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Transplant of hematopoietic stem cells in childhood: where we are and where we are going

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Abstract

Over the past decade, relevant improvements and refinements have significantly changed the indications, technique and results obtained with allogeneic transplantation of hematopoietic stem cells (HSC) in childhood. In this review the most important innovations that have characterized the practice of HSC transplantation in childhood during this decade will be discussed. We will analyze the clinical and biological advantages or disadvantages which characterize most typically HSC transplantation procedure in terms of the source of these cells (bone marrow, peripheral blood, placental blood). A fundamental turning point in the history of allogeneic transplantation of HSC is represented by the use of placental blood, which was first employed in 1988. Autologous, peripheral blood progenitor cells are increasingly being used as a source of HSC following high-dose therapy for malignant disease, because of the ease of collection and the markedly faster kinetics of engraftment in comparison with bone marrow. In particular, over the past decade, due to the much faster recovery of all hematopoietic lineages in comparison with bone marrow and due to the short duration of antibiotic therapy and hospitalization, also in pediatric patients, autotransfusion of circulating hematopoietic progenitors is rapidly replacing autologous bone marrow transplantation after high-dose chemotherapy for lymphomas and solid tumors. On the contrary, due to concerns in pediatric patients related to the use of hematopoietic growth factors in a healthy donor, allograft of peripheral blood progenitor cells is not routinely used. Since indications for allogeneic HSC transplantation that had already been well established in the recent past have been complemented by others and a relevant number of disorders are no longer considered to be eligible for allograft, the evolution in the indications for allogeneic transplant of HSC in childhood will be discussed. Likewise, biotechnological, social and organizational refinements which have allowed the greatest advances of allogeneic HSC transplantation in this decade will be analyzed, as well as some still open bioethical question regarding this procedure.

Key words: bone marrow transplantation, hematopoietic stem cell trasnplantation, peripheral blood transplantation, placental blood transplantation, transplant of hematological disorders in childhood, bioethics and transplantation

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To deal as objectively as possible with the transplantation of hematopoietic stem cells (HSC) in childhood, which has so many implications, we have divided its scope into separate questions. This seems to us to be the most practical way to clearly examine those that are currently the most vigorously debated problems concerning the transplantation of HSC in childhood. Since it is unfeasible for us to discuss all of the innovations that have characterized the practice of HSC transplantation during this decade, turning it into one of medicine's most stimulating fields, we will concern ourselves exclusively with allogeneic transplantation, with only a few remarks on autologous transplantation purely for the sake of indispensable comparisons.

We feel that it is highly important to answer the following questions:

- which steps of HSC transplantation history are still particularly significant today?
- which clinical and biological advantages or disadvantages characterize most typically HSC transplantation procedure in terms of the source of these cells (bone marrow, peripheral blood, placental blood)?
- which biotechnological, social and organizational refinements have allowed the greatest advances in this decade?
- in what way has there been an evolution in the indications for allogeneic transplant of HSC in childhood?
- which issues continue to be particularly critical, with an eye to future developments, especially in terms of *human cost*?
- in what way might one find a bioethical perspective in HSC donation?

Which steps of HSC transplantation history are still particularly significant today?

Thirty years after the first two successful bone marrow transplants (BMT) in children, aged 5 months and 2 years and affected by severe combined immunodeficiency (SCID) and by Wiskott-Aldrich syndrome respectively,[1,2] it is possible – and, we believe, worthwhile – to compile a sober inventory of data and dates, also because – as the saying goes – a medical science without history is like a man without his memory.

The year 1968[1,2] thus introduces the fascinating, and in some respects dramatic, scenario of a thera-

py which is one of the major triumphs of clinical medicine and typically has the goal of producing *biological chimeras*, an expression which has been effectively borrowed from Homeric mythology to designate an individual in whom the individual's own cell lines (the *self*, therefore, in the original meaning of this term according to Burnet[3]) live alongside foreign cell lines that are *non-self* (and are so by definition in the case of transplantation from an allogeneic donor). Creating this biological chimera implies forcing a law of nature which is fundamental in preserving the biological uniqueness and integrity of every individual.

Once the immune system has formed (around the 12th-14th week of gestational age), and even more so once it has matured further (in human beings, from the 5th-6th month of gestational age), it in fact tends to physiologically reject foreign cell lines. To achieve engraftment of an allogeneic transplant, it is therefore necessary to contrast this rejection reaction or at best prevent it from occurring. However, in the case of HSC transplantation it is also necessary to contrast a symmetrical and opposite reaction, which is responsible for so-called graft-versus-host disease (GVHD), a disorder which is supported by the donor immunocompetent cell lines that are present in the transplanted bone marrow (or in the cells of peripheral or placental blood) and antigenically do not tolerate the tissues of the recipient.

Accordingly, the creation of a biological chimera began by using bone marrow from an HLA-compatible related donor. However, the autosomal and codominant genetic induced limitation of this condition, which causes only 25-30% of individuals requiring BMT to have an HLA-identical family donor, inevitably entailed the need to expand the pool of donors possibly suitable for transplantation. This was done by using compatible unrelated donors.[4-7] At the same time, steps were taken to establish *registries* of HLA-compatible unrelated donors suitable for subjects without an HLA-compatible relative in which allogeneic HSC transplant was indicated, whilst additional information was gradually acquired regarding the importance of the exact match of HLA system antigens between the donor and the recipient in terms of the risk of rejection and of development of GVHD.[8,9] The most significant dates of this organizational aspect are 1974, when Mrs. Shirley Nolan founded the first registry of volunteer unrelated bone marrow donors; 1985, when the *National Marrow Donor Program* (the largest registry, currently including almost 3,000,000 donors) was established in the United States; and 1988, when the *European Blood and Marrow Transplantation* (EBMT) group, encouraged by Jon van Rood, took the initiative of collecting and counting the phenotypes of all donors in the registries around the world.[10-15] There are now almost five million donors in the various international registries (more than 200,000 in the Italian one, see also Figure 1), and transplantation from an unrelated donor is used increasingly even, and we are tempted to say especially, in children.[16-18]

Data reported to the BMT registry of the *Italian Association for Pediatric Hematology and Oncology* (AIEOP-BMT) clearly show that only 2.5% of allogeneic transplants were performed with unrelated HSC transplants in 1990-1991; yet already in 1992-1993 this share had increased to 10% and by 1994-1995 the number of unrelated transplants comprized 24% of all allogeneic transplants (see also Figure 2).

Meanwhile, the range of diseases for which HSC transplantation had become the therapy of choice (or the only feasible therapy) had expanded rapidly with respect to the indication given by the initial historical experiences. A listing by category of the diseases that could be treated by allogeneic transplantation, compiled 10 years ago (Table 1a),[19] would have to be sig-

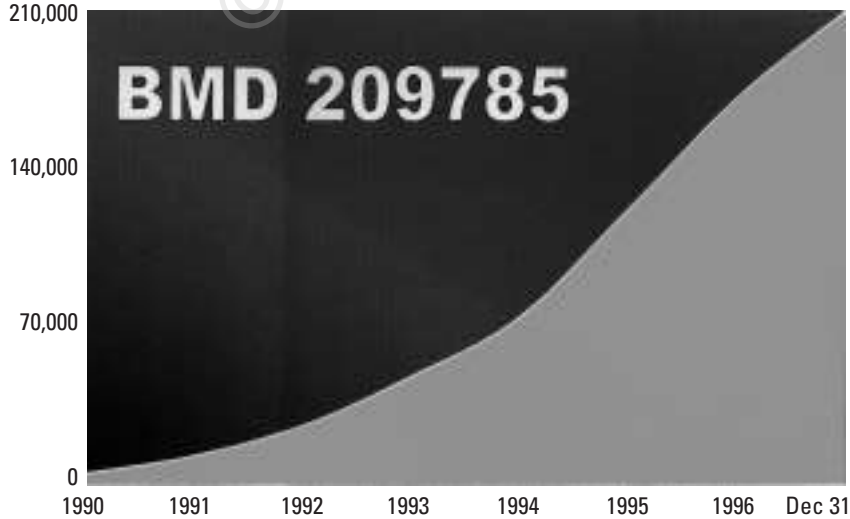

Figure 1. Increase in the number of unrelated bone marrow donors observed in the Italian Registry. BMD = bone marrow donors.

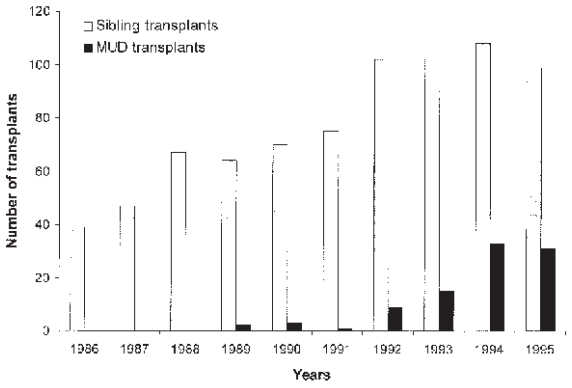

Figure 2. Absolute number of allogeneic transplants of HSC performed in children from 1986 to 1995 reported to the AIEOP-BMT registry and subdivided according the type of donor employed. A significant increase in the number of allograft performed from an unrelated volunteer can be observed in the last four years.
MUD = matched unrelated donor.

nificantly rewritten (see also Table 1b) in view of the progress made in the hematological, oncological and immunological field, both in terms of the benefits that can be achieved by allogeneic transplant of HSC and in terms of the risks linked to this procedure. Significant and sometimes dramatic improvements have in fact been achieved, albeit to different degrees for different diseases, with allogeneic transplant of HSC over the last 10-15 years. Clear proof of this is given by data related to pediatric patients affected by severe combined immune deficiency (SCID) and severe aplastic anemia (SAA).[20,21] In the first group, the cumulative probability of survival in patients treated by BMT from an identical sibling, which was estimated at roughly 60% until 1982, has risen above 95% since 1983.[20] When analyzing the role of allogeneic transplant of HSC in patients with SCID, it is opportune to mention the recently described possibility of performing in utero allograft.[22] For patients with SAA, the increase in disease-free survival has been from 49% in the period 1970-1980 to 70% in the period 1981-1983 and to 81% over the next five years (1984-1988).[21] There have been less significant improvements in patients with acute lymphoblastic leukemia (ALL) given an allogeneic BMT from an HLA-compatible relative: in these patients, according to data provided by the AIEOP-BMT registry, the cumulative probability of leukemia-free survival was 42% in the period between 1985 and 1990 and increased only to 50% in the period 1991-1995 (unpublished results).

A fundamental turning point in the history of allogeneic transplantation of HSC occurred in 1988, when transplant of umbilical cord blood cells (UCBC) was used for the first time.[23] Actually, there had been other attempts to surrogate BMT with the transplantation of HSC of other origin and particularly HSC from peripheral blood, but as regards allogeneic transplantation this practice is not yet applied commonly in pediatrics.

Briefly, the history of the transplantation of circulating progenitor cells (CPC) began in 1976, when Richman and colleagues noted that during the phase of recovery following aplasia due to chemotherapy

Table 1a. Diseases (subdivided according to their pathogenesis) considered to be eligible for an allogeneic bone marrow transplantation in 1988.

Malignant	*Nonmalignant*	
	Acquired	*Congenital*
Acute lymphoblastic leukemia	Severe aplastic anemia	Immunodeficiencies[1]
Acute myeloid leukemia	Paroxysmal nocturnal hemoglobinuria	Hematologic defects[2]
Chronic myeloid leukemia		Bone defects[3]
Non-Hodgkin lymphoma		Mucopolysaccharidoses[4]
Selected solid tumors		Lipidoses[5]

[1]Immunodeficiencies: severe combined immunodeficiency, chronic mucocutaneous candidiasis, others; [2]hematologic defects: Fanconi's anemia, Diamond-Blackfan anemia, thalassemia major, sickle cell disease, Glanzmann thromboasthenia, chronic granulomatosis disease, Chediak-Higashi syndrome, Wiskott-Aldrich syndrome; [3]bone defects: osteopetrosis; [4]mucopolysaccharidoses: Hurler syndrome, Hunter syndrome, Maroteaux-Lamy syndrome, others; [5]lipidoses: metachromatic leukodystrophy, Gaucher disease, other lipidoses (modified by Burgio GR, Nespoli L, Porta F, Bonetti F.[19]).

Table 1b. Diseases considered for the time being eligible for an allogeneic transplant of HSC in childhood.

Acute lymphoblastic leukemia	Severe combined immunodeficiencies (SCID)
Acute myeloid leukemia	Autosomal recessive variants X-linked SCID
Chronic myeloid leukemia	HLA-molecule deficiency Reticular dysgenesis Omenn syndrome
Myelodysplastic syndromes	Adhesion leukocyte deficiency syndrome
Severe aplastic anaemia	Immunodeficiency with hyper-IgM
Fanconi anemia	
Dyskeratosis Congenita	Wiskott-Aldrich syndrome
Thalassemia major	Chediak-Higashi syndrome
Sickle cell disease	Familial hemophagocytic lymphohistiocytosis
Selected cases of Diamond-Blackfan anemia	Malignant osteopetrosis
Paroxysmal nocturnal hemoglobinuria	Selected variants of lysosomal and peroxisomal storage disorders

there was a clearly demonstrable and significant increase in $CD34^+$ cells in peripheral blood, expressing a mobilisation of hematopoietic stem cells.[24] Twelve years later (1988), Duhrsen achieved a considerable increase in these cells in cancer patients after treatment with recombinant human granulocyte-colony stimulating factor (rHuG-CSF).[25]

In childhood autologous transplantation, CPC have been mobilized into peripheral blood and collected on a large scale by leukapheresis after treatment with hematopoietic growth factors (HGF), administered as a single agent or, more frequently, as an enhancement of chemotherapy mobilization.[26-28] Autologous CPC are increasingly being used following high-dose therapy for malignant disease, because of the ease of collection and the markedly faster kinetics of engraftment in comparison with bone marrow.[29] In particular, over the past decade, due to the much faster recover of all hematopoietic lineages in comparison with bone marrow and due to the short duration of antibiotic therapy and hospitalization, also in pediatric patients, autotransfusion of CPC is rapidly replacing autologous BMT after high-dose chemotherapy for lymphomas and solid tumors.[30] Recently, CPC have been considered as an alternative to bone marrow for allogeneic transplantation and this procedure is being used increasingly in adults.[31,32] Although there is no definitive proof from controlled clinical studies, allogeneic transplant of CPC has some undisputed advantages in comparison with BMT: suffice it to say that for the recipient the duration of neutropenia and of thrombocytopenia is reduced, and that for the donor the trauma of harvesting marrow from the bone, with the associated inevitable anesthesia, is eliminated and that hospitalization may not be indispensable. However, since the release of large numbers of hematopoietic progenitors into the circulation of healthy donors requires the use of HGF, the major concerns in pediatric practice, at least today, are the unknown long-term consequences to the donor of the administration of these cytokines, which have been proved capable of stimulating the proliferation of both normal and malignant stem cells. Uncertainty about the long-term safety of using HGF limits the wide diffusion of this technique in childhood, where the related donor is also a minor in the great majority of cases. Moreover, the problem of the vascular access of the donor for leukapheresis further limits the use of CPC (HSC) for allogeneic transplantation in childhood. Therefore, it is not surprising that at present allogeneic transplants of CPC in children are usually performed either when the donor is an adult or when a second transplant from a minor is required because a previous marrow engraftment has failed.[33]

It is beyond doubt that in pediatrics, as mentioned earlier, the most significant alternative to BMT (which is now used routinely) is still UCBC transplantation, which was introduced by a report by Gluckman et al. concerning a 5-year-old child affected by Fanconi's anemia and given an allogeneic transplant of HSC using the cord blood of a healthy, HLA-compatible sibling.[23] Ten years after the transplant, the child is alive and has a completely normal full blood cell count.

In the first ten years that have passed since this first experience, over 600 UCBC transplants have been performed both from an HLA-compatible family donor and from an unrelated donor, often with disparity for one or more antigens of the major histocompatibility complex in the latter case.[34-36] In fact, the reported low incidence of acute and chronic GVHD has promoted the establishment of large cord blood banks in Europe and the USA, where at present more than 12,000 cord blood units have been collected and typed for the HLA system.[37-39] Improvements in the methods used for cell collection, manipulation and freezing have allowed a rapid increase in the use of CB progenitor cells, which are now extensively employed for allogeneic transplantation. During these ten years, the initial reservations regarding any possible damage to the *donor* neonate, concerning injuries such as periventricular hemorrhages or possible induction of anemia (both of which would have been secondary to an early ligation of the umbilical cord), have been overcome.[40,41] The applicability of the procedure even to adults has been demonstrated;[35] however, the possibility that the cells obtained from a cord may be quantitatively insufficient to allow quick engraftment of the transplant in most adult transplanted patients has been considered repeatedly and is still its greatest limitation in terms of routine use.[34] Profiles and problems in terms of organization and administration, affecting storage, handling and cryopreservation of placental blood but also related to the purposes of its use (allogeneic transplant versus a hypothetical future autologous transplant due to an intervening need of the donor) have been debated repeatedly.[40] This brief mention of UCBC transplantation would seem to be an appropriate ending for our sober history.

Which clinical and biological advantages or disadvantages characterize most typically HSC transplantation procedure in terms of the source of these cells (bone marrow, peripheral blood, placental blood)?

The reservations we outlined regarding allogeneic transplantation of CPC in pediatrics limit the utilization of this technique for the time being and accordingly cause it to be at the very least far less frequently feasible for practical application than has ever occurred over 30 years for BMT and 10 years for transplants of UCBC. Accordingly, even today this fact still prevents one from considering these three transplantation techniques (BMT, transplantation of CPC and UCBC) on a par for comparison.

In addition to the already-mentioned possibility to perform an allogeneic transplant of CPC in childhood either in the rare cases in which the compatible donor

is an adult (and therefore donates CPC by his own free choice or due to the need, arising from the condition of the recipient, to achieve rapid hematological recovery) or when a second transplant from a minor is required because of failure of previous bone marrow engraftment, it is necessary to also briefly mention the use of CPC in the particular condition of a transplant from a partially compatible adult donor.

In this situation, due to the genetic disparity between the donor and the recipient (i.e., transplants using HLA-partially matched family donors) and to the procedures of T-cell depletion, the risk of graft rejection is markedly increased. Since the engraftment of donor hematopoiesis is considered to be a dynamic phenomenon that depends on competition between both immunocompetent and progenitor cells of the donor and the recipient, the use of cytokine-mobilized CPC offers a unique possibility to enormously increase the number of donor HSC infused. Therefore, as suggested by data reported by Aversa *et al.*,[42] this technique, by permitting the infusion of a high number of CPC, could also be a way to optimize the chance of donor hematopoietic engraftment in children without an HLA-compatible related or unrelated donor who need a transplant. However, the considerable reservations that still exist as to the immunologic recovery of these patients (with the consequent risks of infectious complications or of relapses of the neoplastic disease) confine allogeneic transplantation of T-cell depleted CPC to the role of a *heroic* therapy, whose application in day-to-day clinical routine, despite being highly desirable, is far from being truly established at present in the child as well as in the adult.

As regards the two most widely used practices (BMT and transplantation of UCBC), we recently proposed a comparative summary of their advantages and disadvantages, as shown in Tables 2 and 3.

More generally, it seems reasonable to admit that the application goals of the two procedures are roughly similar, in that the diseases that can be cured by one procedure can also be treated by the other. It seems just as reasonable to acknowledge that an HLA-compatible family bone marrow donor will always be easier to find than a family UCBC donor when the need arises. The timeliness implied by having cord blood available generally entails that a new child has been *programmed*, hoping that it will be HLA compatible with respect to the sibling who needs the transplant. This is in any case an ethically controversial problem that follows the pattern of BMT from a programmed donor (applicable in each instance only after a scrupulous reflection) and cannot be presented in purely utilitarian terms, despite being motivated by a form of bioethics that is applied within the family.[43] The fact that extemporaneous application of UCBC transplantation from a programmed donor is far more *within reach* than an equally *programmed* BMT is also one of its precise ethical limitations (see also Table 3).[40,44,45]

Table 2. Advantages associated with transplant of umbilical cord blood cells in comparison with bone marrow transplantation.

For the recipient

- Prompt availability (with less time wasted between identification of an unrelated donor and stem cell transplantation)
- Reduction of time required to identify an unrelated HLA-compatible donor
- No risk of donor refusal
- Reduced risk of both acute and chronic GVHD
- Possibility of performing transplant using 1 or 2 antigen HLA-disparate donor
- Low risk of viral contamination (i.e. HCMV, EBV), with consequently low risk of transmission of infectious disease

For the donor

- Ease and safety of collection, without the risks associated with general anesthesia (required for marrow harvesting)
- Lower incidence of psychological problems related to the figure of the child-donor and to possible transplant failure

Modified by Burgio GR and Locatelli F.[40]

Table 3. Possible disadvantages associated with transplant of umbilical cord blood cells in comparison with bone marrow transplantation.

For the recipient

- Increased risk of graft failure
- Routine applicability only in patients with a body weight of less than 30 kg (?)
- Delayed platelet and neutrophil recovery
- Possible reduction of the graft-versus-leukemia effect (?)
- Absence of adoptive transfer of specific immunity towards infectious agents due to fetal immune immaturity and lack of previous antigenic exposure
- Increased risk of transmission of inherited disorders
- Possible risk of transmitting viral diseases (in particular AIDS), not identifiable if cord blood collection occurred during the period of serological conversion

For the donor

- Ethical problems associated with donation (i.e. increased propensity to conceive a child to save a child)
- Possible unavailability of donor's own cord blood stem cells if he/she subsequently should develop a disorder requiring transplantation of hematopoietic progenitor cells

Modified by Burgio GR and Locatelli F.[40]

As regards transplantation from an unrelated donor, it is fair to say that up to now BMT and UCBC transplantation are mutually complementary and have corresponding advantages and disadvantages, and that this will certainly continue to be the case until access to banks for easily finding placental blood is very widely available. The choice to privilege one kind of transplant rather than the other will be made on the basis of parameters related both to the

patient (such as HLA antigens, kind of disease, fragility of the clinical and hematological condition, body weight) and to the donor (such as the degree of HLA compatibility with the recipient and, for placental blood, the number of available cells). It is in fact beyond doubt that the shorter time needed both to locate a suitable donor and to perform the transplant and the absence of donor attrition are significant advantages of unrelated UCBC transplantation.[37,39] Likewise, the data collected up to now clearly show that due to the reduced immune reactivity of cord blood cells, UCBC transplantation from familial donors and from unrelated donors is associated with a reduced risk of acute and chronic GVHD.[34-36] The reduced risk of severe immune reactions has allowed the performance of UCBC transplantation between unrelated subjects with, in some cases, disparity for 2-3 HLA antigens; by comparison, a complete HLA match between recipient and donor is mandatory for successful unmanipulated BMT between unrelated subjects.

Whilst prompt availability, the possibility to perform transplants across the HLA barrier and the reduced risk of GVHD clearly tilt the balance toward UCBC transplantation between unrelated subjects, the number of unrelated BM donors is, as mentioned, far higher than the cord blood units currently stored. Moreover, there are still significant reservations concerning a possible delay in the kinetics of hematological recovery, the risk of graft failure in higher-body-weight subjects, and the graft-versus-leukemia (GVL) effect of placental blood. In fact, even though no specific study has addressed these issues, the available data indicate that particularly in patients given the lowest number of cord blood cells the kinetics of myeloid and platelet recovery after UCBC transplantation seems to be delayed in comparison with BMT.

The dose of cord blood progenitor cells necessary to ensure early and sustained hematopoietic engraftment and favorable clinical outcome is still not precisely defined. Wagner *et al.* claimed that the lowest dose of cord blood nucleated cells reported capable of resulting in complete and sustained engraftment in a recipient of a family UCBC transplantation is 1×10^7/kg of recipient body weight.[35] However, recently the *Eurocord Transplant Group* documented that a dose of nucleated cells available before thawing lower than 3.7×10^7/kg of recipient body weight was highly predictive of both graft failure and poor survival after UCBC transplantation.[34] It should be noted that it is seldom possible to have such a number of cells available in the case of an adult patient or of a child older than 10-12 years. In fact, since the average leucocyte count in placental blood is about 10×10^6/mL and the average volume of donated blood is about 80 mL, the average number of nucleated cells in one cord blood unit before thawing may reach 800×10^6. Therefore, in patients with a body weight above 30 kg it is not reasonable to expect to be able to transplant a number of cells higher than 3.7×10^7/kg of recipient body weight. The reported low incidence of GVHD might also be a major drawback to the use of cord blood as a source of stem cells for allogeneic transplantation in leukemic patients. In fact, since the role of allogeneic lymphocytes in the control and/or eradication of malignancy is clearly established, the absence of the component of GVL activity associated with GVHD[46] could constitute a theoretical concern in leukemic subjects given UCBC transplantation. However, currently available data do not allow us to conclusively establish whether UCBC transplantation really predisposes patients to an increased risk of leukemia relapse.

Which biotechnological, social and organizational refinements have allowed the greatest advances in this decade?

It is not by chance that we have considered the goal of allogeneic transplant of HSC – regardless of the source of hematopoietic progenitors chosen to perform it (especially cord blood or BM) and of the indication for which it is used – to be the creation of a biological chimera in which hematopoietic (and immunocompetent) cell lines of the donor live alongside the cells that constitute the recipient's organs and tissues.

This goal is obviously attained when one achieves maximum mutual tolerance of donor and recipient cell lines and prevents graft rejection and GVHD. Prerequisites to this goal are as follows: a) the possibility now available to acquire knowledge, at the highest possible analytical level, concerning the HLA antigens and alleles of both donor and recipient (allowing to choose the most *similar* donor, especially in trans-plantation between unrelated subjects); b) the choice of conditioning regimens which are targeted specifically for the disease to be treated; c) the adoption of GVHD prevention and treatment strategies capable of reducing the impact of this complication on transplant-related morbidity and mortality; and finally d) the application of all the resources capable of favoring the engraftment and expansion of the cell lines that derive from HSC transplant whilst of course protecting the transplanted patient with respect to the various infectious and hemorrhagic complications that threaten him or her during the first months after transplant.

We are therefore referring both to the pivotal issues of HSC transplantation and to *support therapies*, in the broadest sense of this expression, and we believe it is useful to delve into these issues briefly.

Advanced knowledge of antigens and alleles of the HLA system, which must be taken into account particularly in transplantation between unrelated individuals, have been based in recent years on the introduction of molecular biology techniques related to tissue typing. The enormous increase in the number of

class-I and class-II alleles of the HLA system that can be recognized by DNA typing methods with respect to those identified by serological typing (see also Table 4), together with a better knowledge of the role played by some antigens (i.e., locus C and DQ of the HLA system), have amply demonstrated the extreme polymorphism of the human histocompatibility complex and allowed an understanding of the higher incidence of complications having an immune-mediated pathogenesis (GVHD and graft rejection) observed after allogeneic transplant of HSC from unrelated donors.[10,12,14,15,47,48] More refined HLA typing methods have also been complemented by functional assays, such as those based on the frequency of cytotoxic T-lymphocyte precursors and of helper T-lymphocyte precursors, which in some experiences have been shown capable of predicting, with satisfactory reliability, the risk of developing severe GVHD.[49-51] Not surprisingly, over the last five years the improvement of HLA-typing methods and the use of the above mentioned functional assays have been matched by a considerable reduction in transplant-related mortality and in the risk of developing the most severe forms of acute GVHD.[10,12,14,47,48,52] This progress on the biotechnological level had to be matched, to allow their best use, by corresponding expansions in the structures meant to find unrelated donors.

As mentioned, this need has been met on the one hand by creating international registries of bone marrow donors, albeit for the time being with a regrettable inadequate representation of some ethnic minorities in these registries, and on the other hand by the planning of *banks* for collecting placental blood. This has led to significant advantages for both procedures; for BMT from unrelated donors, in particular, there has been an increase in the probability (currently estimated at 40-50%)[14] of preliminarily identifying a donor in time and a reduction in the time required to complete the search.

As regards conditioning regimens, the two main goals of this decade have been to identify therapies having the least possible incidence of side effects and to devise strategies capable of increasing the myeloablative and particularly antineoplastic effectiveness of pre-transplant therapy. A typical example of the tendency to identify strategies associated with lower acute or late toxicity has been the progressive reduction in the use of radiation therapy, particularly in SAA patients. This is done in view of the high incidence of second neoplasms in these patients that burdened this treatment with respect to conditioning regimens based on using only cyclophosphamide, possibly associated with anti-lymphocyte globulin (ALG).[53,54] Although its clinical application is still only in the earliest stages, the use of radiolabeled monoclonal antibodies specifically targeting hematopoietic cells (such as the one conjugated with ^{131}I, which targets the CD45 leukocyte antigen), refined by the Seattle group,[55] is worth mentioning as a model for selectively increasing the antineoplastic effectiveness of the pre-transplant preparative regimen.

Table 4. Number of alleles recognized by serological and molecular techniques at each HLA locus.

	Serological typing	*Molecular typing*
Class I		
HLA-A	18	87
HLA-B	35	191
HLA-C	8	44
Class II		
HLA-DRB1	14	189
HLA-DQB1	6	32

As regards pharmacological prevention of GVHD, the introduction of cyclosporin-A (Cs-A) was welcomed with particular and well-grounded favor in the early 1980s. However, only in the last 10 to 15 years, have the refinement in administering patterns and most of all its association with short-term methotrexate allowed the achievement of a significant reduction in the incidence and severity of GVHD.[56,57] Also, one cannot avoid mentioning the introduction, in recent years, of other molecules such as FK506 (although as yet untested in the pediatric age group and certainly not free from significant toxicity),[58,59] which may lead to further improvements in the prevention and treatment of this complication and whose importance as the main cause of transplant-related mortality has been boosted by the increasing use of unrelated donors.

Assistance to the transplanted patient, in terms of support therapy, has always had a crucial role in determining the success of the transplant. The most significant advances achieved during this decade are the post-transplant use of hematopoietic growth factors, such as rHuG-CSF or recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF), which have proved capable of accelerating granulocyte recovery, reducing the period of aplasia;[60-63] improvements in treating early infectious complications, especially in relation to the early diagnosis of viral infections (particularly due to cytomegalovirus), and in their presymptomatic therapy;[64-66] the possibility of effectively contrasting hepatic venocclusive disease by means of the tissue activator of plasminogen or with defibrotide.[67-69]

One of the greatest advances of this decade in allogeneic transplantation of HSC has certainly been the increasing understanding (and the consequently acquired importance) of the fundamental role of the donor's immune system in achieving a good outcome for the transplant. This has brought about adoptive immunotherapy approaches, which have a particularly important place among biotechnological refine-

ments. One of the most sophisticated examples of these true cellular-engineering strategies is undoubtedly the infusion of specific clones of T-lymphocytes which are cytotoxic for cytomegalovirus. These clones have proved capable of preventing the development of interstitial pneumonia after allogeneic HSC transplantation,[70] which is a particularly severe complication that had been calculated to occur in approximately 5-10% of transplanted children and even more frequently in patients transplanted from an unrelated donor.[71] An equally important and equally elegant (in its application) success has been yielded by the infusion of cell lines or specific clones of cytotoxic T lymphocytes, which have been found capable of preventing the development, or of inducing the disappearance, of EBV-induced lymphoproliferative diseases, whose highest incidence is found in patients subjected to transplantation from unrelated volunteers or from a partially-matched family donor, particularly when T-cell depletion procedures are applied.[72] Gene marking studies have shown the persistence of these donor-derived EBV-specific cell lines for a few months after infusion and their reappearance after periods of apparent nonidentifiability during episodes of viral reactivation. Among adoptive immunotherapy approaches, the infusion of donor leukocytes aimed at inducing a new hematological remission in patients affected by a relapse of Philadelphia positive chronic myeloid leukemia after allogeneic transplant of HSC is mentioned for last, since it can be applied at a later stage than other methods and is sometimes burdened by complications which endanger the patient's life (development of GVHD and pancytopenia).[73-75] Yet it is worth mentioning as last but not least because this infusion allows the possibility of further remission in approximately 70% of these patients and is one of the most effective demonstrations of the importance of the immune-mediated GVL effect in the success of allogeneic transplantation of HSC. Future goals will be to extend the applicability of this approach also to patients with acute leukemia experiencing a relapse and most of all to devise strategies capable of separating the GVL effect from the undesirable development of GVHD conditions, ideally by using clones or cell lines that are leukemia-specific.[76]

In what way has there been an evolution in the indications for allogeneic transplant of HSC in childhood?

Significant advances in therapies cannot arise without being accompanied by equally significant changes in their indications.

The application strategy of transplants has certainly not stagnated; its indications have changed since its inception. Constant improvement of transplant results and of the outcome obtained for example with chemotherapy in acute leukemias or with treatments based on the use of HGF and interferons in immunodeficiencies or with immunosuppressive therapy in SAA allow to continuously revise and update the indications of the various therapeutic options for the various diseases and in the different stages of each disease, being true to the goal of providing patients with better therapies associated with the lowest possible side effects.

The indications for allogeneic HSC transplantation that had already been well established in the recent past have been complemented by others (for example, gradually through the years, those for some metabolic disorders, immunodeficiency syndrome with hyperIgM, sickle-cell anemia). On the other hand, the availability of rHuG-CSF has radically changed the course of some congenital neutropenias (such as Kostmann's syndrome),[77] thus making transplantation, especially in the absence of an HLA-identical donor, no longer the first choice. Likewise, the combined use of cycles of antibiotic prophylaxis and of recombinant human interferon-gamma has resulted in a significant improvement in the duration of survival and quality of life in patients with chronic granulomatous disease.[78] To the same extent, the availability of β-glucosidase and of bovine adenosine deaminase has been found to be an effective treatment in children having no HLA-compatible family donor and affected by Gaucher's disease and by SCID secondary to ADA deficiency,[79] respectively.

Both of these opposite developments in the use of allogeneic transplant of HSC, toward an expansion of its indications and toward its limitation, are grounded in the advances of current therapeutic strategies. The reason for extending the indications is the goal of creating new *chimeras*, with a life-saving effect, for diseases that were thought to be nontransplantable. The basis for limiting the indications of HSC transplantation is not only a more complete understanding of the risks related to transplantation (especially when the donor is not an HLA-compatible sibling) with respect to its possible expected success, but also an advancement which is far more significant because it relates to improvements or therapeutic innovations whose effects are more advantageous than transplant procedures, to the point of eclipsing them, at least as elective indications.

Typical examples (many more could be made) of increasingly elaborate criteria for choosing transplantation over alternative treatments are SAA and acute promyelocytic leukemia (APL), for which one choice is made rather than the other on the basis of biological characteristics of the disease which have been demonstrated to be predictive of possible failure of conventional treatment and on the basis of the kind of donor that is available.

In greater detail, in SAA about 80% of pediatric patients can be definitively cured thanks to allogeneic BMT from an HLA-identical sibling.[80] In patients lacking a compatible relative, by virtue of the combined use of Cs-A, ALG and rHuG-CSF, the results of

immunosuppressive treatment have improved dramatically in recent years, since very high survival percentages (on the order of 60-90% 2-3 years after the onset of the aplasia) have been achieved.[81,82] These values make this therapeutic approach competitive with respect to transplantation, especially for patients having no compatible family donor, in which disease-free survival is significantly lower (with percentages no higher than 30%) with respect to allogeneic transplant from an HLA-identical sibling.[16,83] On the contrary, the main factors that still make one prefer allogeneic BMT from an HLA-compatible family donor in patients with SAA over immunosuppressive treatment are related to the frequent dependency on the administration of Cs-A in many patients in order to maintain good granulocyte and platelet counts, to the possibility of relapses of marrow aplasia even after months of substantial normality of the hematological condition or in any case of independence from transfusional support, and to the risk of developing secondary neoplasms (in particular, myelodysplastic syndrome and acute myeloid leukemia), which is significantly higher in individuals subjected to immunosuppressive treatment.[54,84]

Likewise, in APL, combined treatment with all transretinoic acid and cytostatic therapy can cure a large percentage of patients without having to resort, at least as a first step, to high-dose chemotherapy with the related necessary support of HSC.[85,86] On the other hand, it has been clearly demonstrated that patients in which the presence of the PML-RARα fusion gene can be documented by molecular-biology methods at the end of the combined treatment will almost invariably undergo hematological relapse[87] and therefore, if an HLA-compatible sibling is available, must be subjected to allogeneic transplant of HSC in first hematological remission. Moreover, subjects who experience leukemia relapse and have a negativized transcript of the PML-RARα fusion gene at the end of the second reinduction and consolidation treatment can benefit, in a high percentage, from an autologous HSC transplant, whilst if they show the presence of the above mentioned transcript they will relapse even with this transplant approach and therefore require an allogeneic transplant of HSC from an unrelated volunteer.[88]

In recent years, certainly also in view of the reduction in transplant-related mortality and morbidity associated with the use of CPC,[89] there has also been a considerable extension of autologous HSC transplant to include solid neoplasms (being actually applied far more significantly in medical oncology of adult patients) and possibly disorders outside the classical domains of pediatric hematology, immunology and oncology such as autoimmune diseases with particularly severe prognosis (for example systemic sclerosis with lung involvement and systemic lupus erythematosus with renal and cerebral involvement).[90-92]

Which issues continue to be particularly critical, with an eye to future developments, especially in terms of human cost?

Any allogeneic HSC transplant entails risks and can be burdened by potentially very serious human costs. Especially because of this (without ignoring the burden of financial costs), the changes in the therapeutic scenario that we examplified in the previous section are more than justified. These changes also justify in general the evolution that characterize many therapies over time, whenever equally effective alternatives with lesser undesirable effects and therefore with lower costs are proposed.

In addition to the risks inherent to short-term complications, which heavily affect the mortality related to the transplantation of allogeneic HSC, there are also late sequelae, whose importance and impact is being defined more and more precisely.[93,94] Since the first successful applications, the number of pediatric patients that have become long-term survivors after transplant of allogeneic HSC has grown gradually. Accordingly, more and more attention has been paid to the quality of life of the cured patients. Undoubtedly, long-term complications are a subject of particular interest for the pediatrician, in view of the long life expectancies of children cured by transplant and of the particular susceptibility to iatrogenic damage of an organism during growth.

We would also like to mention here the drama that accompanies the onset of a second neoplasm induced by chemotherapy or radiation therapy, although we have already done so in relation to the gradual limitation of the use of radiation treatments. Many studies have in fact demonstrated that patients subjected to transplant of HSC can develop secondary neoplasms with a significantly higher incidence than control populations of the same age and sex.[53,93-96] These neoplasms, mostly malignant solid tumors, are more frequent in patients subjected to transplant when young (further demonstrating the extreme importance of the issue for pediatricians)[95] and have often been reported with an average onset period of approximately 5 years after transplant. Their onset mechanism consists of the carcinogenic role of ionising radiation by using total body irradiation (TBI), the mutagenic action of some cytostatic drugs, the immunosuppression treatments that persist in some of these patients (not coincidentally, patients with chronic GVHD that have a particularly high risk), some genetic predisposition characteristics of patients (subjects with Fanconi's anemia are particularly at risk, probably due to their peculiar chromosomal instability, which is secondary to defective DNA repair processes), and finally a history of chemotherapy and radiation therapy (cranial radioprophylaxis, which was once used widely in the front-line treatment of patients with ALL or non-Hodgkin's lymphoma, has clearly been shown to have a crucial role in determining the onset of neoplasms affecting the central ner-

vous system).[53,93,96,97]

Mention must also be made of the infertility that many *cured* patients pay as the price for a transplant.[98] We judge this biological cost to be far less heavy than the cost of a second neoplasm, but at least in terms of quality of life, the inability to procreate might often be perceived as a considerable limitation. Of course, the refinement of intervention strategies (i.e. collection and cryopreservation of germ cells at various stages of maturation and possibly, when feasible, shielding of the gonads in patients subjected to radiation treatment) aimed at avoiding these costs will be a worthy guideline toward achieving a more favorable restoration of the health, in the long term, of *ex-children* who had required a transplant. The reduction of iatrogenic human costs is one of the constant goals to be sought in improving any therapy, especially in the case of *frontier* therapies.

In this regard, a fine example of success achieved in remedying an iatrogenic damage related to the pre-transplant conditioning regimen observed in a considerable percentage of children subjected to HSC transplant is the possibility to correct severe reductions in growth rate by means of a replacement treatment based on using growth hormone (GH).[94] Especially in patients given TBI during myeloablative therapy (and even more so in patients subjected to cranial radiation prophylaxis during front-line treatment), it is in fact possible to observe a considerable slowing of the growth rate, mostly caused by deficits in the production of GH which are in turn caused by damage to the hypothalamic-pituitary axis.[99] Timely replacement hormone treatment with recombinant human GH has proved able to significantly correct growth impairment, thus permitting the achievement of the expected normal genetic height.

The effect of an HSC transplant in terms of social costs related to the quality of life is very difficult to quantify, since a particularly large number of variables affects this parameter: the age of the child, the structure of the family to which he belongs, its socioeconomic level, the level (including psychosocial aspects) of the structure that accommodates the patient and the duration of the patient's stay in this structure, not to mention the variables of the course of the post-transplant period. In any case, it is evident that any progress aimed at optimizing transplant technique, in its broadest sense, from conditioning regimens to support therapies, will also help to reduce these costs significantly.

In what way might one find a bioethical perspective in HSC donation?

The donation, from a living being, of an organ (a kidney) or of part of an organ (the liver), programmed by the spontaneous and free will of an aware adult, is an act of deserving altruism; in other words, an act of solidarity at the highest level. It fully belongs to the domain of a bioethics of high worth. Kidney transplantation from a minor donor is instead burdened by considerable reservation. Demonstration of a *best interest* of the donor for psychological reasons are difficult to accept. The informed consent of a child, even of a young adolescent, is inherently uncertain and usually tainted by a lack of ponderation; moreover, it can be deeply biased by suggestions inspired by adults. Transplantation of an organ (the kidney) from a child donor has also been considered as *involuntary altruism*, with a well-founded and subtle critical attitude.[100] Its pertinence to the area of unquestionable bioethical feasibility is burdened by these serious considerations.

For bone marrow, which is harvested without compromising the psychological and physical integrity of the adult or child donor, except for the minimal theoretical risks related mainly to anaesthesia, there are few doubts as to its full pertinence to bioethical domain. This is a true donation from a living being, which is based on a deep sense of solidarity that the donor has or will acquire in the future; in any case, in-depth reflections concerning consent, promoted in the child and/or developed together with the child or on his behalf, now leave little room for doubt as to the ethical merit of this procedure.[44]

This conclusion can also be applied to the transplantation of HSC derived from cord blood: of course, any thought regarding consent can be promoted only by informing the parents. Yet one cannot say that BMT and UCBC transplantation inspire the same bioethical considerations.[40]

Although in any case the umbilical cord and its blood constitute a structure which by nature is to be disposed of, nonetheless it *belongs* to the neonate.

Once the enormous value of its life-saving potential in terms of transplant became known, the question arose of whether it is appropriate or not to reserve placental blood for an allogeneic transplant and thus automatically deprive the donor of any future chance to use it if an autologous HSC transplant becomes necessary. In the opinion of the vast majority, this ethical dilemma is solved positively (i.e., choosing use in allogeneic transplantation); however, ahead of this issue there are many others that have been debated, also in the light of other ethical problems, above all the need to validate by means of several tests (some of which involve the mother) the *usability* in allogeneic transplantation of this particular blood, which must be found to be free of all risk of transmitting infectious diseases and genetic diseases carried by HSC. Subjecting the blood of the neonate (and/or of the mother) to tests that have not been requested by the parents and are not part of any neonatal screening would be an arbitrary act unless *consent* to perform them is requested and unless once this consent is obtained, maximum respect for privacy is ensured.

However, if a genetic or infectious disease were to be identified, it would have to be reported to the

mother or to the parents. This would inevitably cause deep emotional turmoil, especially if the disease had not been suspected. In the case of incurable diseases, such anxiety would not only be devastating but also useless.

On the other hand, the practice of UCBC transplantation has overcome these reservations. Thanks to an appropriate and substantial banking policy, it has become a life-saving resource, like BMT has been and still is in full.

Both of these procedures can in fact be said, in summary, to meet all the requirements of bioethics: *autonomy* (parents must be fully able to express informed consent and the same must be true, with particularly critical attention, for a minor of an age allowing involvement); *nonmaleficence-beneficence* (in the cost or risk/benefit balance, the latter will abundantly prevail), and *justice* (owing to all the safeguards related to the fairness of proceeding, which must be respected thoroughly). Indeed, it is obvious that even in the complex and multifaceted domain of justice, HSC transplants must meet all the requirements of ethical acceptability.

When answering an ethical and bioethical question, one must think and ponder critically whilst deeply respecting and considering the opinions and orientations of others. Less caution might be required in answering practical questions, such as the first five that we have proposed in this review. Yet, we would like to give even these answers the value of an orientation rather than consider them resolute indications. The swift evolution of the issues at hand suggest this approach.

Besides, one must always bear in mind Dante's words: *doubt, like a shoot, stems from the root of the truth*.

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