

Preimplantation Hla Typing- Practical Tool for Stem Cell Transplantation Treatment of Congenital and Acquired Disorders

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Abstract

It is well known that to achieve an acceptable engraftment and survival in stem cell therapy, an HLA identical stem cell transplant is strongly required. However, the availability of the HLA matched donors even among family members is extremely limited, so pre-implantation HLA typing provides an attractive practical tool of stem cell therapy for children requiring HLA matched stem cell transplantation.

The present experience of PGD for HLA typing of over one thousand cases shows that PGD provides the at-risk couples with important prospect not only to avoid an inherited risk of producing the offspring with congenital disease, but also to establish an unaffected pregnancy, which may benefit the affected member of the family with hemoglobinopathies, immunodeficiencies and other congenital or acquired bone marrow failures.

Despite ethical issues involved in pre-implantation HLA typing, the data presented below show the increasing attractiveness of this option for the couples with affected children requiring HLA compatible stem cell transplantation. So the couples at risk of having children with congenital bone marrow disorders have to be informed about presently available option not only for avoiding the birth of affected child, but also for selecting a suitable stem cell donor for their affected siblings.

Keywords: Preimplantation HLA typing; PGD; Stem cell transplantation; Hemoglobinopathies; Immunodeficiencies; Aneuploidy testing

Introduction

Preimplantation HLA matching could not be indication for prenatal diagnosis because it is not acceptable to terminate a normal pregnancy only because the fetus is HLA unmatched. However, preimplantation genetic diagnosis (PGD) for this purpose is perfectly acceptable because only a limited number (ideally one) of the embryos after hypostimulation are replaced, so this embryo (embryos) may represent unaffected one with a perfect match for affected siblings at need for a transplant. The world's first case of preimplantation HLA typing was introduced in combination with mutation analysis for Fanconi anemia (FA), with the objective of establishing an unaffected pregnancy yielding a potential donor progeny for transplantation in an affected sibling [1,2]. FA is one of the severe congenital disorders requiring stem cell transplantation from the family member, because any modification of the conditioning is too toxic for these patients leading to a high rate of transplant-related mortality, representing also the world's first disease, that cord blood stem cell transplantation has been introduced [3]. This severe autosomal recessive disorder is characterized by inherited bone marrow failure, congenital malformations and an increased predisposition to the development of leukemia. It is genetically heterogeneous, involving different complementation groups (FANCA, FANCB, FANCC, FANCD and FANCE) [4-6]. Bone marrow transplantation is the only hope, as it restores definitively hematopoiesis in FA patients, with the most important requirement being HLA identical stem cell transplantation from a sibling, to avoid late complications due to severe GVH [7,8].

The results of this first case demonstrated feasibility of preimplantation HLA matching as part of PGD, with a prospect for its application to the other inherited conditions, such as thalassemias and other congenital disorders, also requiring an HLA compatible donor for bone marrow transplantation. PGD for HLA testing provided a realistic option for the couples desiring to avoid the birth of an

affected child, together with the establishment of a healthy pregnancy, potentially providing an HLA match for an affected sibling. As will be described in this paper, preimplantation HLA testing is currently also applied as a primary indication, i.e. in cases not requiring mutation testing, such as for couples having affected children with leukemia or other cancers, awaiting for an HLA compatible donor with no success for years. These new indications make PGD a genuine alternative to conventional prenatal diagnosis, providing patients with important prospect not only to avoid an inherited risk without facing termination of pregnancy, but also to establish a pregnancy with particular genetic parameters to benefit the affected member of the family.

Preimplantation HLA Typing with and without PGD

Our experience on PGD with HLA typing is presented in Table 1, showing that among conditions requiring HLA compatible stem cell transplantation, hemoglobinopathies is the major indication, representing the commonest autosomal recessive diseases in Mediterranean region, Middle East and South East Asia.

Hemoglobinopathies

Hemoglobinopathies, including thalassemia and sickle cell disease, are autosomal recessive conditions affecting the production of beta-globin chains resulting in a severe anaemia, which makes the patients transfusion dependant starting from six months after

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Disease	Patients	Cycles	# of Embryo Transfers	# embryos Transferred	Pregnancy	Birth
Thalassemia/ Sickle Cell Disease	51	149	82	130	20	15
FANCA,FANCC, FANCD2,FANCF, FANCI, FANCI	17	53	34	52	7	4
WAS	2	2	2	4	1	1
X-ALD	2	5	1	1	0	0
Hyper IgM	5	8	6	9	3	2
HED+ID; IP	2	9	6	8	2	3
DBA	3	5	3	6	2	2
Krabbe	1	1	1	2	1	2
Chronic Granulomatous Disease	1	3	3	5	1	1
TOTAL	85	228	131	209	34	30

Table 1: Experience in PGD with HLA typing.

birth, so bone marrow transplantation is the only and most efficient option for radical treatment of these patients. At present more than 400 different mutations have been described in β -globin gene, located in chromosome 11 (11p15.5), causing congenital anaemia of variable severity. Prenatal diagnosis has been applied widely for over three decades resulting in considerable reduction of new cases of thalassemia to up to 70% in many populations, including such large countries in the Eastern Mediterranean region, as Greece, Turkey and Iran [9-11]. A considerable progress has been achieved also in treatment of these conditions by bone marrow transplantation [12], the application of which is mainly limited to the availability of HLA matched stem cells, making PGD an attractive option for couples with thalassaemic children. PGD for thalassemia has been already provided for 17 years [13-15], so HLA typing is presently offered in the same framework, allowing couples not only avoiding the birth of another child with thalassemia, but also producing an unaffected child as a potential HLA matched donor for thalassaemic sibling.

In our experience, of a total of 293 PGD cycles for 161 couples at risk for producing offspring with thalassemia, 144 cycles were performed for HLA typing. Polar body (PB) or embryo biopsy was used to detect thalassemia mutations, and embryo biopsy was also used for HLA testing, in order to identify the embryos containing the maternal and paternal chromosomes 6 identical to the sibling with thalassemia, as described in detail elsewhere [16-18].

In brief, HLA typing was based on testing of closely linked polymorphic short tandem repeat (STR) markers located throughout HLA region [19-22], including D6S426, D6S291, Ring 3 CA, TAP1, G51152, D6S2447, LH1, DN, D6S273, 9N-2, TNF a,b,c,d; 62, MIC A, MIB, D6S276, D6S439, D6S1624, D6S265, D6S510; D6S248, RF, MOG a,b,c,d, D6S 258, D6S306, D6S464, D6S299, D6S46. The applied strategy provided a 100% HLA match, because the embryos with the same paternal and maternal chromosomes 6 as in the affected siblings were pre-selected. The chances to identify unaffected embryos fully matched to thalassaemic siblings is 18.75%, as for other autosomal recessive conditions, based on 25% chance of HLA match and 75% chance of having unaffected embryo (see below).

Of more than two dozens of different beta-globin gene mutations tested, the most frequent ones were IVSI-110 mutation -100 cases (33%), followed by IVS I-6 -39 cases, IVSII-745 - 23 cases, Codon 8 - 20 cases, IVSI-1 -18 cases, and codon 39 and IVSI-5 -16 cases each [18]. Among other mutations were IVSII-2, Codon 5, Codon 6, Codon 41/2, E121K, -29 (A-G) -87, R30T, Cap 1, deletion 69 kb and deletion 13.4 kb. Mutation testing resulted in detection and transfer of 476 unaffected embryos (approximately, 2 embryos per transfer) in 240 (81.9%) of 293 clinical cycles, yielding 67 (27.9%) unaffected pregnancies and birth of 70 thalassemia-free children [18]. PGD for thalassemsias currently

represents 15% of our overall experience of thousands of PGD cycles performed for single gene disorders [23].

A total of 149 of PGD cycles for heamoglobinopathies in our series, were performed in combination with HLA typing, which allowed detecting and transferring unaffected HLA matched embryos in 82 of them (Table 1). Of 824 embryos with conclusive results for testing of beta-globin gene mutations and HLA type, 602 (73.0%) were predicted to be unaffected carriers or normal, of which only 130 (21.5%) appeared to be HLA identical to the affected siblings, which is not significantly different from the expectation. The transfer of these embryos resulted in 20 unaffected HLA identical pregnancies and birth of 15 healthy children. Umbilical cord blood was collected at birth of these children, or bone marrow obtained at 1 year of age, and transplanted or pending, resulting in a successful hematopoietic reconstitution in all of them.

With the current progress in treatment of hemoglobin disorders, PGD may have an increasing impact on the decision of the well treated patients to reproduce. In fact, the life expectancy of the patients with hemoglobin disorders has been dramatically improved with the increasing success rate of radical treatment by stem cell transplantation [24]. However, the further impact of this treatment will depend on the availability of HLA identical donors.

PGD for HLA typing has currently been applied as an efficient tool for couples at risk in many PGD centres to ensure having thalassemia-free children HLA identical to the affected siblings, to serve a potential donor for stem cells for transplantation treatment. This currently is available for a wider application in those communities where thalassemia is highly prevalent, and will improve the access to HLA matched bone marrow transplantation of thalassemia.

For example, in one of the largest series of PGD for thalassemia with HLA typing performed in Istanbul, Turkey, 236 PGD cycles were performed resulting in birth of 70 thalassemia-free children. Of 48 children transplanted (in addition to thalassemia, children with 9 other different conditions was transplanted), successful outcome was observed in 44 of them with a total hematopoietic reconstitution, while the graft failure occurred only in 4 of them [25-27].

Immunodeficiencies

Preimplantation HLA typing appeared to be of great utility for severe congenital immunodeficiencies (SCID), as without compatible bone marrow transplantation the patients with SCID cannot survive. HLA matched stem cell transplantation improves or completely replenishes the immune system, so PGD is an obvious alternative for inherited forms of SCID, to ensure the birth of unaffected children, who may then also serve as a potential stem cell donor progeny for the affected siblings. Our accumulated experience of PGD for SCID includes PGD for ataxia telangiectasia (AT), Omen syndrome

(OMS), FA, hyperimmunoglobulin M syndrome (HIGM), X-linked adrenoleukodystrophy (X-ALD), Wiscott-Aldrich syndrome (WAS), X-linked hypohidrotic ectodermal dysplasia with immune deficiency (HED-ID) and other few conditions listed in Table 1 [28,29].

The largest group was PGD with HLA typing for FA – 53 cycles, followed by HED-ID – 9 cycles, HIGM – 8, X-ALD and Diamond-Blackfan anemia (DBA) – 5 each, chronic granulomatous disease -3, WAS -2, and Krabbe disease -1. This initially also included the world's first case of PGD for OMS, mentioned, which resulted in transfer of two unaffected embryos, yielding the birth of healthy twins. Because in this case, the affected sibling died early in childhood, there was no need for HLA typing, but the couples with previous OMS children will definitely be potential candidates for performing PGD with HLA typing to provide also an identical HLA donor progeny for stem cell transplantation [28].

The other disease requiring PGD is Ataxia Telangiectasia (AT), which is a progressive, neurodegenerative childhood disease that affects the brain and other body systems. A weakened immune system makes the patients susceptible to recurrent respiratory infections. Although the currently used symptomatic and supportive treatment, including high-dose vitamin regimens, physical and occupational therapy and gamma-globulin injections to supplement a weakened immune system may be helpful, the prognosis is very poor, patients still dying in their teens. The case of PGD for AT was reported previously for a Saudi family with 3 affected children [30].

As mentioned, bone marrow transplantation is also the only treatment for FA, as it restores hematopoiesis in FANCA patients. However, because any modification of the conditioning is too toxic for these patients, similar in FANCC, leading to a high rate of transplant-related mortality, the HLA identical cord blood transplantation from a sibling is particularly valuable, to avoid late complications due to severe GVH, as mentioned above. Of 17 couples at risk for producing a progeny with FA, in addition to two carriers of IVS 4+4 A-T mutation in FANCC gene, three were carriers of FANCD2, FANCF, FANCI, FAMCCJ, and FANCA gene mutations. Overall, 52 unaffected HLA matched embryos were transferred in 34 cycles, resulting in seven unaffected pregnancies and 4 FA free and HLA matched children, as potential donors for their siblings, with successful transplantation treatment described above..

Five cycles were performed for X-linked Adrenoleukodystrophy (X-ALD), which affects the nervous system and the adrenal cortex, with three main phenotypes. One of them manifests between ages four and eight as attention deficit disorder, followed by progressive impairment of cognition and behaviour, vision, hearing and motor function leading to total disability within two years. The other phenotype, called adrenomyeloneuropathy, manifests in late twenties as progressive paraparesis, sphincter disturbances and hearing loss, while the third - presents with primary adrenocortical insufficiency by 7-8 years of age. The disease is caused by mutations of ABCD1 gene, with more than 200 different mutations reported by the present time, which may be detected by PCR and direct sequencing, except for large deletions identified by Southern blot analysis. Carrier screening and prenatal diagnosis is available and the same method may be applied for PGD with simultaneous HLA typing.

Of special interest is preimplantation HLA typing for hyperimmunoglobulin M Syndrome (HIGM), which is a rare immunodeficiency characterized by normal or elevated serum IgM levels, with absence of IgG, IgA and IgE, which results in an increased

susceptibility to infections, manifested in the first few years of life, and a high frequency of autoimmune hematologic disorders, accompanied by gingivitis, ulcerative stomatitis, fever, and weight loss. HIGM is caused by mutation in the CD40 ligand gene (CD40LG), located in chromosome Xq26, which leads to a defective CD40 ligand expression resulting in the failure of T cells to induce IgE synthesis in interleukin-4-treated B cells. Although a regular administration of intravenous immunoglobulins may be used for treatment, the best results were obtained by HLA matched bone marrow transplantation, which makes PGD the method of choice for those who cannot find an HLA match among their relatives.

Eight PGD cycles were performed for 5 couples with HIGM, one with C218X mutation in exon 5 of CD40 ligand gene (CD40LG), 3 with maternal mutations C218X exon 4 c.437_38 ins A, and one with exon 4 c.397 ins T. The maternal mutations were analyzed by PB1 and PB2, followed by HLA and aneuploidy testing in biopsied blastomeres. Of 8 cycles PGD for HLA performed, 9 unaffected HLA matched embryos were transferred in 6 cycles, resulting in 3 clinical pregnancies and birth of 2 healthy babies, as potential donors of HLA compatible stem cells for their siblings.

The first attempt of cord blood transplantation from one of the babies did not result in acceptable engraftment, so the second transplantation was performed one year later, using bone marrow mixed with the remaining portion of the cord blood sample, which provided better results in achieving successful engraftment and reconstitution of the sibling's bone marrow, and resulting in a total cure of the patient.

Wiscott-Aldrich Syndrome (WAS) and X-linked Hypohidrotic Ectodermal Dysplasia with Immune Deficiency (HED-ID) are the other lethal X-linked immune deficiency, in which lymphocyte dysfunction result in severe infections, and increased risk of lymphoproliferative malignancies. While supportive therapy may increase survival rate, the only hope for avoiding early mortality is bone marrow transplantation. Intravenous immunoglobulins and prophylactic antibiotics may be useful in improving clinical status, but bone marrow transplantation is required to prevent early mortality.

A total of 11 cycles were performed for these conditions, in which 12 embryos were detected to be unaffected and HLA matched (8 for HED-ID and 4 for WAS), and transferred in 8 cycles, resulting in birth of 4 unaffected babies (3 free of HED-ID and 1 free of WAS), confirmed to be HLA matched to affected sibling. Cord blood from this child was collected and transplanted to the affected sibling with HED-ID, resulting in a complete cure.

The presented data show the usefulness of PGD for SCID, as there is no effective treatment for these conditions other than stem cell transplantation. PGD provides the couples at risk with the option to avoid the affected pregnancy and have a progeny free of SCID. If there is already an affected child in family, PGD with HLA typing makes also possible to have an access to the HLA identical stem cell transplantation through selection and transfer of those unaffected embryos which are also present HLA match to the sibling.

Preimplantation HLA typing as a sole indicator

As presented in Table 2, in addition to 228 PGD for HLA cycles, 98 cycles were performed for preimplantation HLA matching without testing for causative gene. These couples were wishing to have another child anyway, but requested that these children may also be a potential cord blood donor for the affected siblings with leukemia, or sporadic

PREIMPLANTATION TESTING	Patients	Cycles	# of Embryo Transfers	# embryos Transferred	Pregnancy/ Birth
HLA TESTING ONLY	46	98	65	99	24/19
HLA + MUTATION	85	228	131	209	34/ 30
T O T A L	131	326	196	308	58/49

Table 2: Preimplantation HLA typing with and without PGD.

DBA (with no gene mutations identified), requiring bone marrow transplantation or cord blood transplantation treatment [31].

There was no difference in performing preimplantation HLA testing without PGD, except limiting the analysis of the day 3 or day 5 embryo to only HLA typing with the sibling requiring stem cell transplantation, using a multiplex hemi-nested PCR system. A haplotype analysis for father, mother and the affected child was performed for each family prior to preimplantation HLA typing, using a set of polymorphic STR markers located throughout HLA region. This allowed detecting and avoiding misdiagnosis due to preferential amplification and ADO, potential recombination within the HLA region, and a possible aneuploidy or uniparental disomy of chromosome 6, which may also affect the diagnostic accuracy of HLA typing of the embryo.

In a total of 98 clinical cycles from 46 couples performed with a primary indication of HLA typing, 99 HLA matched embryos were pre-selected for transfer. Proportion of embryos predicted to be HLA matched to the affected siblings was 21.5%, not significantly different from the expected 25% (Table 3). The transfer of 99 HLA matched embryos in 65 clinical cycles, resulted in 24 singleton clinical pregnancies and 19 HLA matched children born. These results suggest that testing of available number of embryos per cycle allows preselecting a sufficient number of the HLA matched embryos for transfer to achieve a clinical pregnancies and birth of an HLA matched progeny. The first successful transplantation with stem cells obtained from these children was achieved in sibling with sporadic form of BDA, resulting in a complete cure [31].

Presented data show feasibility of preimplantation HLA matching for families with affected children with bone marrow disorders, who may wish to have another child as a potential HLA matched donor of stem cells transplantation treatment of the affected sibling. As seen from our data, HLA matched embryos were preselected and transferred in all cycles, resulting in clinical pregnancies and birth of HLA matched children in almost every second transferred cycle.

The results also demonstrate the prospect for the application of this approach to other conditions, requiring an HLA compatible donor for stem cell transplantation. This provides a realistic option for those couples who would like to have another child anyway, as they may potentially provide an HLA match progeny for an affected sibling. In addition to leukemias and sporadic forms of DBA, the method may be applied for any other condition, such as for couples having affected children with different cancers awaiting an HLA compatible donor with no success for years. These new indications make preimplantation testing a genuine alternative to conventional prenatal diagnosis, providing patients with important prospect not only to avoid an inherited risk without facing termination of pregnancy, but also to establish a pregnancy with particular genetic parameters to benefit the affected member of the family.

Limitations and Future Prospect of PGD for HLA Typing

Presented data demonstrate that PGD for HLA typing may become a practical option, available for a wider application in order

to further improve the radical treatment for congenital and acquired bone marrow failures by stem cell transplantation. Despite the high rate of preferential amplification and ADO in PCR analysis of single blastomeres, potential recombination within the HLA region [16,29], and a high rate of mosaicism for aneuploidies at the cleavage stage, the technique is highly accurate in preselecting of HLA matched embryos for transfer.

A relatively high frequency of recombination in the HLA region is one of the major limitations of PGD for HLA typing, which may affect not only the accuracy of preimplantation HLA typing, but also the outcome of stem cell transplantation. In our experience, of 1713 embryos tested for HLA, 1634 (95.5%) were non-recombinant, 52 (3%) with maternal, and 27 (1.5%) with paternal recombination [16,29]. Therefore, haplotype analysis prior to initiation of the actual cycle is strongly required, so that the couples may be informed about their possible options. For example, the maternally derived recombination may become obvious after PB1 analysis, without which maternal haplotypes cannot be established, while the paternal haplotypes may be identified through sperm typing. The major problem in performing PGD for HLA may be faced when the preparatory testing identified the sibling being with maternal recombination, so it could be unrealistic to identify the exact match, and the couples should be informed that only relatively close match may be possible, which may be discussed with paediatric haematologist in the pre-selection process of the embryos for transfer.

Despite the need for further improvement of the technique as mentioned, the present experience shows that the couples with affected children requiring HLA compatible stem cell transplantation have a realistic option to undergo IVF and PGD with a combined preimplantation HLA typing, so to have an unaffected HLA matched child as potential donor of compatible stem cells for sibling.

The other important limitation is that the majority of patients requesting preimplantation HLA typing are of advanced reproductive age, so the outcome of the procedure has not yet been sufficiently high, many patients still undergoing two or more attempts before they become pregnant and deliver an HLA identical offspring. So testing for age-related aneuploidy may appear useful for improving the reproductive outcome of preimplantation HLA typing, which will also minimize the risk of delivering a child with chromosomal disorders, providing reassurance for patients who are usually concerned about their pregnancy outcomes.

Aneuploidy testing is currently offered as an integral component of preimplantation HLA typing to the patients of advanced reproductive age, performed in increasing number of preimplantation HLA typing cycles combined with or without PGD in our experience. Although the chances of pre-selecting unaffected HLA matched embryos that could be also euploid is quite low, our preliminary results of the reproductive outcome comparison between the groups of combined PGD/HLA with and without aneuploidy testing showed a significant difference (see below). Despite transferring a lower number of embryos, the pregnancy rate was higher in the aneuploidy testing group, suggesting the potential utility of aneuploidy testing in preimplantation HLA typing, allowing the avoidance of transfer of those HLA identical embryos that

HLA MATCH Only - $\frac{1}{4}$ (25%)
Autosomal Recessive or X-linked Free + HLA MATCH - $\frac{3}{4} \times \frac{1}{4} = 3 / 16$ (18.75%)
Autosomal Dominant Free + HLA MATCH - $\frac{1}{2} \times \frac{1}{4} = 1 / 8$ (12.5%)
Autosomal Recessive or X-linked Free + HLA MATCH + ANEUPLOIDY-Free - $\frac{3}{4} \times \frac{1}{4} \times \frac{1}{2} = 3 / 32$ (9.4%)
Autosomal Dominant Free + HLA MATCH + ANEUPLOIDY-Free - $\frac{1}{2} \times \frac{1}{4} \times \frac{1}{2} = 1 / 16$ (6.25%)

Table 3: Chances for Detection of Disease Free and HLA Match Embryo in Preimplantation HLA Typing.

are chromosomally abnormal, which are destined to be lost anyway either before or after implantation. Alternatively, incidental transfer of aneuploid embryos in the absence of chromosomal testing should lead to implantation and pregnancy failures in preimplantation HLA typing cycles, or may compromise the pregnancy outcome through spontaneous abortions.

The addition of aneuploidy testing expected to identify at least 50% chromosomally abnormal embryos in patients of advanced reproductive age, will be also lowering the probability of detecting the embryos for transfer by half. In fact, the mean number of embryos for transfer was approximately 1.0, on the average, which also reflects the lower probability of identification of HLA matched unaffected embryos free of aneuploidy, taking into consideration the average number of available embryos with results, usually much lower in women of advanced reproductive age (under 10 embryos on the average in our experience). With one in two embryos expected to be aneuploid, one in four HLA matched and three of four unaffected in autosomal recessive conditions, the overall probability of finding of a suitable embryos for transfer could not be expected to be higher than 1 in 10 embryos (Table 3) [29]. So with the availability of only under 10 embryos on the average with conclusive results in our material, only one HLA matched unaffected euploid embryo may have been expected to be available for transfer, assuming also that not all embryos develop to the status acceptable for transfer, which is, of course, may be considered below the optimal number of embryos to be replaced to ensure a clinical pregnancy and birth outcome. However, with present tendency of limiting the transfer to only one blastocyst, to avoid multiple pregnancies, the availability of a single euploid embryo for transfer is quite sufficient to obtain a clinical pregnancy and birth of an HLA identical progeny for stem cell transplantation for the affected siblings.

Therefore, patients should be fully aware of limits of the expected successful outcome of the above testing, which was shown to result in pre-selection and transfer of the HLA matched unaffected embryos in only 13.7% of the embryos tested, which is even a bit lower than may have been predicted. Despite such a relatively moderate success rate, the number of PGD requests in combination with HLA typing has been increasing overall, with the recent emergence of considerable proportion of cases, involving preimplantation HLA typing without PGD.

Conclusions

Preimplantation HLA typing opens an important possibility of PGD application for stem cell therapy. Because of limited availability of the HLA matched donors even among family members, this approach appeared to be attractive for couples with children requiring HLA matched bone marrow transplantation.

It is well known that to achieve an acceptable engraftment and survival in stem cell therapy requires the finding of an HLA identical stem cell transplant. However, there remain a large number of patients

for which no HLA matched family member exists, so the search is extended to haplotype matched unrelated donors, despite resulting in severe complications in more than half of patients [32].

Due to the small number of children per family, only one third of patients are able to find an HLA identical sibling, which may further be improved by 3% using an extended family search for a matched related donor with one or two identical ancestral haplotypes [33]. In the remaining patients the only resort is the identification of a matched unrelated donor, which might be maximized by establishing national registries. These registries allow overcoming to some extent the genetic heterogeneity in the populations, which may affect the frequency of unique haplotypes, so improving the in donor selection process.

Presented data show that couples undergoing preimplantation HLA typing may be expected to require a repeated cycle to be able to preselect and transfer HLA matched embryos. Even with probability of selecting only one HLA matched embryo from five tested, an acceptable pregnancy rate was observed, despite transferring only one or two embryos on the average, suggesting the usefulness of preimplantation HLA matching as part of PGD. The data provide a realistic option for the couples desiring to establish a pregnancy potentially providing an HLA match progeny for treatment of the affected family members. However, preimplantation HLA typing raises important ethical, legal and social issues.

The present overall world's experience of PGD for HLA typing of over one thousand cases, resulting in birth of more than two hundred HLA matched children, shows that PGD provides patients with important prospect not only to avoid an inherited risk without termination of pregnancy, but also to establish a pregnancy with particular genetic parameters, which may also benefit the affected member of the family. With introduction of aneuploidy testing, this may also expand the practical application of preimplantation HLA typing to patients of advanced reproductive age, allowing the improvement of their chances to become pregnant and deliver an HLA matched progeny for stem cell transplantation in the affected siblings. This also makes possible to apply this approach to HLA compatible stem cell transplantation for older affected siblings, which has already been performed in our experience for the 14-years old sibling with thalassemia, resulting in a successful donor cell engraftment with neither acute nor chronic GVHD [34].

In conclusion, despite ethical issues involved in preimplantation HLA typing [35-37], the presented results show the increasing attractiveness of this option for the couples with affected children requiring HLA compatible stem cell transplantation. It is also important that no embryo is discarded based on the results of preimplantation HLA typing, as all unaffected embryos are frozen for future use by the couple. So the couples at risk of having children with congenital bone marrow disorders have to be informed about presently available option not only for avoiding the birth of affected child, but also for selecting a suitable stem cell donor for their affected siblings, which may presently be the only hope for treating of siblings with congenital or acquired bone marrow failures.

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