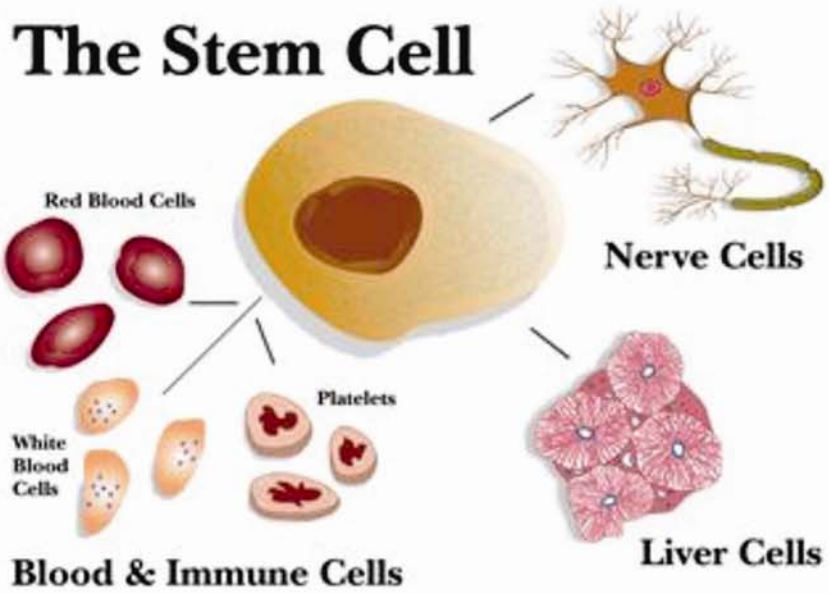
National Bioethics Committee

Protocol / Guidelines for Stem Cell Research / Regulation in Pakistan



Pakistan Medical Research Council

Shahrah-e-Jamhuriat, Off, Constitutional Avenue, Sector G-5/2, Islamabad

Tel: 92-51-9207386,9216793, Fax: 92-51-9216774. E-mail: pmrc.rdc@gmail.com, www.pmrc.gov.pk

These guidelines have been developed by the National Bioethics Committee, Pakistan and adopted by the Human Organ Transplant Authority

Protocol/Guidelines for Stem Cell Research/Regulation in Pakistan

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1. INTRODUCTION

The ability to cultivate a special class of cells known as stem cells and the possibility to use them as therapeutic tools has ushered in the era of what is known as regenerative medicine. All across the world research and clinical applications of stem cells involving human subjects are regulated by well established international guidelines with country specific details mandated by religious and social mores.

1.1 Origin and Properties of Stem Cells:

During the development of multi-cellular organisms a fertilized egg undergoes repeated cellular divisions to produce a mass of unspecialized cells known as embryonic stem cells.

They are uncommitted primordial cells which ultimately give rise to adult stem cells most of which differentiate into characteristic cells of organs and tissues. Stem cells are defined by their ability to keep dividing and renewing their population and thus are not exhausted. In contrast some of the differentiated specialized cells often do not divide and once damaged are depleted.

Potential of stem cells to renew and differentiate offers exciting possibilities to reverse tissue and organ damages caused by metabolic and degenerative diseases and aging.

1.2 Where are Stem Cells Found?

Gametes/ blastocysts/ fetal tissues/ placenta/umbilical cord cells/ adult tissues serve as sources of stem cells.

1.3 Classification of Stem Cells according to their Differentiation Potential

a) ***Totipotent* Stem Cells*:*** These are stem cells with absolute developmental plasticity which can give rise to *all cell types* that are found in an embryo, fetus or developed organism including the trophoblast and the placenta. The *zygote* and the *cells of the very early stage (i.e. the 2-cell stage) are totipotent*.

b) ***Pluripotent* Stem Cells:** as the zygote undergoes further mitotic divisions a mass of cells develops. This mass consists of unspecialized cells that can give rise to most, but not all, the tissues necessary for fetal development. Further specialization gives rise to multipotent cells that are committed to give rise to cells that have a particular function (e.g. multipotent cells committed to giving rise to the red cells, white cells and platelets).

c) ***Unipotent Stem Cells*** These self renew as well as give rise to a single mature cell type; e.g., spermatogenic stem cells. They can proceed only along one developmental pathway.

d) ***Induced Pluripotent Stem Cells (iPS):*** A recently developed technique has made it possible to develop multipotent stem cells from adult skin cells by genetic reprogramming (Yamanaka, 2006). The iPS resemble embryonic stem cells in their ability to differentiate into myriad of cells that constitute tissues and organs of the body and offer the advantage that they can be developed and put back in the same individual from whom they were derived. They therefore escape immune surveillance.

1.4) Sources of Stem Cells are as follows:

There are many sources of stem cells and appendix 1 details some of the issues related to stem cell research.

i) Adult Stem Cells: Derived from peripheral blood, tissue or bone marrow
ii) Cord Blood Cells: Derived from placenta
iii) Embryonic Stem Cells: Derived either from blastocysts or foetal tissues

1.4.1 Adult Stem Cells:

Adult bone marrow cells have been used for more than a decade. Stem cells in adult tissue are often multi-potent and can produce many, but not all cell types. These cells can be multiplied but do not have an unlimited capacity for renewal like embryonic stem cells. Adult stem cell therapy (ASCT) by qualified and experienced staff using appropriate validated technology, has become well established in certain hematological disorders such as very severe aplastic anemia, chronic granulocytic leukemia and thalassaemia major (well chelated, Pessaro category 1). ASCT has also been used for other hematological conditions such as relapsed childhood acute lymphoblastic leukemia and acute myeloid leukemia, but its use is not established as standard of care. Research is ongoing to determine whether these adult stem cells can also be made to differentiate into other tissues but in contrast to ASCT in hematological disorders, the use of such cells for non-hematological indications is experimental. The same applies to emerging techniques of modification of adult stem cells such as retro-differentiation, which are experimental at this stage.

1.4.2 Cord Blood Cells:

Cord blood stem cells (CSC) are also obtained from aborted fetal tissue and umbilical cord blood and have shown to be successful in reconstitution of bone marrow in children in many disease conditions. These stem cells and those from the adults are pluripotent in nature. They can be multiplied and maintained in culture and do not have unlimited capacity for renewal like the embryonic stem cells. Research is ongoing to determine whether these cells can also be made to differentiate into other tissues. Use of fetal stem cells will reduce the amount of foetal tissue used for various therapies. **TERMINATION OF PREGNANCY FOR OBTAINING FOETUS FOR STEM CELLS research or for transplantation will not be permitted.**

1.4.3 Embryonal Stem Cells:

These are pluripotent and have the capacity to develop into any cell of the body. These are obtained from the very early stages of the embryonic development probably up to 2-4 cell stage in humans i.e. within 5-7 days of conception. While scientists believe that these cells can be directed in the laboratory to differentiate into any cell or tissue to treat many diseases affecting various tissues and organs, these applications are still experimental. The main source of embryonic stem cells is currently IVF clinics dealing with infertility treatment, where SPARE OR SUPERNUMERARY embryos (at a very early stage) may be available for these purposes. **However no embryo should be CREATED for the sole purpose of obtaining stem cells.**

1.5 Stem Cells at the Center Stage of Biology and Medicine:

The journey of a stem cell as it traverses the trajectory of its life reveals the molecular and genetic events underlying growth, differentiation and development from a single fertilized egg to a complex multicellular organism. It also provides understanding of disease, aging and death.

Stem cells have generated a new medical paradigm known as *stem cell therapies.* Some of these such as bone marrow transplant (BMT) using adult hematopoietic stem cells are well established. Certain cell based treatments for ophthalmologic and musculoskeletal conditions are also being used. Geron Corporation, one of the leading stem cell companies is awaiting FDA clearance for the first ever trial of treatment for spinal cord injury. But treatment modalities for other diseases such as Parkinson's and Alzeimer's , immuno-genetic conditions and stroke and cardiac repair are still at the stage of animal studies and are unlikely to develop into routine bed side therapies in foreseeable future.

Promise and Problems: on the one hand there is no doubt that the yet far from fully realized potential of stem cells holds great promise for many life threatening and debilitating diseases and on the other they have been hyped as magic bullets for rejuvenating aged human skins. Claims abound as to the effectiveness of skin treatment with stem cells without proof of the principle. There is a need to regulate the diverse aspects of stem cell research and therapy where the immense power to cure and rejuvenate is harnessed and possible harm is avoided.

2. THE NEED FOR NATIONAL REGULATORY AUTHORITY AND INSTITUTIONAL OVERSIGHT AND MONITORING COMMITTEES

Stem cell research and its applications pose *serious ethical, social, legal and safety concerns* and call for exceptional care and vigilance particularly when it comes to *human embryonic stem cells (hES) derived from embryos.*

Research and therapy with adult stem cells is not embroiled in serious controversies but unrelenting watchfulness is indicated. Detailed National and institutional guidelines should be formulated to protect patients and donors from possible harm. It is extremely important that persons drafting guidelines are professionals with adequate understanding of scientific, ethical, legal and social aspects of SCRT and that all stakeholders are represented. As the field is new and rapidly advancing it is equally important that once the guidelines are developed they are reviewed at appropriate intervals.

It is imperative to develop mechanisms for implementing guidelines/Regulatory Frame Work and to invest the Implementing Authority with adequate powers to punish violations.

2.1Human Organ Transplant Authority (HOTA) for Cell Based Research & Therapy:

The guidelines have to be in accordance with religious sensitivities and cultural norms of our people.

PMRC should remain the main Regulatory Authority in the area of stem cell and related medical research. HOTA will set up an advisory subcommittee of the National Bioethics Committee in Research (NBCR) for cell based Research & Therapy. Given the wider application of such technologies by sector other than the Ministry of Health such as educational and research institutions and Ministry of Science & Technology, the HOTA Sub-Committee will have additional representation from these sectors. The membership of this HOTA Sub-Committee will be derived from the membership of the National Bioethics Committee for Research and shall also consist of additional co-opted members representing the fields of reproductive health, hematology and representatives of all organ transplant societies/ institutions.

All centers performing stem cell research and therapy should be registered with the HOTA for accreditation, on the basis of their technical competence (in stem cell collection procedures, enumeration, cryopreservation, stem cell viability studies) and ethical review and oversight procedures.

All proposals involving stem cells of any source for research or non-approved therapy (see Appendix 1 for approved therapeutic indications), should be cleared by Human Organ Transplant Authority (HOTA) Sub-Committee, through the National Bioethics Committee.

2.2 Scope:

The HOTA will have the responsibility to examine the scientific, technical, ethical, legal and social issues in the area of cell based research and therapy.

2.3 Scientific/Technical Issues:

All proposals, from public or private sector, for research or non-approved stem cell therapy (SCT) should be placed before the HOTA Sub-Committee for approval after due clearance through appropriate institutional ethical review committee and scientific peer review process.

2.4 Ethical, Legal and Social Issues:

Institutional Ethics Committees should keep in view the ethical, legal and social issues and should adhere to the ethical guidelines as per the Pakistan National Guidelines for Human Research (2006), the Helsinki Declaration (2000), the CIOMS guidelines (2002) as well as the WHO publication on “Genomics and Global Health” (2003

3. GUIDELINES

3.1 Adult Stem Cells:

While the approved use of specific adult stem cells does not pose major ethical problems at present their use must be considered experimental except for specific hematological indications. As indicated above, all centers doing stem cell treatment and research should register with HOTA. Bone marrow, peripheral, blood, skin, limbal cells are some of the tissues in use for procuring adult stem cells.

- No commercial sale or transaction for stem cell use or research will permitted
- All clinical use or therapeutic trails of stem cell must be conducted by institutions and qualified specialists who must be registered with HOTA.
- Proper informed consent procedures are to be followed as given in international ethical guidelines.
- Standard Operating Procedures, as outlined in the Appendix I, should be followed for procurement, cataloguing, source identification, storage and preservation.
- In vivo Studies: Experimental work on stimulation of adult stem cells also has tremendous future. However, approval from HOTA must be sought for carrying out all such experiments.

3.2 Cord Blood Stem Cells:

For using umbilical cord blood from a live fetus or a neonate it must be ensured that no harm should occur to the fetus or the neonate. Since the exact timing of the clamping of the umbilical cord has a significant impact on the neonate and early clamping may cause an abrupt surge in arterial pressure resulting in cerebral intraventricular haemorrhage, particularly in premature neonates, normal clamping protocol (Appendix II) will be followed when collecting foetal blood for transplantation. There is a risk that the neonate donor may need his or her own cord blood later in life. Parents will be informed of the risks of donation and a written consent will be obtained from them on behalf of the fetus. Cord blood stem cells are generally used for hematopoietic stem cell transplantation. For any other form of therapy detailed protocol has to be submitted for approval.

The following points should be specifically considered while collecting umbilical cord blood for banking:

- No harm should occur to the fetus or the neonate.
- Exact timing of the clamping of umbilical cord should be defined. Early clamping may lead to cerebral haemorrhage. Normal clamping protocol should always be followed.
- Parents should be correctly informed regarding risks and benefits involved.
- Free informed consent should be obtained from both parents. If there is disagreement between the parents, the mother's wish shall prevail.
- ID card should be issued for voluntary donation to enable access/benefit in future in case required for self/relatives.

Cord blood stem cell banking is permissible. However, all Cord Blood Banks should be registered as per guidelines applicable to the blood banks. Commercial exploitation of stored blood should be regulated strictly. Special care must be taken in collection, processing and storage of umbilical cord stem cells to avoid transmission of infections.

Maternal screening should be carried out for transmissible infections. Purpose of banking should be clearly explained to couples interested in storing cord blood. The ideal use of these cells at present is for allogenic hematopoietic stem cell transplantation. Expansion of umbilical cord stem cells for transplantation in adult and use for non-hematopoietic indications is still in exploratory phase. When it comes to registries and banking, the commercial aspects pose additional problems. The advertisement related to collection of samples should be carefully looked into with respect to conflict of interest, utility of samples, accessibility and affordability

3.3 Fetal Stem Cells/tissue:

These can be processed from spontaneously aborted fetus or from fetuses obtained from hospitals. International guidelines for fetal tissue transplantation should be followed. DNA fingerprinting of the cell line should be preserved and it is advised to keep it in cell repository. Generally fetal stem cell use is presently experimental. Any therapeutic fetal cell transplantation will not be permitted at present and this possibility will be examined at an appropriate time later. **Termination of pregnancy should not be sought with a view to donate fetal tissue in return for possible financial or therapeutic benefits. Informed consent to have a termination of pregnancy and the donation of fetal material for purpose of research or therapy should be taken separately. The wishes of the mother will prevail in case of any difference of opinion.**

3.4 Embryonic Stem Cells (ES Cells):

No Embryo should be generated for the sole purpose of obtaining stem cells. Only surplus or spare or supernumerary embryos **(under 16 weeks gestation),** can be used with the permission of the couple from IVF clinics. Cell lines generated should be registered. At present only research program relating to *in vitro* induction of differentiation into various cell lines will be cleared by the NRC on case to case basis. Any therapeutic trial will be examined in detail before approval.

Reproductive cloning will not be permitted on ethical grounds. Human cloning is not permitted for the purpose of creating a new individual.

3.5 Monitoring Mechanism:

The HOTA will be authorized to make site visits as required and receive annual reports of cleared projects. These annual reports should be submitted in appropriate format for further continuation of the project by the 10^{th} month of commencement of the project decision and review should be communicated in 4-6 weeks. Any violation of guidelines would be strictly dealt with and procedures will be established to enforce such regulations and penalties in the event of violation through the PMDC and the Ministry of Health.

3.6 Commercialization and Patent Issues:

Established stem cell lines can have considerable commercial value as wide ranging potential benefits for large number of patients is possible. Patent issues need wider discussions and public debates should be held on who should be the beneficiary and what type of patents can be taken. Exploitation of Pakistani biological material by foreign commercial interests is not permitted.

3.7 IPR Protection should be accorded to commercially utilizable materials and procedures. Benefits of commercialization should be extended to community including patients, researchers and institutions engaged in research and applications.

3.8 Regulation of Stem Cell Lines:

All cell lines should be registered with HOTA. All proposals for therapeutic trial should be cleared by this committee before submitting to national or international funding bodies.

3.9 International Collaboration:

National guidelines of respective countries should be followed and all research protocols for sponsored research must be cleared with appropriate ethical review committees in the sponsoring country. Collaboration will be permitted only after the joint proposal with appropriate MOU is approved by the National Research ethics Committee following clearance by the HOTA. No export of cell lines *per se* will be permitted.

3.10 Generation of Embryonic Stem Cells from Non-Human Sources:

Embryonic stem cells for experimental purposes cal also be obtained from sources such as rodents. Primates, domestic animals, farm animals etc. Research in these areas should be encouraged but is strictly experimental and must undergo similar ethical review and clearance.

3.11 Check list to be submitted to the Human Organ Transplant Authority (HOTA) for Any Proposal

a. Title of the proposal.
b. Institution concerned.
c. Investigators' name with brief bio-data and relevant publications.
d. Source of funding.
e. An assurance signed by the responsible institutional head that the pluripotent stem cells were derived from human embryos in accordance with the guidelines.
f. Informed consent document duly signed by appropriate individuals.
g. Brief summary of the proposed work.
h. IRB/IEC clearance (if sponsored, ethical review and clearance from appropriate ethical review committees in the sponsoring countries).
i. Certificate that no undue inducements/ incentive is provided for donation of embryo.
j. Separate consent for infertility treatment and donation of embryos to be taken.
k. Certificate that only spare embryos are being used. Private sector involved in such research should also come under the purview of this committee and it should comply with due safeguards and standards and submit all proposals for clearance.

3.12 Permissible Research on Stem Cells

a. Use of human stem cell lines derived from hES, hEG, hSS or fetal/adult stem cells is permitted for *in vitro* investigations.
b. *In vivo* studies of human cell lines and their differentiated derivatives are allowed in small **non-primate** animals at various stages of development (embryonic, fetal, postnatal, adult). ***The animals derived from these experiments shall not be allowed to breed*** especially when there is a possibility that human cells could significantly contribute to development of gonads and/brain.
c. Animal studies for pre-clinical evaluation of efficacy and safety of human stem cell lines is also allowed as per above mentioned criteria.
d. Informed consent from donors is required for:
    - in- vivo animal studies when using stem cells from donors of bone marrow, peripheral blood, umbilical cord blood, skin, limbal cells, dental cells, bone cells, cartilage cells or any other organ (including placenta)
    - Establishment of fetal/adult hSS and new hES cell lines from spare, supernumerary embryos.
    - Establishment of Umbilical Cord Stem Cell banks

3.13 Clinical Grade Stem Cells for Biomedical Research and Therapy

- Clinical grade stem cells are classified as therapeutics and are required to be produced under international GMP/GTP conditions.
- The cells should be well characterized about their *stemness* and *safety.*

3.14 Prohibited Research

- Creation of a zygote by IVF, SCNT or any other method with the specific aim of deriving a hES cell line for any purpose.
- Introduction of hESCs into non-human primate blastocysts
- *in vitro* culture of any intact human embryo for longer than 14 days or until formation of the primitive streak begins, whichever occurs first
- Breeding of animals that have had hESCs introduced into the germ line
- Germ line genetic engineering or reproductive cloning.

- Transfer of human blastocysts generated by SCNT or parthenogenetic or androgenetic techniques into a human or non-human uterus.
- Animals in which any of human stem cells have been introduced at any stage of development should not be allowed to breed.
- Research involving directed non- autologous donation of any stem cells to a particular individual is also prohibited.
- Any research involving implantation of human embryo into uterus after in vitro manipulation, at any stage of development, in humans or primates.

Ref: Ethical Guidelines for Biomedical Research in human Subjects". 2000, 2006 ICMR

4. Application of blood and marrow transplantation (BMT)

Introduction:

The clinical use of progenitor cell transplantation technology has been instrumental in the treatment and cure of thousands of patients with haematological and non haematological malignant disorders. However, the procedure still carries significant morbidity and mortality. It is also at risk of commercial exploitation and unethical practices. The setting up of centres for BMT must be strictly regulated and their working should be periodically and objectively monitored.

The following guidelines are for the infrastructure of these centres:

4.1 BMT Centre

This centre must be located in a tertiary care hospital which will provide a wide range of support and patient care services including critical care management, haemodialysis, advanced imaging facilities, cardiac support facilities, infection surveillance and management facilities. Dedicated areas, according to workload will be earmarked for BMT patients in these hospitals.

The BMT Centre will have

- A clinic to perform pre-donation physical examination and specialized tests (X-ray, ECG, Echo etc)
- Indoor facilities which must have dedicated operation theatres and isolation rooms with state-of-art ventilation facilities.

The BMT Centre must have harvesting facilities for bone marrow and progenitor cells. There should be atleast two cell-separators, facilities for quantitative and qualitative evaluation of harvested progenitor cells and facilities for cryo preservation and storage of progenitor cells.

The Centre must have direct access to the following accredited facilities.

- HLA Typing Laboratory
- Microbiology Laboratory
- Biochemistry Laboratory
- Blood Transfusion Laboratory
- Histopathology Laboratory

4.1.1 Essential Personal

a. Medical Director

The centre must be under the control of a medical doctor who has accredited post-graduate qualification in haematology, a minimum of 15 years experience in laboratory and clinical haematology and demonstrable knowledge and interest of BMT.

b. Transplant Physician

At least two transplant physicians should be full time employees of the centre. They must be medical graduates and have accredited post-graduate qualification in haematology. They must have 10 years experience of clinical haematology, have worked under the supervision of a transplant physician for atleast 2-years at a centre doing a minimum of 10 BMT per year. They should have exposure to atleast 10 bone marrow harvesting procedures.

c. Program Coordinator

Master in Social Sciences, having working knowledge of statistics, computers and record keeping.

I. Support Medical Faculty

1. Anesthetist
2. Pulmonologist
3. Gastroenterologist
4. Nephrologist
5. Cardiologist
6. Neurologist
7. Infection Disease Physician

II. Support Services

1. Pharmacy
2. CSSD
3. Nutritionist
4. Social Worker

III. Monitoring of Services

The services of the centre must be supervised by a regulatory body which will include;

1. Medical Director
2. Program Coordinator
3. Legal Expert
4. Financial Advisor
5. Ethical Service Advisor
6. Social Service Advisor
7. Women's Representative
8. Donor Representative

IV. Accreditation of the Centre

It should be mandatory for all BMT centres to allow access to inspectors for the purpose of accreditation. The inspection team, to be constituted by HOTA, must ensure that the centre is located in an institution at a fixed physical site, is equipped and staffed as per regulation, and is not involved in unethical commercial practices. The centre must maintain a record of donors of blood, aphaeresis progenitor cells and bone marrow. It should be carrying out management activities including education, counseling, rehabilitation of patients and should be capable of dealing with medical screening and confidentiality issues.

Appendix I

5. GUIDELINES FOR THE COLLECTION, PROCESSING AND STORAGE OF HUMAN BONE MARROW, PERIPHERAL STEM CELLS FOR TRANSPLANTATION

5.1 General Principles:

Each stem cell transplant unit shall establish, document and maintain an effective and economical quality system to ensure and demonstrate that adequate and appropriate standards of work are maintained.

5.2 Procedural Step Related to Bone Marrow Harvest:

Donor suitability must be ensured. The donation may be an autograft or an allograft from a related or unrelated donor. The allogenic donor should be counseled and a full medical examination carried out to establish that the donor is fit for anesthesia. Routine haematological, biochemical and virological parameters are checked. A chest X-ray and ECG should be performed. Written informed consent must be obtained from the donor before the recipient commences pretransplant conditioning. It is a cardinal principle that unrelated donors should be anonymous, unpaid and not pregnant.

The donor is admitted, night before harvest to the transplant centre or hospital experienced in marrow harvests. The donor receives a unique donor identification number (DIN) and this must be assigned to the marrow, both primary and secondary collection packs and all the sample tubes used.

5.3 Marrow Collection:

Before the start of harvest the identity of the donor must be checked. Collection of marrow should be by aseptic technique into pyrogen-free containers with sufficient anticoagulant for the quantity of marrow to be collected and appropriate for the subsequent processing. The container label should state the amount of anticoagulant and the maximum amount of marrow that can be collected and required storage temperature. The marrow should be anticoagulated with ACD-A unless preservative free heparin is requested by the transplant centre. In the latter case marrow should be returned to the patient within 12 h. The anticoagulant solution must be clear, free from deposit.

Harvest should only be undertaken by trained medical staff. Personnel required are an anesthetist; operators to harvest marrow, haematological scrub nurse and theatre staff. Harvest lists should be dedicated or at the beginning of the operating list. The volume of marrow withdrawn from the donor must be controlled. The volume taken should be such that a target cell count appropriate to transplant is reached.

5.4 Records:

A record of the total volume of marrow removed from the donor must be documented in the patient's notes. The most efficient way of measuring the volume in plastic bags is by weight of 1 ml. Of marrow is 1.06g; a unit containing 405-495 ml should therefore weigh 430-522 g plus the weight of the container and its anticoagulant.

5.5 Operating Procedure:

The anaesthetized patient is positioned on the operating table with pelvis supported to make the iliac crest prominent. The choice of harvest needle is one of personal preference and can very from conventional needles for diagnostic aspiration from iliac crests through to designed harvest

needles with holes along the lateral aspect of the shafts (e.g. (Islam). No more than 5 ml of bone marrow should be collected into a 20 ml syringe containing preservative free heparin and the needle then repositioned after each aspiration. It is usually possible to take marrow at several different depths from one site. The needle is then withdrawn and resited, samples being taken as widely as possible along the posterior iliac crest. If an inadequate cell count is obtained from both posterior iliac crests the patient should be turned over and further aspirates taken from the anterior iliac crests and the sternum.

5.6 Marrow Processing:

A closed system is preferred in which the syringe is emptied directly into 500ml or 1L bags containing anticoagulant. Marrow should be filtered in accordance with the harvest center's routine practice to remove fat, aggregates, clots or bone spicules if it is not processed further by centrifuge or sedimentation.
During and after harvesting samples of marrow can be obtained from the bag and nucleated cell count carried out to ascertain the anticipated volume needed to produce engraftment.

5.7 Estimation of Marrow Dose at Harvest:

The present recommended '**dose** 'of nucleated cells is expressed per kilogram of recipient body weight.

- I Autografts: minimum dose 1.5 x 108 cells /kg.
- ii. HLA identical sibling allografts for aplastic anaemia: minimum dose 3 x 108 cells /Kg (Storb et al, 1977).
- iii. HLA identical sibling for leukemia haemoglobinopathies and inborn errors of metabolism: minimum dose 1.5-2 x 108 cells/kg
- iv. Unrelated donor allograft: minimum dose 2-3 x 108 nucleated cells/Kg
  A minimum number of marrows (nucleated cells) per kilogram recipient body weight should be stated by each transplant unit to permit engraftment. The weight of the recipient must be ascertained.

5.8 Post-Harvest Marrow Processing:

In allogeneic transplantation the completed barrow bag is rendered airtight, labeled and its contents may then be infused intravenously into the recipient where both donor and recipient are ABO compatible or cryopreserved. If volume reduction is required buffy coat cells can be separated and transferred to a second sterile pack. When there is no major blood group incompatibility between donor and recipient then a cell fraction known to contain the repopulating stem cells and low in haematocrit should be obtained by density separation.

If removal of T cells is required to prevent graft versus host disease the volume of the donation is reduced before incubation with monoclonal antibodies. An appropriate method is to use blood cell washer or cell separator. Processing also may be modified to collect donor red cells for autologous reinfusion.

Autologous marrow can be similarly separated to leave a buffy coat or further processed to a mononuclear cell fraction which may be 'purged 'before storage. Purging involves incubation of the marrow with most commonly the cyclophosphamide antimetabolite, 4 hydoxy-per-oxy-cyclophosphamide (4 H-C; Kaizer et al., 198) or with monoclonal antibodies. Monoclonal antibodies may also be used for positive selection of putative progenitor stem cells (e.g. anti-CD34 antibodies). The purged / unpurged marrow is then cryopreserved (see below). Biological methods of purging such as long-term bone marrow culture are still experimental.

5.9 Peripheral Blood Stem Cell Collection:

Haemopoietic cells are present at low concentration in steady-state blood. Such cells can be mobilized from the bone marrow into peripheral blood during the recovery phase from myelosuppressive chemotherapy or following administration of haemopoietic growth factors or by a combination of the two. They are collected by leukapheresis on a blood cell separator set to obtain sufficient mononuclear cells for engraftment. A suggested value is $7x\ 10^8$ mononuclear

cells/kg but this will vary according to the centre and whether myeloablative treatment is given. Some centers use lower values of 2×10^8 mononuclear cells /kg.

Mobilized blood progenitor autografts are usually associated with very rapid haemopoietic reconstitution. Timing of the leukapheresis may be guided by the appearance of CD34 positive cells is the blood, or by surrogate markers such as white blood cell and platelet counts.

During recovery from myelosuppressive therapy a white count greater than 1×10^9/1 and platelets greater than 70×10^9 /l are generally taken as the time to initiate leukapheresis but absolute counts will very with the chemotherapy regime and whether or not growth factors are employed.

Mobilized blood stem cells collections are usually assessed by their CD34 positive cells or CFU-GM. 'Threshold doses' need to be determined by each centre by the recommended minimum number of CFU-GM and is usually in the range 5-20 $\times 10^4$/kg (Craig et al., 1992). It is usual that the donor is an autograft recipient and that the material is stored by cryopreservation.

5.10 Nucleated Cell Collection:

The technique for procurement of the nucleated cell layer rich in stem cells depends on the cell separator machine used. The aim is to collect the buffy coat interface between plasma and red cell. The total nucleated cell volume collected is determined from the total blood volume of the donor ascertained from their height/ weight prior to apheresis. It is recommended that in adults 7-15 /l is processed. Where the nucleated cell yield is greater than 100×10^9/l dilutions in plasma are needed to prevent aggregation during the process of freezing (see Storage).

When the collection is complete the residual volume of blood is returned to the donor.

Appendix II

6. GENERAL SPECIFICATION FOR IDENTIFICATION, STORAGE AND TRANSPORTATION OF BONE MARROW AND PERIPHERAL BLOOD STEM CELLS

6.1 Identification:

The unit containing stem cells must be labeled with the name of the product, the donor's name and hospital number, unique donation number, date of collection, the presence and type of anticoagulant and additive media if any, ABO and Rh D group and the volume of the product.
Storage (Rowley & Davis.1990; Reiman & Sacher, 1991)

An SOP should be written to include the following: a designated storage area: a procedure for quarantine of bone marrow and peripheral blood stem cells: a procedure for validating the conditions of storage achieved in any given storage area. This should include temperature control and preventation of microbiological contamination. If, as a result of microbiological screening, the donor is positive for any of the mandatory microbiological marker (table 1) then that unit should be stored in isolation to avoid cross contamination of other units.

6.2 Storage Unfrozen:

Unmanipulated bone marrow and peripheral blood stem cells may be stored unfrozen for up to 72 h at 4 + 2˚C. The anticoagulant conventionally used in ACD. Heparin is unsuitable. Storage at 4˚C will, however, reduce leukocyte viability and cellular aggregation may occur.

6.3 Preparation of Frozen Marrow and Peripheral Blood Haemopoietic Cells:

Haemopoietic cells in bone marrow are enriched and concentrated by differential centrifugation into a buffy coat. Further enrichment using a density gradient may be required. Peripheral blood cells are not normally concentrated. The cell concentration frozen should be less than 100 $*10^9/1$ (see above Nucleated Cell Collection).

The above products are cryopreserved using one of the established cryoprotectants, for example 10 or 15% v/v dimethyl sulphoxide (DMSO) or 5% v/v DMSO plus 6% w/v hydroxyethyl starch (HES). DMSO should be added slowly to its diluent's to avoid a rise in temperature since desired final concentration of DMSO is isotonic in molar terms. This requires the addition of albumin solution (HAS) is used additional salts or a suitable impermeable sugar are necessary (Pagg. 1984). The diluent is then cooled on ice to 0-4˚C, DMSO added and the cryoprotectant mixed with an equal volume of haemopoietic cells.

6.4 The Freezing Process:

A feedback-controlled cooling machine (controlled rate freezer) will provide reproducible, standardized cooling conditions. The cooling program should be one that has been shown to be effective (e.g. 2 ˚C/ min to -30˚C, followed by 4˚C/min to -70˚C with adequate control of freezing plateau: Gorin, 1986). Passive cooling methods may also be effective providing that they produce acceptable cooling profiles (Makino et al., 1991). The bags in which the marrow is cooled should be made from plates ton produce a thickness of about 3 min to facilitate heat transfer. Temperature should be recorded in a control bag and the data kept with the processing records.

Peripheral blood cells may be cryopreserved by the same methods.

Once below -70˚C the bone marrow and peripheral blood stem cells should be transferred for storage. Such deep frozen is fragile and container bags may fracture.

6.5 Thawing of Frozen Bone Marrow/Peripheral Blood Cells:

1. Contamination of the marrow/peripheral blood bag with water bath fluid is to be avoided and it is essential that double bags are used.

2. Thaw in a water bath at 37-40°C with gentle agitation. Observe carefully for rapid expansion of the bag during thawing which will suggest that liquid nitrogen has leaked into the bag during storage. If this occurs release the pressure immediately by puncturing the bag with a sterile needle.

3. DMSO toxicity is temperature dependent (Goring 1986). It is therefore important to remove the bags from the waterbath soon as the last ice has melted and not to allow the marrow to reach the waterbath temperature. Keep the thawed marrow cool until administration and infuse within 5 min of complete thawing. Current practive is not to remove the DMSO before injection into the patient (Rowley & Anderson, 1993). Premedication of the patient with steroid/ antihistamine is recommended.

4. Any thawing incidents should be documented and reported to the clinician in charge of the recipient who will decide whether action is required.

6.6 Testing:

Testing of frozen products may be performed utilizing 1-or 2-ml aliquots of material frozen at the same time under conditions that are as close as possible to the bulk product. It should be noted that small ampoules will not cool at the same rate as large bags. Assay may be performed to determine short-term progenitor growth post-cryopreservation.

6.7 Specific Laboratory Procedures Related to Provision of Haemopoietic Cells for Engraftment Serology:

The ABO and RhD blood groups of all donors should be performed as set out in the Guidelines for Compatibility Testing in Hospital Blood Banks (Boulton et al, 1987).

6.8 Cell Counting:

The minimum information required is the total nucleated cell count obtained at stem cell harvest. If counts are corrected for peripheral blood dilutions, this should be clearly indicated to avoid confusion with uncorrected nucleated counts. Such counts should be performed on a cell counting machine by validated methods or performed manually. The volume of bone marrow or peripheral blood harvest is best calculated by weight.

6.9 Cell Viability

The quality of frozen cells can be assessed by the trypan blue exclusion test or automated techniques using a flow cytometer and propidium iodide. These results do not correlate with in vitro growth, but given indication of consistency of the technique.

6.10 Red Cell Depletion:

Red cell depletion of the donor graft is strongly recommended in ABO mismatched allogenic transplant. This can be carried by HES sedimentation or differential centrifugation (Braine et al., 1982: Ho et al., 1984). An alternative is to plasma exchange the recipient and/or to give A/B antigen rich secretor plasma prior to the transplant.

6.11 Microbiology and Virology:

The sterility of the bone marrow/ peripheral blood product at various stages should be ascertained using liquid and semi-solid culture medium. This is especially important prior to freezing and at the final stage of processing after freezing before the product is infused into the patient. A pilot tube is thawed and cultured. The clinician should be informed if the cultures are positive. Donor bone

marrow can transmit infectious disease and all donors should have the mandatory advisable and desirable tests (shown below:) carried out and where possible the serological tests repeated at not less than 90 days from the first screening sample:

a. Mandatory

HBsAg (hepatitis B surface antigen)
HIV 1+2 antibody (human immune deficiency virus)
Anti-HCV (Hepatitis C virus antibody)
VDRL or equivalent test for syphilis

b. Advisable

CMV antibody
Toxoplasma gondii antibody

c. Desirable

HSV (herpes simplex antibody)
HZV (herpes zoster antibody)
HTLV-1 (Human T-Lymphotrophic virus)

Appendix III

7. PRE-REQUISITES FOR A STEM CELL TRANSPLANTATION CENTRE

7.1 Standards for collection, processing and storage of cells for clinical use:

For the consistence and reliable results International standard procedures should be adopted. These standards are designed to provide minimum guidelines for facilities and individuals performing collection, processing and storage of cells for clinical use or providing support services for such procedures. These standards are not intended to include all procedures and practices that a facility or individual should implement if the standard of practice in the community or governmental laws or regulations establish additional requirements. Each facility and individual should analyze their practices and procedures to determine whether additional standards may apply.

Protocols shall be developed, implemented, and documented for the validation or qualification of significant products of facilities, processes, equipment, reagents, labels, containers, packaging materials, and computer systems.

For this purpose training of the staff in renowned stem cell/transplantation centers is recommended. There shall be procedures for biological, chemical, and radiation safety, as appropriate, and a system for monitoring training and compliance.

7.2 Personnel

1. There shall be a Collection Facility Head / Officer in-charge an individual with a doctoral degree, qualified by postdoctoral training or experience for the scope of activities carried out in the facility. This individual is responsible for all technical procedures and administrative operations of the collection facility. This individual should participate regularly in educational activities related to the field of cell collection and / or processing.

2. The Collection or Processing Facility Head / Officer in-charge with a doctoral degree, n shall have at least one year's experience in the collection procedure. This individual shall have performed or supervised at least 10 collection procedures of each type that are to be carried out at the facility.

3. There shall be a Collection Facility Medical Head/Director who is a physician licensed in the jurisdiction in which the facility is located. This individual is directly responsible for the pre-collection evaluation of the donor, final approval of the prospective donor for the collection procedure, conduct of the collection / processing procedure, care of any complications arising from collection and compliance of the collection facility with these Standards.

4. There shall be adequate numbers of trained support personnel available at the facility where the collection is performed.

5. The training, continued education and continued competency for the performance of operations shall be documented.

7.3 Ethical issues:

- Informed consent from the donor shall be obtained and documented by a licensed physician or other health care provider familiar with the collection procedure before the high dose therapy of the recipient is initiated.
- The procedure shall be explained in terms the donor can understand, and shall include information about the significant risks and benefits of the procedure and tests performed to

protect the health of the donor and recipient and the rights of the donor to review the results of such tests.

- The donor shall have an opportunity to ask questions and the right to refuse to donate.
- In the case of a minor donor, informed consent shall be obtained from the donor's parents or legal guardian in accord with applicable law and shall be documented.

7.4 Laboratory facilities for cell processing

The facility responsible for cell processing shall be of adequate space and design for the intended procedures.

- The operation of the facility shall be divided into defined areas of adequate size for each operation to prevent improper labeling and/ or contamination of the product.
- The facility shall be operated in a manner to minimize risks to the health and safety ofemployees, patients, donors and visitors.
- The facility shall have written policies and procedures for infection control, biosafety, chemical and radiological safety, emergency response to worksite accidents, and waste disposal.
- Instructions for action in case of exposure to communicable disease or to chemical, biological and radiological hazards shall be included in the safety manual.
- Decontamination and disposal techniques for medical waste shall be described. Human tissue shall be disposed in such a manner as to minimize any hazard to facility personnel or the environment in accordance with applicable governmental laws and regulations.
- Eating, drinking, smoking, the application of cosmetics or the insertion or removal of contact lenses shall not be permitted in work areas.
- Gloves and protective clothing shall be worn while handling human specimens. Such protective clothing shall not be worn outside the work area.
- There shall be adequate equipment for the procedures performed at the facility.
- The facility shall be maintained in a clean and orderly manner as established in SOPs.
- The facility shall be secure to prevent the admittance of unauthorized personnel.

7.5 Equipment

Equipment used in the processing, testing, freezing, storage, transportation, and transplantation of products shall be maintained in a clean and orderly manner and located so as to facilitate cleaning, calibration and maintenance.

- Each collection facility shall be operated in a manner to minimize risks to the health and safety of employees, donors, volunteers, and patients. Suitable environment and equipment shall be available to maintain safe operations.
- Environmental control lab facilities of international standards are must. Equipments used in the collection of products shall be maintained in a clean and orderly manner and located so as to facilitate cleaning, calibration and maintenance.
- The equipments shall be observed, standardized and calibrated on a regularly scheduled basis as described in the SOPs Manual and according to the Manufacturer's recommendations.
- Sterilization equipments shall be designed, maintained and used to ensure the destruction of contaminating microorganisms.
- Refrigerators and freezers used for the storage of specimens, cell products, blood products, human tissues, or reagents shall not be used for any other purpose.

7.6 Consumables

- Reagents used in collection of products shall be of appropriate grade for the intended use and shall be sterile.
- Procedures for production of in-house reagents shall be validated.
- Each supply and reagent used in the collection of the product shall be examined visually for damage or evidence of contamination as it comes into inventory and this review shall be documented. Such examination shall include inspection for breakage of seals, abnormal color and expiration date.

- All supplies and reagents used in the collection of products shall be stored in a safe, sanitary, and orderly manner.
- Lot numbers and expiration dates of reagents and disposables shall be recorded.
- Supplies and reagents should be used in a manner consistent with instructions provided by the manufacturer.

7.7 Tissue processing

Human tissue refers to cells obtained from any living or cadaveric human donor or organ. The cell processing facility shall have written policies and procedures addressing all appropriate aspects of the operation including processing; emergency and safety procedures; donor and patient confidentiality; quality management and improvement; errors, accidents and adverse reactions; corrective actions; personnel training; competency assessment; outcome analysis; audits; labeling; storage, including alternative storage if the primary storage device fails; transportation; expiration dates; release and exceptional release; disposal of medical and biohazard waste; equipment and supplies; maintenance and monitoring; cleaning and sanitation; and a disaster plan.

- All open cell handling procedures must be performed in class 100 environment.
- More than minimal manipulation of products should only be performed in a clean-room environment. Environmental monitoring of such rooms must be performed and documented.
- There shall be a written request from the recipient's physician before processing is initiated.
- Processing of cellular therapy products shall be performed according to protocols defined in the facility's SOPs.
- Methods for processing shall employ aseptic technique and be validated to result in acceptable cell viability and recovery.
- There shall be written documentation of an interim assessment of donor suitability for the collection procedure by a qualified person immediately prior to each collection procedure.
- For donors of peripheral blood aphaeresis products, a complete blood count, including platelet count, shall be performed within 72 hours prior to the first collection and within 24 hours before each subsequent aphaeresis.
- For progenitor and other adult cell collection, methods for collection shall employ aseptic technique and shall use procedures validated to result in acceptable progenitor cell viability and recovery.
- The collected cells shall be packaged in a closed sterile container / transfer packs approved for human cells and labelled.
- Bone Marrow shall be filtered to remove particulate material prior to final packaging, distribution or transplantation using sterile filters that are non-reactive with blood.

7.8 Labelling control

- Labelling operations shall be conducted in a manner adequate to prevent mislabelling ofproducts that shall include the following quality management elements:
- Container labels shall be held upon receipt from the manufacturer pending review and proofing against a copy approved by the Collection Facility In-charge or designee to ensure accuracy regarding identity, content, and conformity.
- Stocks of unused labels representing different products shall be stored in an orderly manner to prevent errors. Stocks of obsolete labels shall be destroyed.
- A system of checks in labelling procedures shall be used to prevent errors in translating information to container labels.
- All labelling shall be clear and legible and printed using moisture-proof ink.
- Labels shall be affixed or attached firmly to the container.
- The proper name and significant modification(s) shall be noted on the label.
- Products that are subsequently re-packaged into new containers shall be labelled with new labels as appropriate. Records to allow tracking of products including collection or processing facility identity, unique numeric or alphanumeric identifier, collection date and time, product identity, donor and recipient information on the original container shall be maintained.
- When the label has been affixed to the container, a sufficient area of the container shall remain uncovered to permit inspection of the contents.

- The product label shall be complete. Not applicable (NA) may be used when appropriate.

7.9 Stem cell storage and transportation facilities:
Cell collections shall be handled and discarded with precautions that recognize the potential for transmission of infectious agents. Issues of donor health that pertain to the safety of the collection procedure shall be communicated in writing to the collection facility staff.

Prospective donors shall be evaluated by medical history, physical examination by a trained physician and laboratory testing for the risks of the collection procedure including the possible need for central venous access and/or mobilization therapy for collection of blood cells and anaesthesia for collection of marrow. This evaluation shall be documented.

7.10 Cryopreservation

Sample aliquots of the product, cryopreserved and stored under the same conditions as the product, should be available for testing for 5 years.
Cryopreservation procedures shall be included in the cell processing facility's SOPs and shall describe:

- The name and freezing criteria of the cell product or aliquot.
- The cryoprotectant solution and its final concentration.
- Cryopreservation container & acceptable range of product volume for reproducible cryopreservation.
- Acceptable range of nucleated cell concentration of the final product after cryopreservation.
- Cooling rate and product temperature at endpoint of controlled cooling. The cooling rate achieved shall be recorded, if a rate-controlling device is used.
- Acceptable temperature range for storage.

7.11 Storage

- Materials that may adversely affect cell products shall not be stored in the same refrigerators or freezers.
- For products immersed in liquid nitrogen, procedures to minimize the risk of microbial cross-contamination of products shall be employed.
- Refrigerators and freezers for product storage shall have a system to monitor the temperature continuously or at least every 8 hours.
- For products fully immersed in liquid nitrogen continuous temperature monitoring is not required. There shall be a mechanism to ensure that levels of liquid nitrogen in liquid nitrogen freezers are maintained.
- Storage devices for products or reagents for product processing shall have alarm systems that are continuously active. The alarm systems shall have audible signals.
- If laboratory personnel are not always present in the immediate area of the storage device, a remote alarm device shall be required at a location staffed 24 hours a day.
- Alarms shall be set to activate at temperatures or an unsafe level of liquid nitrogen to allow time to salvage products.
- There shall be written instructions to be followed if the storage device fails. These instructions shall be displayed in the immediate area containing the storage device.
- Alarm systems shall be checked periodically for function.
- Additional storage devices of appropriate temperature shall be available for product storage if the primary storage device fails.
- The storage device shall be located in a secure area. Locking capability for the device or the storage location should be used when the area is unattended.

7.12 Transportation

Procedures for transportation of the collected product shall be designed to protect the integrity of the product being shipped and the health and safety of facility personnel.

- The primary product container shall be placed in a secondary container and sealed to prevent leakage. The outer shipping container shall be thermally insulated and shall conform to the regulations regarding the mode of transport.
- Frozen or non-frozen products that leave the facility or are transported on public roads shall be shipped in an outer shipping container.
- The outer shipping container should be made of material adequate to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.
- Cryopreserved products with an indicated storage temperature below -80°C shall be shipped in a validated liquid nitrogen “dry shipper” that contains adequate absorbed liquid nitrogen to maintain temperature at least 48 hours beyond the expected time of arrival at the receiving facility.
- The product shall be shipped to the processing laboratory at a temperature defined in the SOP Manual.
- The transit time should be minimized. If the intended recipient has received high-dose therapy, the product shall be hand-carried by a suitably informed courier in the passenger compartment. There shall be plans for alternative transport in an emergency.
- The products should not be passed through X-Ray irradiation devices designed to detect metal objects. If inspection is necessary, the contents of the container shall be inspected by hand.

Appendix IV

8. TRANSPLANT REGISTRY FORM
STEM CELL TRANSPLANT FIRST REPORT

FORM-16(c)
Refer Rule 10(4)

Transplant Centre
Name of Physician
Recipient Name
Recipient's Age/Sex
Recipient's Father's Name
Recipient's CNIC number/Form B
Address
Donor's name (if applicable)
Donor's Age/Sex
Donor's CNIC number/Form B
Donor's Father's name
Relationship of recipient
Indication for transplant
Source of stem cells

i) Adult stem cells:
   a. Bone Marrow
   b. Blood
   c. Tissue

If source is blood or tissue please mention by what process stems are harvested.

Is this harvesting procedure	experimental	YES	NO
	accepted norm	YES	NO

ii) Cord Blood Cells:
iii) Embryonal stem cells: Derived either from blastocytes or foetal tissues

Date of Procedure_____ Date reported:_____

Signature: _____ Signature: _____

_____ _____
Head Transplant Centre Member Evaluation Committee

Name: Name:

Note: In case donor/recipient is a married woman,
Name of husband as well as father will be endorsed.

Fax/Mail to Administrator, Monitoring Authority
Transplantation of Human Organs& Tissues,
House-80, Street-23, F-10/2, Islamabad.
On the day of Transplantation on Fax: 051-0266107
followed by a copy by Courier/post.

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