

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN INDIA

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Introduction

India's first successful allogeneic bone marrow transplantation (BMT) was done on 20th March 1983 at Tata Memorial Hospital. VK (File No AP 14703) was then just a nine-year-old girl. Her disease (Acute Myeloid Leukemia) disappeared, she went on to complete her studies and currently (2002) she is enjoying a happy married life while being gainfully employed. A perfect example of cancer cure!

Our country has come a long way since then even in the field of hematopoietic stem cell transplantation (HSCT). We now have more than 15 centres that have used this potentially curative mode of therapy in upwards of 800 patients. In fact we have treated patients not only from India but also from neighboring countries like Sri Lanka, Nepal, Pakistan, Bangladesh, Africa and the Middle East.

Even with our limited insight, we appreciate today that haematopoiesis is a complex process with an elegantly simple underlying concept. A finite population of self-renewing pluripotent stem cells in the bone-marrow can multiply and generate the entire spectrum of mature blood cells including erythrocytes, platelets, granulocytes (neutrophils, eosinophils, basophils), monocytes/macrophages and lymphocytes. Pluripotential stem cells replenish themselves as well as differentiate in to multipotential progenitor cells. These subsequently irreversibly commit to specific lineage-restricted hematopoietic cells. Under the control of a variety of factors that intricately regulate the process of haematopoiesis, the daily WBC production in the normal adult reaches 3.5×10^{10} cells.

HSCT is a procedure based on a very simple principle. First a patient is identified who has a potentially lethal disease (e.g. acute myeloid leukemia or beta thalassaemia major). This disease should be such that it can be eradicated by high dose chemoradiotherapy (called conditioning). Such treatment is highly toxic and will invariably

lead to permanent destruction of hematopoietic stem cell - i.e. the body's blood forming cells. Thus the potentially curative therapy can only be used if new normal hematopoietic stem cells can be infused and made to work in the patient's body. Such normal blood stem cells are harvested from a donor [Currently there are three distinct sources of such stem cell i.e. bone marrow, peripheral blood (usually after mobilization from the bone marrow) and umbilical cord blood] and reinfused into the patient (after being given the potentially curative chemo-radiotherapy). The conditioning will destroy all normal hematopoietic marrow cells to make physical space available for the new cells to engraft as well as lead to immunosuppression to prevent graft rejection. The patient is then nursed in a protective environment till the newly infused normal marrow cells starts functioning and the patient has engrafted (which usually takes about three to four weeks). Thereafter the patient is discharged and potentially cured of his ailment!

Thus, HSCT is a unique mode of therapy in the field of oncology that

1. it is the most intense form of treatment followed by medical oncologists.
2. it exposes the entire human body to potentially life threatening consequences because this is the only hope of cure.
3. it Rts the only transplantation procedure which does not require a surgeon.

BMT FACILITIES

Upto 1995 a total of 320 centres worldwide had done more than 66,000 transplants with the longest survivor period being 22 years. Tata Memorial Hospital was the first to start BMT in India in 1983. It was also the first centre to do Umbilical Cord Blood Transplantation in 1996. Today there are many centres offering this facility (Tata Memorial Hospital, Mumbai; Christian Medical College, Vellore; Institute Rotary Center Hospital, New Delhi; Adiyar Cancer Centre, Madras; Apollo Speciality Hospital, Chennai; Tata Rural Cancer Centre, Barshi; R&R Army Hospital, New Delhi; Apollo Hospital, Hyderabad; Cancer Care Trust Hospital, Indore; Inlaks Hospital, Pune; Gujarat Cancer & Research Institute, Ahmedabad; Sanjay Gandhi Postgraduate Institute

of Medical Sciences, Lucknow; Armed Forces Medical College and Command Hospital (SC), Pune; etc).

The field of HSCT is constantly evolving. The focus is on making the procedure safer as well as more effective. Hence centres in India are continuously refining their infrastructure and techniques. For instance, at Tata Memorial Hospital, the dedicated BMT Unit has been upgraded to now having six rooms (three HEPA filtered isolation rooms with positive pressure atmosphere and three reverse barrier nursing rooms). Each room acts like a second home for the patient who is usually in the ward for two to three months. Hence they are provided facilities like central air conditioning, music system, TV with cable, telephone, exercise bicycle, monitoring with CCTV etc. The aim is to give the most sterile atmosphere as well as make the patients as comfortable as possible.

Most hospitals also have a dedicated team of well qualified and experience staff for their BMT Units. Both the nurses and doctors have received training from some of the best centres in the world and are currently capable of developing additional human resource in India by imparting state of the art training to colleagues.

TYPES OF HEMATOPOIETIC TRANSPLANTATION

The main component of bone marrow which ensures hematopoietic regeneration is the pluripotent stem cell - which has been characterised by immunophenotyping (identification of the cell surface protein/ antigen CD34+ will demarcate cells represent a heterogeneous group including the most primitive blood forming stem cell). Depending upon the donor, the transplantation is called allogeneic, autologous or syngenic. In each case, the source of these hematopoietic stem cells could be the bone marrow, peripheral blood or umbilical cord blood as mentioned above.

In allogeneic bone marrow transplant (allo BMT) the normal hematopoietic stem cells are derived from a HLA matched donor. The donor may or may not be related to the patient (matched sibling donor BMT or matched unrelated donor BMT). In syngenic BMT the donor is an HLA identical twin sibling. In autologous BMT (ABMT) the patient's own stem cells are harvested during remission and used for

hematopoietic rescue after supralethal chemoradiotherapy. The mechanism by which allo BMT is supposed to cure malignancy is by the anti cancer effect of drugs as well as by the immunological reaction between graft and cancer cells known as graft versus leukemia effect (GVL). This second mechanism is important to eradicate minimal residual disease. Evidence now clearly shows that the disease free survival may be more in patients who develop GVHD because of the accompanying GVL.

INDICATIONS

BMT has been performed in India for a large no of conditions. Common indications are shown in Table I. The five most frequent ones include CML, Thalassaemia Major, Aplastic Anemia, Multiple Myeloma and Acute Leukemias. Development of infrastructure and human resource has lead to more and more patients being given the benefit of this potentially curative mode of therapy.

Table I : Common Indications for Hematopoietic Transplantation

A) MALIGNANT

- i] Hematological
 - AML (1st remission; except APML)
 - ALL (2nd remission)
 - ALL (1st remission for high risk cases)
 - CML
 - CLL (young)
 - MDS
 - Non Hodgkin's Lymphoma (relapsed / high risk)
 - Hodgkin's disease (multiple relapsed)
 - Multiple Myeloma

- ii] non hematological
 - Breast Cancer
 - Neuroblastoma
 - Testicular Cancer
 - Ovarian Cancer
 - Intracranial neoplasms
 - Lung Cancer
 - Malignant Melanoma
 - Renal Cell Carcinoma (metastatic)

B) NON-MALIGNANT GENETIC DISEASE

Thalassaemia major
Severe combined immunodeficiency (SCID)
Sickle cell disease
Kostmann's syndrome
Chronic granulomatous disease
Chediak-Higashi syndrome
Diamond-Blackfan syndrome
Congenital aplastic anemia
Fanconi's anemia
Osteopetrosis
Hurler's syndrome
Lysosomal storage disorders
Wiskott Aldrich Syndrome

C) NONMALIGNANT ACQUIRED DISEASE

Severe Aplastic Anemia

DONOR

The primary requirement for performing allo BMT is the availability of HLA matched sibling or unrelated donor. HLA antigens are genetically determined cell surface molecules, which are responsible for development of immunological response against the foreign antigens (necessary for protecting the human beings from infections). For allo BMT, typing of Class I and II group antigens is mandatory. The HLA phenotype of an individual is determined using monoclonal antibodies (serological methods) or molecular methods like restriction fragment length polymorphism (RFLP) and sequence specific oligonucleotide probes (SSOP). These techniques are currently available at the most active HSCT centres in India.

In other countries, the establishment of voluntary bone marrow registries (not yet been established in India due to cost constraints) has made it possible to get a HLA matched unrelated donor. Thus potentially curative therapy becomes available to a larger group of eligible patients. India has taken the initiative to set up an umbilical cord blood bank at Sir Hurkosondas Hospital, Mumbai. They already have collected and cryopreserved about 4,000 collections. Tata Memorial Centre (TMC), Mumbai has pioneered the use of umbilical

cord blood for hematopoietic transplantation in India. They have devised an indigenous closed system for sterile collection of umbilical cord blood costing only a few hundred rupees. This replaces a commercially available cord blood collection bag that would cost thousands of US dollars. The technology for cryopreservations of such stem cells has also been set up, refined and standardized at TMC (controller rate freezing in liquid nitrogen as well as snap freezing at 70 °C; DMSO as cytoprotectant). This technology was also transferred to Command Hospital (SC), Pune for wider utility. Today, umbilical cord blood transplantation has been performed at four centres in India. In the future, it is also expected to have wider application.

However, the high incidence of treatment related complications and mortality with unrelated BMT must be an integral part of treatment decision making. Age is also a limitation as allo BMT is usually not recommended beyond the age of 55 years. This led to the development of the concept of ABMT. It was initially restricted to solid tumors where the bone marrow was free of disease. The autologous reinfusion of hematopoietic cells helped in rescue from the otherwise lethal myelosuppression of the potentially curative chemo-radiotherapy given from the primary tumor. ABMT has been successfully used in Ca Breast, Neuroblastoma, Germ cell tumors, Ovarian Ca., Ca Lung and even Melanoma. Subsequently this concept was also expanded to include hematological malignancies as well. This is because they also demonstrate a steep dose-response relation to anti cancer drugs. Infusion of autologous marrow (harvested during remission) allows escalation of dose intensity beyond conventional limits upto potentially curative levels. At first glance ABMT for leukemia appears to be irrational. This paradox was resolved when it was demonstrated in rat model that marrow treated in vitro can reconstitute hematopoiesis without recurrence of disease (Purging).

For hematological malignancies, the requirements for the performance of ABMT are:

1. Residual disease in the patient must be capable of being eradicated.
2. Marrow autograft should have sufficient number of normal repopulating marrow stem cells and very few malignant cells.

3. Facilities and technology for marrow processing, cryopreservation and intensive supportive care.

HEMATOPOIETIC STEM CELLS IN CIRCULATION

Presence of circulating CD 34 +ve stem cells in humans has been known since 1971. However their number is too small to be of clinical use for transplantation (being less than 0.5% in adults). With the advent of recombinant human hematopoietic growth factors, we now have a method to mobilise them (make them come out of the marrow into circulation) in sufficient number for clinical use. Initially PBSCTs were performed only in the autologous setting. Now many centres, including in India, are also performing allogeneic PBSCTs. As an alternate to mobilised PBSCH, AIIMS, New Delhi has also used unprocessed whole blood transplantation for multiple myeloma. The ideal mode of counting CD34+ve cells is by flowcytometry currently available at most transplant centres in India. About 4×10^6 CD 34+ve cells/ kg body weight of the recipient (minimum of 3×10^6 CD 34+ve cells/ kg) must be collected so that there is a good chance of success.

The advantages and disadvantages of PBSCT are shown in Table I.

Table I : Unique Characteristics of PBSCT

ADVANTAGES	DISADVANTAGES
1. No need of anaesthesia	1. Need to use growth factors
2. Out patient procedure	2. Need for apheresis system
3. Less painful	3. Theoretically increased chances of GVHD
4. Rapid recovery from myelosuppression	
5. Cost-effective	
6. More GVL effect (?)	

PRE TRANSPLANT WORKUP

At the transplant centre, he undergoes investigations to establish baseline organ function status. Tests are carried out to ensure that the patient is fit to undergo the BMT procedure and that he does not have any active infection. The blood groups of the patient and the

donor need not be the same. Patients also receive oral antibiotics and antifungal agents to reduce the risk of complications

MOBILIZATION

We commonly use either growth factors alone or a combination of chemotherapy followed by growth factors for collecting enough stem cells. Commonly used chemotherapy is mini ICE (all 3 drugs for 3 days). Growth factors are usually used after a gap of 8 days on an OPD basis in the dose of 5 to 10 ug/kg SC twice a day till peripheral blood shows adequate CD 34 +ve cells ($> 5/\text{ml}$). Usually one or two harvest sessions processing 12 to 16 litres of blood are enough to yield $> 5 \times 10^9/\text{L}$ of mononuclear cells or $> 2 \times 10^6/\text{L}$ of CD34 +ve cells. The harvested stem cells are then cryopreserved using 7.5 to 10% DMSO (final concentration) in RPMI and a liquid nitrogen or 800C freezer (which would qualify as graft manipulation). Cells stored in this manner will maintain adequate viability and can be thawed subsequently for clinical use up to 5 yrs and 6 months respectively. Care has to be taken at the time of thawing. The DMSO that acts as a cytoprotectant at very low temperatures will actually damage the stem cells at room temperature. Hence the cells must be reinfused immediately after thawing. The physical process of cryopreservation and subsequent thawing is more toxic to the malignant cells and also acts to eliminate contamination by disease cells.

IN VITRO MANIPULATION

Harvested hematopoietic stem cells can, and have been regularly, given back to the patient without any manipulation. However, selection of only some of the collected cells can help in improving the success of BMT.

Purging GVHD : One of the important causes of post transplant morbidity and mortality is graft versus host disease. The donor's T lymphocytes (present in the harvested cell) recognize the patient's body as 'foreign' and mount an immune response. Purging (removing) these T lymphocytes will reduce the risk of GVHD and the risk of life threatening complications.

Purging Residual Disease: Cells collected for transplantation can also be contaminated with residual diseased cells that remain alive and can lead to disease recurrence. This led to the developments of techniques to eradicate residual disease (leukemic) cells from the harvested cells. This can be achieved by chemotherapeutic drugs (4 OH- cyclophosphamide, mafosfamide, etoposide, etc) or exposing them to other toxins. It can also be done by positive selection of the good / desirable cells (enrich CD 34 +ve cells. The techniques available include CELLector flasks, Immuno-magnetic beads and Avidin-Biotin columns. At Tata Memorial Hospital, Mumbai, use of CD34 coated flasks (CELLector flasks) was shown to result in adequate (two to three log) removal of lymphocytes with acceptable recovery of CD34 positive stem cells. The use of immuno-magnetic beads is gaining increasing popularity. In diseases having a specific genetic abnormality (like CML), anti-sense (against bcr-c.abl chimeric mRNA) can also be used for in vitro purging.

IN VITRO EXPANSION

An important limitation of cord blood harvesting is the lack of control on the volume collected. This results in restricting its use for patients who are less than 40 kg in weight (usually children). To surmount this problem, the harvested cells can be 'grown' and expanded in the laboratory. This is still considered experimental. The success of cloning in animals has rekindled interest in expansion outside the body.

CONDITIONING

The aim of conditioning therapy is to eradicate the host clone (normal as well as malignant), to create space for host stem cells and to cause immunosuppression (so that the new infused cells are not rejected). The conditioning chemotherapy used generally depends upon the underlying disease and may consist of chemotherapy plus radiation or chemotherapy alone.

Recently the concept of non-myeloablative conditioning has been studied. It is believed that after allogeneic transplantation, the patient often has both host and donor cells surviving - to form a chimera. Whether such a patient is cured or not, depends on the efficiency of

the donor lymphocytes in getting rid of the residual diseased cells - rather than their destruction by the conditioning. If such is the case, we can reduce the intensity of conditioning (making it non myeloablative). This will reduce the complications, shorten time to hematopoietic recovery and also has the potential to decrease cost. Such a conditioning however has the possibility of increasing the risk of relapse. Our preliminary data indicates that with non myeloablative conditioning, the immediate treatment related mortality is reduced but at the cost of increased day 100 morbidity and mortality (mainly due to lung related problems).

COMPLICATIONS

The most common complications of the procedure may be due to:

1. Toxicity of chemotherapy e.g. Hepatic veno-occlusive disease, haemorrhagic cystitis, convulsions.
2. Myelosuppression e.g. infections, bleeding.
3. Immunosuppression e.g. viral, bacterial or fungal infections.
4. Graft Versus Host Disease in allogeneic transplantations only.

Infections are the most important cause of morbidity and mortality during the post transplant period. The patients are susceptible to bacterial, viral and fungal infections as well as parasitic infestations. This is because of factors like myelosuppression, immunosuppression, tissue damage, indwelling venous access devices, use of parenteral nutrients, etc. Development of graft versus host disease depresses the host immunity further and increases the chances of infections. The infections follow a set pattern in the post BMT period and can therefore be anticipated. The post BMT period is characterized by three phases viz. a) Phase of aphaasia b) Early immune recovery and c) Late immune recovery. The phase of bone marrow aphaasia lasts for 3-4 weeks and has the additional problem of disrupted mucocutaneous barriers. During this period bacterial infections are common. After the first 2 weeks, incidence of fungal infections is also high. During the phase of early immune recovery, which lasts for 3-4 months, the blood counts are normal. At this time the patient is susceptible to infection with CMV and fungi like aspergillus and candida. The phase of late immune recovery starts 6 months after

the marrow reinfusion and may not be complete for 1-2 years. Development of chronic GVHD during this period significantly delays the immune recovery. Here reactivation of varicella zoster, infection with capsulated bacteria like *S.pneumoniae*, *Neisseria*, *H.influenzae* and mucocutaneous candidiasis are commonly encountered. In order to reduce the incidence of infections, prophylactic use of antibacterial, antifungal and antiviral agents has been tried. Isolation of patient in HEPA filtered rooms with laminar airflow and reverse barrier nursing helps in preventing acquisition of new pathogens.

Graft versus host disease is an immunologically mediated reaction. It occurs in two forms: acute and chronic. Acute GVHD generally develops within the first 4 to 5 weeks post transplant. It generally affects gut, liver and skin. The incidence and severity of acute GVHD is directly related to the degree of HLA mismatch. The overall incidence of this complication in a HLA matched sibling donor transplant is 40% with 10-15% patients developing severe acute GVHD. It is one of the major causes of post transplant mortality in the first 100 days. Acute GVHD prophylaxis consists of combination of cyclosporine with methyl prednisolone and / or Methotrexate This reduces the incidence of severe and life threatening acute GVHD. Additional measures include its treatment include ALG, monoclonal antibodies and thalidomide.

Chronic GVHD resembles an autoimmune disorder. It is characterized by involvement of skin, gut, musculoskeletal system, eyes, liver and oesophagus. Fibrosis and sclerosis of involved tissues is a characteristic feature. Treatment is with steroids and cyclosporin. Thalidomide has been tried and found to be effective in preventing the recurrences of the disease. Other measures used include UV radiation and phototherapy.

GENE THERAPY

In the last 12 years there have been more than 1300 publications in gene therapy. IN the BMT Setting, gene therapy can be used for gene replacement, gene addition or gene transfer. In the first one the missing gene is replaced (e.g. beta thalassaemia, SCID, Fanconi's anemia). In gene addition the whole emphasis is on normal cells where a new gene is added to confer a survival advantage (Darwin's theory).

For example the hematopoietic cells can be made more resistant to the therapeutic consequences of myelosuppression. Gene transfer is used for several indications like repair of mutations. For instance replacing mutated p53 with the normal functioning counterpart in lung cancer leads to a higher response rate (mechanisms involved in this and other potential beneficial effects include increased tumor sensitivity, host sensitivity and host response to therapy as well as gene marking).

Certain drugs need activation by enzymes before they become active. The classical examples are gancyclovir, which requires phosphorylation with thymidine kinase. Cells that are transfected with genes for the corresponding enzymes will specifically become susceptible to the toxic effects of these drugs and can be targeted *in vivo* for selective elimination. In the hematopoietic transplantation setting its immediate application would be in the use of donor lymphocyte transfusions. Such DLT are commonly used for cytogenetic or clinical relapse following allogeneic BMT for its graft v/s leukemia effect. However it has the potential to cause life threatening GVHD and aplastic anemia. Such donor lymphocytes can be transfected with the thymidine kinase gene. Thereafter if they result in unacceptable toxicity, they can be eliminated by treating the patient with gancyclovir.

Another important role of gene therapy is in conferring resistant to the side effects of potentially curative therapy (chemo or radiation). This can be done using *pgp* (MDR 1).

Currently clinical trials using gene therapy and hematopoietic transplantation are ongoing in the following:

1. Gene marking (no therapeutic benefit to patient)
2. ADA SCID
3. X SCID
4. Chronic Granulomatous Disease
5. Goucher's Disease
6. Fanconi's Anemia
7. MDR, MGMT, DHFR, HIV-1

COST

BMT is an expensive mode of therapy upto Rs. 10 to 12 lakhs. With the current emphasis on cost effectiveness, it is important to know how results can be improved by the judicious application of new/modified techniques. Tata Memorial Hospital, Mumbai has devised a Transplantation Events and Activity Template that addresses this issue. Events included hospitalization, fever, hypotension, etc and activities included conditioning, ward rounds, blood collection, etc. All these were evaluated for the time spend, consumables utilized and their cost. Taking the data from patients undergoing autologous transplantation for acute myeloid leukemia (AML), the median (and range) cost of transplant was calculated. Impact of alterations in the treatment plan (example use of growth factors) can be extrapolated onto the template and predict changes in cost. It has been shown that such an approach has shown saving of 10 to 20 % of the cost by using growth factors to hasten hematopoietic reconstitution or for harvesting stem cells from the peripheral blood. This approach is independent of geographical borders the template having been utilized for data from both developing (India) as well as developed (UK) countries.

FUTURE

Since the humble beginning in 1983, facilities for HSCT have expanded well in India. The BMT Unit at Christian Medical College, Vellore has also been designated as center of excellence by ICMR. However, the current national capacity is suboptimal to meet the needs for India's population (with an estimated incidence of 8,00,000 new cancer patients/year).

Existing centres need to focus on the development of regimen/strategies tailored to our needs. For instance, cancers like CLL and MM are diagnosed very early in our population. They are in the most productive phase of their lives and are fit to tolerate dose intensive therapy. Hence they should be subject to strategies that lead to a curative outcome.

State of the art techniques like in vitro manipulation for positive selection of CD 34+ve cells will reduce the incidence of GVHD as well as tumor cell contamination. The untapped potential of umbilical cord blood awaits systematic evaluation and utilization.

Hematopoietic stem cell transplantation is by no means an ideal mode of therapy. It is currently the only available curative mode of treatment for several oncological ailments. However, in the future, Gene therapy and stem cell research is expected to change the way we cure patients completely changing the application of HSCT.