Content of Attach A - **DONATION OF HAEMATOPOIETIC STEM CELLS**

(V9 1/7 gen. 2015)

This document is not intended in substitution Attach A but as a possible support. **It is reminded that under Italian current legislation the potential blood stem cell donor must be able to understand (including forms) and communicate in Italian**.

Haematopoietic stem cells

Haematopoietic cells, which are produced by the bone marrow and released into the blood, derive from progenitor cells, called stem cells, which are known to be totipotent, i.e., they reproduce themselves very quickly and can differentiate into various other type of cells.

Progenitor cells are rather scarce but, besides their incredible reproductive activity (they generate 200-400 billion new cells every day), they are able to regulate their own reproduction so that the number of cells remains constant over the course of a person's life, regardless of whether some cells are withdrawn (donation).

These cells can be collected either from the bone marrow, from the peripheral blood, following mobilisation with growth factors and from blood of cord at the birth.

What is haematopoietic stem cell transplant?

Haematopoietic stem cell transplants (HSC) have proven to be one of the most useful therapeutic strategies for the treatment of both malignant haemopathies (e.g.: acute, chronic, myelogenous or lymphoblastic leukaemias) and for inherited ones (e.g. Thalassemia Major), for which current, conventional treatment provides either insufficient results or none at all.

By transplant, we mean the process of replacing sick or non-functioning bone marrow with healthy stem cells which have the ability to regenerate all blood cells, thus reconstituting normal haematopoietic and immunologic activities.

The transplant can be *autologous* (HSC transplant from the same patient after appropriate treatment) or *allogeneic* (HSC transplant from a healthy donor). In this case a donor, whose genetic characteristics (tissue compatibility) are similar to the recipient's, must be found.

An allogeneic HSC transplant basically consists of two steps:

–the first step is to destroy the patient's existing marrow cells by the use of special drugs and/or radiation;

–the second step is to reconstitute the patient's marrow patrimony by intravenously infusing (much like a normal transfusion) the stem cells provided by an HLA compatible donor. These cells are able to colonise the appropriate bone site and to begin producing normal blood cells.

Why are HSC donors needed?

More than twenty years ago, HSC transplants were only carried out between HLA identical siblings.

However, proof that 80% of the patients suffering from fatal haemopathies could not benefit from such a valid therapy (every year in Italy about 1,000 patients who are eligible for transplant have no compatible donor amongst their brothers and sisters), led haematologists to search for donors outside family. The results that were obtained by using unrelated volunteer donors were quite satisfactory and despite the great difficulties involved in finding subjects with similar genetic characteristics, National Bone Marrow Donor Registries were set up worldwide.

These organisations are actually databases that are connected to each other through an international network and they provide the individual patient with an extremely broad pool of potential donors. Some strategies are needed however, to increase the probability of finding a compatible donor, as due to the high number of possible HLA combinations, this probability ranges from 1/1,000 to 1/100,000, depending on the frequency of the characteristics that are taken into consideration.

In Italy in 1989, several scientific associations dealing with these issues (the Italian Society of Haematology, the Italian Association of Immunogenetics and Transplant Biology, the Italian Association of Paediatric Onco-Haematology, the Italian Society of Transfusion Medicine and Immunohaematology), started up an Italian programme called **"Bone Marrow Donation".** They established the Italian Bone Marrow Donor Registry, which is located in Genoa and is managed by the Tissue Typing Laboratory of the Galliera Hospital, whose activity was officially acknowledged with Law n.52- 6th March, 2001. Its aim is to provide haematological patients who have no ideal donor (HLA identical siblings) with an unrelated volunteer donor, whose immunogenetic characteristics are such that there is a very high chance of treatment success.

What is tissue compatibility?

Every one of us has a genetic set inherited from our parents and that, like our fingerprints, makes us unique. Some of these genes control the expression of structures *(antigens)* that are present on the surface of all the cells in our body.

Thanks to these antigens, which characterise each individual person, the immune system recognises its own normal cells and reacts against any extraneous ones, as well as any of its own cells that have been modified.

In humans, the genes that control the "recognition" of various tissues in the organism constitute, what is called, the HLA System (Human Leukocyte Antigens). These genetic characteristics can be determined directly by testing the DNA encoding for these antigenic products. In case of transplant, these tests (generically called tissue typing or HLA typing), together with other, so-called “functional” tests, are used to establish donor/recipient compatibility. The probability of finding identical HLA is only good amongst siblings (25%), whilst it is extremely rare among unrelated individuals.

How are bone marrow haematopoietic stem cells collected from the donor?

Haematopoietic stem cells from unrelated donors are collected from the bone marrow by repeated injections into the pelvic bones. Therefore, this procedure must be performed under anaesthesia, otherwise it would be too painful.
Although it is usually carried out under general anaesthesia, it may also be performed using epidural anaesthesia administered by a lumbar puncture.

However, general anaesthesia is preferable. On average, collection lasts 30-45 minutes and causes no damage or disability to the donor, as proven by more than 150,000 marrow blood collections carried out worldwide.

However minimal risks do exist (see Attachment H) and are connected to the procedure itself. They may be sub-divided as follows:

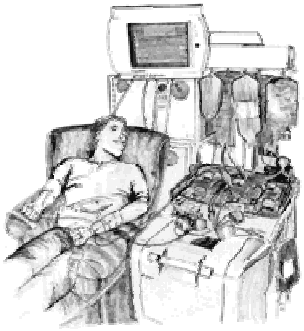
- anaesthesiological risk (connected to the type of procedure that is used and to the anaesthetic that is administered);
- infectious risk (both marrow blood collection sites and infusion sites are susceptible to infection);
- risk of lesions (during marrow blood collection some tissue may be damaged, leading to, for example, sciatica).

Therefore, in order to cope with possible complications resulting from these risks, the donor must not be affected by any severe cardiocirculatory or renal alterations.

On the average, collection takes less than one hour, after which the donor must stay in hospital for 48 hours. Upon awakening, and for a few days afterwards, he/she will feel some pain, although usually quite limited, at the collection site. After three days at most, the pain is practically non-existent. The quantity of marrow blood that is withdrawn varies depending on the body mass of the recipient, but it usually ranges between 700 and 1,000 mL. The body does not feel deprived of this marrow and the collected marrow is spontaneously replaced within 7-10 days. It is wise, however, for the donor to have one or more units of his/her own blood withdrawn one week prior to the collection date. The blood is reinfused in the operating room to balance out the volume of circulating blood. No drug administration is usually required before or after the donation.

On the basis of the above-mentioned points, it is reasonable to assume that the donor will be absent from work for no more than one week.

How are peripheral blood stem cells (PBSC) collected from the donor?



Normally, peripheral blood does not contain enough stem cells for a transplant, thus prior to transplant the amount must be increased.

Therefore, a growth factor called G-CSF (*Growth-Colony Stimulating Factor)* is administered to the donor. G-CSF has been on the market for the last few years as a pharmaceutical formulation and its main characteristic is its ability to make stem cells grow more quickly and then to facilitate their passage into the peripheral blood.

Due to the stimulation that takes place in the bone marrow, G-CSF may cause some mild to moderate discomfort that can easily be controlled by simple analgesics.

The most common disturbances include: mild fever or fever (even up to 38 °C), headaches, bone pain (especially in the pelvic area, back and limbs), fatigue and in some cases, loss of appetite. These symptoms disappear quickly when treatment is discontinued and there should be no side-effects afterwards.

The risk of death associated with PBSC mobilisation and collection (cerebro-vascular events, spleen rupture, or myocardial ischaemia) in younger, healthy subject is very low but, however, does exist. Currently, there is no evidence suggesting that the risk is any higher than the risks involved in bone marrow cell collection.

In 2006, two papers were published reporting an increased risk of production of leukemic cells because of the G-CSF, in familiar donor with a given genetic pattern. All the cases reported are in fact referred to the use of this drug in leukemic patients' relatives who, obviously, have a genetic background and predisposition for onco-haematological diseases.

On the other hand, a paper of march 2007, that examined more than 23.000 familiar donors mobilized through G-CSF, did not evidence any increased incidence of these diseases.

Therefore, according to the currently available studies, there is not any proof that the use of GSFC increases the risk of these diseases.

Extremely rarely a swollen abdomen is reported together with face, legs and feet swelling; it may be also associated with increased urination, difficult breathing and tiredness. If it happens the donor must immediately contact the Donor Centre physician who gave him the G-SFC.

The G-CSF administration is needed to be able to collect stem cells from the peripheral blood, rather than collecting them from bone marrow. The effects of these drugs become evident after 4-5 days of treatment, and in healthy subjects this is the appropriate time to collect the cells.

These procedures are usually very well tolerated, and do not require anaesthesia of any kind.

Currently existing cell separators require all circuits and materials to be sterile and disposable, and two venous accesses (one from each arm) are usually needed. Blood is drawn from the arm, it goes through a circuit and is fed into a centrifuge. The desired cellular components are isolated and then collected in a special bag, while the rest of the blood is reinfused into the other arm. In case only one venous access is available, withdrawal and reinfusion are carried out alternatively through the same arm.

Throughout the whole procedure, which lasts 3-4 hours, the blood that enters the separator must not coagulate, thus it is continuously mixed with an anticoagulant-agent (ACD, i.e., a citric acid, sodium, citrate, dextrose solution). The presence of sodium citrate in the solution may lead to hypocalcaemia, with tingling of the nose, fingers and around the mouth. These symptoms are mild and usually disappear quickly after intravenous administration of calcium containing preparations.

One or two collection procedures carried out on consecutive days may be needed in order to obtain the necessary amount of circulating haematopoietic progenitor cells.

What is requested of the Donor?

The following table summarises and compares what is requested of the donor for the two types of HSC collection.

Required commitments	Donation from marrow blood	Donation from Peripheral blood
Informative session	√	√
Laboratory tests	√	√
Auto-donations of peripheral blood	√	

2 days of hospitalisation	√	
General anaesthesia	√	
Post-donation pain	√	
Injection of G-CSF day 1		√
Injection of G-CSF day 2		√
Injection of G-CSF day 3		√
Injection of G-CSF day 4		√
Possible pre-collection side-effects		√
Willingness to reach the apheresis center once or twice on their own		√
Post-donation rehabilitation (days)	Usually 10-15	Usually 1-2

What does being registered in an HSC Donor Registry imply and what is expected?

At enrolment, the donor must be of age, and preferably younger than 36 years old. Gender is indifferent.

He/she should be healthy, or however, should not suffer from any important chronic diseases affecting the main organs or systems.

His/her characteristics must meet the requisites of the Italian Law on transfusions (Law n.219, 21 October, 2005 “New discipline of transfusion activities and national blood product production”) and relative decrees regarding blood donation (see Attachment G). HSC donation is strictly forbidden in the presence of either Hepatitis B surface antigen (HBsAg) in the blood or of the anti-HIV antibody (which is an index of AIDS-virus infection).

It is reasonable to expect that testing for these infections will be carried out immediately prior to any HSC collection, since the subject may become infected at any point of his/her life. If however, he/she is already aware of the presence of one of the above-mentioned conditions, he/she must not volunteer as a possible donor.

If the potential donor fulfils all the recruitment requirements, he/she is tissue typed for HLA antigens. This analysis is performed by withdrawing a small amount of blood (approx. 10 mL even if

the subject is not on an empty stomach) or collecting another biological sample (saliva/buccal swab). The HLA test is relatively complicated and therefore is only carried out in select, specialised laboratories.

The new donor's genetic data are stored in a computer database and then transferred through the Regional Registry to the National Registry. Subsequently, if a potential match with any patients on a waiting list is found at this step, the donor is called back for further sample collection, in order to carry out genetic testing more in-depth.

It is likely that during the following phases of testing the matching with the patient is not confirmed and therefore the donor will not donate HSC to save that patient's life.

Not to worry! His/her genetic data (which are now more complete and in-depth) will not be wasted. In the future, he/she could be compatible with another candidate for HSC transplant.

The donor has the right to withdraw consent at any time. Should this not occur, he/she will remain enrolled until the age of 55.

How does one become a bone marrow donor?

All one has to do is enquire at any of the many hospital structures that participate in the National *"Bone Marrow Donation"* programme and fill in an informed consent form. He/she will then undergo HLA typing. To receive more information, please contact any of the following centers:

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