR4*-tropic strain) [25]. Nowadays double or treble transplantation of *HSCs* is the worked-off technology allowing avoid limiting factor – insufficient cell number of umbilical cord blood that successfully extend the application of umbilical cord blood *HSCs* transplantation in adult recipients [42, 43].

Cooperative work between numerous umbilical cord blood banks makes it highly probable to create a special storage of cord blood *CCR5 delta32/delta32* specimens which, if need be, can be easily enlarged. It has been estimated that nearly 400,000 criopreserved cord blood specimens are stored worldwide among them 2,000-4,000 *CCR5 delta32/delta32* samples [44]. Nevertheless, there is a need of creating regional inventory *CCR5 delta32/delta32* of umbilical cord blood hematopoietic stem cells for increasing probability of finding compatible units for transplantation in view of the geographic variability of *HLA* alleles distribution. Indicative of this are the data about the prevalence of *HLA* alleles that have been received in this investigation. During a comparative analysis of the distribution of *HLA* alleles in cord blood donors with wild *CCR5* genotype and cord blood donors with *CCR5 delta32/delta32* genotype **(Table 2)**, we have disclosed the high occurrence of the most prevalent *HLA* alleles in the North-West Russia among the individuals of both groups **(Table 3)**.

Thus it would be most reasonable that search for respective specimen of *HSCs* for transplantation should be started in the region of recipient's origin. The suitable candidates for transplantation of cord *HSCs* with *CCR5 delta 32/delta32* would be the HIV-infected individuals for whom transplantation of HSCs in connection with hematologic or other concomitant pathology is indicated. Also transplantation of cord blood HSCs with deletion alleles can be indicative for the *HIV*-infected patients who are resistant to antiretroviral therapy.

Our hypothesis, therefore, is that *CCR5 delta32/delta32* cord blood units found during clinical investigations can be readily proposed for HIV infection cure in the Russian Federation and abroad. On the base of this work the National Cord Blood Inventory of umbilical cord blood with *CCR5 delta32/delta32* genotype will be created in the North-West Federal region of the Russian Federation for HIV infection cure.

CONCLUSIONS

1. FREQUENCY OF *CCR5 DELTA32/DELTA32* OCCURRENCE IN THE NORTH-WEST FEDERAL REGION OF THE RUSSIAN FEDERATION MAKES NEARLY 1 %.

2. HIGH INCIDENCE OF *HLA* ALLELES, MOST WIDELY-SPREAD IN THE NORTH-WEST OF RUSSIA AMONG THE UMBILICAL CORD BLOOD DONORS WITH WILD CCR5 AND AMONG *CCR5 DELTA32/DELTA32* GENOTYPE HAS BEEN ESTABLISHED.

3. THE *CCR5 DELTA32/DELTA32* SAMPLES FOUND AS A RESULT OF THE SCREENING SPEAK IN FAVOR OF THE NEED OF CREATING A NATIONAL REGIONAL STORAGE OF UMBILICAL CORD BLOOD SAMPLES FOR HIV-INFECTION CURE.

REFERENCES

1. *Samson M, Labbe O, Mollereau C, et al.* Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry. 1996; **35(11)**:3362-3367.
2. *Samson M, Libert F, Doranz BJ, et al.* Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996; **382**:722-725.
3. *Liu R, Paxton WA, Choe S, et al.* Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996; **86(3)**:367-377.
4. *Novembre J, Galvani AP, Slatkin M.* The geographic spread of the *CCR5 Delta32* HIV-resistance allele. PLoS. Biol. 2005; **3(11)**: 339.
5. *Libert F, Cochaux P, Beckman G, et al.* The deltaccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. Hum. Mol. Genet. 1998; **7(3)**:399-406.
6. *Lucotte G, Mercier G.* Distribution of the CCR5 Gene 32-bp Deletion in Europe. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. 1998; **19(2)**:174-177.
7. *Kofiadi IA, Rebrikov DV, Trofimov DY.* Raspredelenie allelej genov CCR5, CCR2 i SDF1, associiirovannyh s ustojchivost'ju k VICh-infekcii, v Rossijskih populjacijah [The distribution of alleles CCR5, CCR2 and SDF1, associated with resistance to HIV infection, in Russian populations]. Doklady akademii nauk - Reports of Academy of Sciences, 2007; **415(6)**:842-845.
8. *Garred P, Madsen HO, Petersen J, et al.* CC chemokine receptor 5 polymorphism in rheumatoid arthritis. J. Rheumatol. 1998; **25(8)**:1462-1465.
9. *Kantor R, Bakhanashvili M, Achiron A. A* mutated CCR5 gene may have favorable prognostic implications in MS. Neurology. 2003; **61(2)**:238-240.
10. *Voropaeva EN, Skvortsov NV, Voevoda MI, Tarnowski RV.* Klinicheskoe znachenie delecii gena CCR5 u bol'nyh nehodzhkinskimi zlokachestvennymi limfomami [The clinical significance of the deletion of the CCR5 gene in patients with non-Hodgkin's lymphoma]. Bjulleten' SO RAMN - Bulletin SB RAMS, 2001; 31(2):26-30.
11. *Guyader M, Emerman M, Sonigo P.* Genome organization and transactivation of the human immunodeficiency virus type 2. Nature. 1987; **326**:662-669.
12. *McDougal JS, Maddon PJ, Dalgleish AG.* The T4 glycoprotein is a cell-surface receptor for the AIDS virus. Cold. Spring Harb. Symp. Quant. Biol. 1986; **51**:703-711.
13. *Sattentau QJ, Moore JP.* The role of CD4 in HIV binding and entry. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 1993; **342(1299)**:59-66.
14. *Alkhatib G, Combadiere C, Broder CC, et al.* CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science. 1996;**272(5270)**:1955-1958.
15. *Choe H, Farzan M, Sun Y, et al.* The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell. 1996; 85(7):1135-1148.
16. *Deng H, Liu R, Ellmeier W, et al.* Identification of a major co-receptor for primary isolates of HIV-1. Nature. 1996; 381(6584):661 – 666.
17. *Doranz BJ, Rucker J, Yi Y, et al.* A dual-tropic primary HIV-1 isolate that uses fusin and the betachemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell. 1996; **85(7)**:1149-1158.
18. *Dragic T, Litwin V, Allaway GP, et al.* HIV-1 entry into CD4(+) cells is mediated by the chemokine receptor CC-CKR-5. Nature. 1996; **381**:667-673.
19. *Hutter G, Nowak D, Mossner M.* Long-Term Control of HIV by *CCR5 Delta32/Delta32* Stem-Cell Transplantation. N. Engl. J. Med. 2009; **360**:692-698.
20. *Allers K, Hütter G, Hofmann J.* Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood. 2011; **117(10)**:2791-2799.
21. *Burke BP, Boyd MP, Impey H, et al.* CCR5 as a Natural and Modulated Target for Inhibition of HIV. Viruses. 2013; 6(1):54-68.
22. *Hutter G, Thiel E.* Allogeneic transplantation of CCR5-deficient progenitor cells in a patient with HIV infection: an update after 3 years and the search for patient no. 2. AIDS. 2011; **25**:273-274.
23. *Bray RA, Hurley CK, Kamani NR, et al.* National marrow donor program *HLA* matching guidelines for unrelated adult donor hematopoietic cell transplants. Biol. Blood Marrow Transplant. 2008; **14**:45-53.
24. *Lee SJ, Klein J, Haagenson M, et al.* High-resolution donor-recipient *HLA* matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007; **110(13)**:4576-4583.
25. *Petz LD, Redei I, Bryson Y, et al.* Hematopoietic Cell Transplantation with Cord Blood for Cure of HIV Infections. Biol. Blood Marrow Transplant. 2013; **19(3)**:393-397.
26. *Brunstein CG, Fuchs EJ, Carter SL, et al.* Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially *HLA*-mismatched related bone marrow or unrelated double umbilical cord blood grafts. Blood. 2011; **118(2)**:282-288.
27. *Spellman SR, Eapen M, Logan BR, et al.* A perspective on the selection of unrelated donors and cord blood units for transplantation. Blood. 2012; **120(2)**:259-265.
28. *Gluckman E, Broxmeyer HA, Auerbach AD, et al.* Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an *HLA*-identical sibling. N. Engl. J. Med. 1989; **321(17)**:1174-1178.
29. *Gluckman E, Ruggeri A, Volt F.* Milestones in umbilical cord blood transplantation. Br. J. Haematol. 2011; **154(4)**:441-447.
30. *Chen YH, Xu LP, Liu DH, et al.* Comparative outcomes between cord blood transplantation and bone marrow or peripheral blood stem cell transplantation from unrelated donors in patients with hematologic malignancies: a single-institute analysis. Chin. Med. J. (Engl). 2013; **126(13)**:2499-2503.
31. *Smith AR, Baker KS, Defor TE, et al.* Hematopoietic cell transplantation for children with acute lymphoblastic leukemia in second complete remission: similar outcomes in recipients of unrelated marrow and umbilical cord blood versus marrow from *HLA* matched sibling donors. Biol. Blood Marrow Transplant. 2009; **15(9)**:1086-1093.
32. *Tomblyn MB, Arora M. Baker KS, et al.* Myeloablative hematopoietic cell transplantation for acute lymphoblastic leukemia: analysis of graft sources and long-term outcome. J. Clin. Oncol. 2009; **27(22)**:3634-3641.
33. *Gutman JA, Leisenring W, Appelbaum FR., et al.* Low relapse without excessive transplant-related mortality following myeloablative cord blood transplantation for acute leukemia in complete remission: a matched cohort analysis. Biol. Blood Marrow Transplant. 2009; **15(9)**:1122-1129.
34. *Eapen M, Rocha V, Sanz G, et al.* Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. The Lancet Oncology. 2010; **11(7)**:653-660.
35. *Brunstein CG, Gutman JA, Weisdorf DJ, et al.* Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. Blood. 2010; **16(22)**:4693-4699.
36. *Zhang MJ, Davies SM, Camitta BM, et al.* Comparison of Outcomes after *HLA*-Matched Sibling and Unrelated Donor Transplantation for Children with High-Risk Acute Lymphoblastic Leukemia. Biol. Blood Marrow Transplant. 2012; **8(8)**:1204-1210.

37. *Rubinstein P, Dobrila L, Rosenfield RE., et al.* Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc. Natl. Acad. Sci. USA. 1995; **92(22)**:10119-10122.
38. *McKenna DH, et al.* Umbilical cord blood. Core Principles in cellular therapy. AABB, Bethesda. 2008; **3**:47-72.
39. *Liu H, Rich ES, Godley L, et al.* Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. Blood. 2011; **118(24)**:6438-6445.
40. *Magro E, Regidor C, Cabrera R, et al.* Early hematopoietic recovery after single unit unrelated cord blood transplantation in adults supported by co-infusion of mobilized stem cells from a third party donor. Haematologica. 2006; **91(5)**:640-648.
41. *Sebrango A, Vicuña I, de Laiglesia A.* Haematopoietic transplants combining a single unrelated cord blood unit and mobilized haematopoietic stem cells from an adult *HLA*-mismatched third party donor. Comparable results to transplants from *HLA*-identical related donors in adults with acute leukaemia and myelodysplastic syndromes. Best Pract. Res. Clin. Haematol. 2010; **23(2)**:259-274.
42. *Stanevsky A, Shimoni A, Yerushalmi R, et al.* Double umbilical cord blood transplant: more than a cell dose? Leuk. Lymphoma. 2010; 51(6):975-982.
43. *Scaradavou A, Brunstein CG, Eapen M.* Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. Blood. 2013; **121(5)**:752-758.
44. *Gonzalez G, Park SS. Chen DW, et al.* Identification and frequency of CCR5Delta32/Delta32 HIV-resistant cord blood units from Houston area hospitals. HIV Med. 2011; **12(8)**: 481-486.

The authors indicate no potential conflicts of interest.

Received: February 16, 2014
Accepted: March 11, 2014