

# STEM CELL TRANSPLANTATION IN PEDIATRICS

**Tulika Seth, Sujata Mohanty**

Dept of Hematology, BRA-IRCH first floor, Stem Cell Facility, 1<sup>st</sup> Floor ORBO Complex, AIIMS, New Delhi 110029, India

**Abstract :** Pediatric diseases are unique in that there is a preponderance of genetic and metabolic conditions which afflict this age group. There are aggressive malignancies not amenable to or which have failed conventional therapy. Stem cell therapy technology has evolved to make treatment possible for these difficult disorders. Stem cell treatment can be broadly divided into two groups, Hematopoietic stem transplantation (HSCT) and Stem cell therapy. Bone marrow- or peripheral blood-derived allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling or matched unrelated donor can cure children with thalassemia major, severe aplastic anemia and leukemias. There are several centers which perform HSCT in India, they have been performed for both malignant and non malignant diseases. Post transplant complications occur in both the acute setting with acute graft versus host disease, sinusoidal obstructive syndrome and infections causing early mortality and morbidity. Late complications include chronic graft versus host disease and infections as well as the sequelae of treatment. Advancements in understanding transplant biology and the newer immunosuppressive drugs, have helped to reduce and treat graft versus host disease, routine prophylaxis, better early diagnostic markers for viral infections and immunizations have helped in prompt identification, treatment and prevention of infections. Better chimerism techniques have enhanced post transplant monitoring for early identification of relapse and rejection so that intervention can be delivered. For diseases like thalassemia major and aplastic anemia if transplants are planned, the patient and donor workup needs to be performed early as the transplant outcome is better prior to receiving multiple transfusions. Stem cell therapy is the forefront of regenerative medicine and has been utilized for degenerative disorders. Newer applications of stem cell therapy in congenital and developmental pediatric diseases holds promise for many incurable disorders.

## INTRODUCTION

Pediatric diseases differ from adult conditions in that many genetic and metabolic conditions which are either incompatible with a normal lifespan or cause serious developmental sequelae afflict children. Certain aggressive malignancies also occur only in young children and adolescents; these are frequently not amenable to or have failed conventional therapy. These difficult hitherto untreatable, life threatening conditions have led to the identification of a field of treatment using stem cell therapy. The concept of stem cell therapy has been with us for sometime, now the technology has evolved to make treatment possible for these and many other pediatric disorders. Stem cell treatment can be broadly divided into *two groups*. (1.) **Hematopoietic stem transplantation:** the biology, indications, dosage and complications have been well defined. (2.) **Stem cell therapy:** which is a promising, evolving new modality and the last frontier of a new field of medicine. This is however not yet fully elucidated. We will be discussing these two entities separately in this article.

## HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

Hematopoietic stem cell transplantation is a standard procedure in which progenitor cells that have the capacity to reconstitute normal bone marrow function, are transplanted into the patient. This procedure may utilize hematopoietic stem cells from the same patient or from a donor. Hematopoietic stem cell transplantation was started more than 50 years ago. The first successful hematopoietic stem cell transplants were conducted in the 1960s for primary immunodeficiency diseases and leukemia. These early attempts suffered from high morbidity and mortality. This was due to toxicity related to the conditioning (or preparative) regimen given for the bone marrow transplantation, post transplant infectious complications and a complication unique to HSCT, due to immunological effects of the donor cells on the host body called graft versus-host-disease (GVHD).

Subsequent advances in transplant clinical care and transplant biology

have focused on improvement of conditioning regimens that are tailor made for optimum results in different diseases. The standardization of prophylaxis and awareness of common infections and an understanding of the immune mechanisms associated with the adverse effects (GVHD) and the antitumoral effects of the engrafted hematopoietic stem cells have all led to reduction in transplant mortality and morbidity.

### Sources of stem cells

First, a source of stem cells must be identified for the patient, the sources of hematopoietic stem cells for transplantation are conventional bone marrow, cytokine mobilized peripheral blood stem cells (PBST) and umbilical cord blood hematopoietic stem cells. CD34 (+) is a cell surface marker on hematopoietic stem cells which is used to identify and enumerate these cells. The decision about choice of stem cell origin for the patient is based on multiple factors such as host disease, donor characteristics and most importantly HLA match. Each of these sources of cells has specific advantages and disadvantages, which are taken into account for making the selection (Table 1).

**Table 1:** Summary of the characteristics of hematopoietic sources - bone marrow, peripheral blood stem cells and umbilical cord blood.

Property	Bone marrow	Peripheral blood stem cells	Umbilical cord blood
Collection of stem cells	Needs general anesthesia for donor	Needs granulocyte colony stimulating factor for donor	Collection at time of delivery
HLA matching	HLA match class I and Class II	HLA match class I and Class II	Mismatch of 1-2 HLA-A,B-DR
Engraftment time	14- 18 days	9-11 days	20 days
Risk of acute GVHD	Equal to peripheral blood	Equal to bone marrow	Least
Risk of chronic GVHD	Lower than peripheral blood	Highest	Least
Immunotherapy	Feasible	Feasible	Not feasible
Time to unrelated donor identification, donor attrition donor	Unrelated donor search, requires database, funds and time for search. Donors may refuse	Unrelated donor search, requires database, funds and time for search. Donors may refuse	Cord blood units stored and typed. But small units, expense and logistics of starting bank

GVHD= graft versus host disease

HLA = human leucocyte antigen

### Types of hematopoietic stem cell transplantation

The major types of hematopoietic stem cell transplantation (HSCT) are classified by the source of progenitor cells used in the transplant. Stem cells from the host are called autologous stem cells. *Autologous stem*

cells are usually peripheral blood stem cells, but may be bone marrow cells, stem cells from a donor are allogeneic stem cells, these may be peripheral blood, bone marrow or donated umbilical cord blood. Allogeneic stem cell transplantation has been made possible by the identification and study of Histocompatibility antigens the Human Leukocyte Antigen system. These are antigens which are expressed on the surface of most cells in the body including leucocytes. These antigens are also known as the major histocompatibility complex (MHC) and occupy the short arm of chromosome 6. This genetic region has been divided into chromosomal classes which are matched to find the most closely matched or best suited donor. Class I and II matching are prerequisites to selecting an allogeneic donor. Class I region is made up of *HLA-A*, *HLA-B* and *HLA-C* and Class II is made up of *HLA-DR*, *HLA-DP* and *HLA-DQ* genes. Traditionally, the critical loci for HLA matching are *HLA-A*, *B*, and *DR*. The roles of *HLA-C* and *HLA-DQ* have recently gained importance and are now also being considered while selecting a donor. HLA typing is performed by serology and molecular techniques, this is a rapidly expanding field, with identification of mismatches which are acceptable and those that may result in later severe complications such as graft versus host disease or graft rejection. Killer cell immunoglobulin-like receptor (KIR) typing is now being performed and correlated with GVHD, to study the role of alloreactive natural killer cells. A completely matched sibling donor is considered the ideal donor. However every patient does not have an HLA matched sibling, hence unrelated donor transplant in which the donor is completely matched or has a single mismatch can be performed. In certain circumstances e.g. relapsed leukemia, a greater donor mismatch is tolerated and may even be beneficial.

Another source of hematopoietic stem cells are those collected from the umbilical cord blood. The use of cord blood transplantation has rapidly increased because of ease of collection, prompt availability, absence of donation risk, less GVHD due to increased tolerance to HLA-mismatch<sup>1</sup>. Cord blood banking has become important, these cord blood units are HLA typed and stem cells are quantified. They are available to prospective patients. Use of cord blood as a source is constrained by the quantity of cells available in each cord blood unit. The use of multiple cord blood transplants, in which multiple cord blood units from different donors has expanded their use to larger sized patients and has shortened the time to engraftment<sup>2</sup>. However cord blood banks that store the cord blood only for the donors own use in the future, have limited utility<sup>3</sup>.

*Patients without an HLA matched sibling can try for an unrelated donor search in the voluntary bone marrow donor registries.* However for patients belonging to ethnic minorities, particularly Asians the probability of finding an HLA-matched donor is remote, due to their unique HLA alleles. The time taken to search and donor attrition (refusals, inability to donate etc.) have added further hurdles to expanding this process in India. *Most Indian patients cannot afford the cost of an unrelated marrow search, or develop serious complications before transplantation due to the prolonged time taken to complete such a search.*

The transplant team selects the stem cell source based on availability, dose of stem cells needed for the patient, disease, benefit of graft versus leukemia effect and degree of HLA mismatch and need for rapid engraftment of blood cells. If more than one donor is available then the most compatible donor is chosen by evaluating donor sex, cytomegalovirus (CMV) status, degree of blood group mismatch etc.

Autologous transplantation is a technique of giving high dose chemotherapy in which the hematopoietic system is rescued by returning the patient's own stem cells. This is most useful in chemosensitive

hematopoietic and solid malignancies where the higher than conventional dose therapy is able to eliminate the residual malignant disease, and prevent severe toxicity to the patient who would without the rescue have suffered profound ablation of the bone marrow. The host's peripheral blood stem cells must be collected after conventional treatment to control the disease and mobilized by cytokine granulocyte colony stimulating factors. Every effort to ensure that the stem cell product is free of tumor is done since the major complication after autologous transplant is relapse of the original disease. This procedure does not have the immunological complications seen with allogeneic transplants and immunosuppression is not required as the reconstituted immune system is that of the original host.

### Indications

Pediatric hematopoietic stem cell transplants are performed for *malignant and non malignant diseases*. Common malignant diseases for which transplant is indicated are *advanced solid tumors* and *high risk leukemia* patients when a HLA matched sibling donor is available e.g. acute myeloid leukemia, Philadelphia positive Acute Lymphoblastic leukemia (ALL), Juvenile myelomonocytic leukemia (JMML), Infants acute lymphoblastic leukemia (ALL) with 11q23 rearrangement and chronic myeloid leukemia (CML) in children. Patients who have graft versus host disease (GVHD) experience improved relapse-free survival. Children with acute promyelocytic anemia t(15;17), and those with inv(16) and t(8;21) fare well with chemotherapy these patients are not treated with up-front transplant regimens<sup>4,6</sup>. Even in CML and Ph positive ALL with better chemotherapy and newer agents like imatinib and dasatinib some children may do well even without transplant.

Other children for whom hematopoietic stem cell transplantation may be a good option include those who have experienced induction failure or early relapse within 18 months of diagnosis<sup>7</sup>. In children with relapsed leukemias, the response rates to transplant vary with time to relapse, response to the relapse chemotherapy protocol and type of relapse (see Table 2a and b).

**Table 2 a** Indications for Autologous transplantation in pediatric patients

Malignant disorders	Non malignant disorders
Neuroblastoma	Trials for collagen vascular and severe autoimmune disorders
Relapsed lymphomas	
Germ cell tumors	
Certain indications for-	
Chronic myeloid leukemia,	
Acute myeloid leukemia	
Acute lymphoblastic leukemia	
In clinical trials for advanced malignancies e.g. Brain tumors, other pediatric solid tumors.	

**Table 2b** Indications for Allogeneic hematopoietic transplantation in Pediatric patients

Malignant Conditions	Non malignant conditions
Acute myeloid leukemia	Hemoglobinopathy- e.g. Thalassemias , sickle cell disease
Acute lymphoblastic leukemia	Aplastic anemia, Fanconi anemia
Infant Acute lymphoblastic leukemia	Storage disorders
Philadelphia positive Acute lymphoblastic leukemia	e.g. Adrenoleukodystrophy
Juvenile chronic myelo monocytic leukemia	Hurler syndrome
Myelodysplastic and myeloproliferative disorders e.g. Chronic Myeloid Leukemia	Krabbe disease
In clinical trials for advanced malignancies-	Primary Immunodeficiency diseases
e.g. Relapsed lymphomas.	Macrophage and granulocyte disorders
	Kostmans, hemophagocytic syndromes
	Osteopetrosis

Transplantation for *non malignant conditions* are performed for *hemoglobinopathies* e.g. *thalassemia major*; *aplastic anemia* and a variety of immunodeficiencies and genetic disorders. Since the transplants entail correction of gene defect or supply of absent hematopoietic stem cells/ substrate only allogeneic transplants can be performed for these conditions (see table 2b). Children who have received multiple transfusions (Thalassemia major and aplastic anemia) are at risk for rejection and other serious complications of transplant. Hence early referral for matched sibling related transplant is of benefit. In children with immunodeficiencies and genetic disorders the conditions needs to be identified promptly and the children transplanted from a healthy sibling or by using maternal haplo-identical stem cells to save the life of the child and prevent serious morbidity. Severe auto immune diseases such as systemic lupus erythematosus have also been successfully auto-transplanted.

**Transplantation Procedure**

**a) Conditioning**

The initial step for transplantation of hematopoietic stem cells involves selection of the conditioning regimen. This is the name given to the combination of chemotherapy, immunosuppressants, radiation therapy or radio labeled monoclonal antibodies given prior to infusion of the hematopoietic stem cells. The role of conditioning is to make space in the bone marrow for the new stem cells, remove the pathologic cells from the host and provide immune suppression to prevent rejection and graft versus host disease. This conditioning varies in the drugs chosen, doses of chemotherapy and inclusion or exclusion of radiotherapy dependent on the disease entity for which transplantation is being performed.

**b) Collection of stem cells**

- (i) *Harvesting bone marrow*: Stem cells are obtained from the bone marrow by repeated aspirations of the posterior iliac crests of the donor under general anesthesia. Collection of the required adequate cell dose can be a difficult procedure in small sibling donors. The collections can be of unprimed bone marrow or primed with granulocyte colony stimulating factors (G-CSF).
- (ii) *Peripheral blood stem cell collection*: The bone marrow stem cells can be mobilized into the peripheral blood and collected via leukocytapheresis. Along with increasing the number of cells, G-CSF also causes the release of proteases that degrade the proteins that anchor the stem cells to the marrow stroma, causing their release into the peripheral blood. Autologous stem cells are collected post recovery after a cycle of chemotherapy with hematopoietic growth factors like G-CSF. In allogeneic transplant the healthy matched donor is given 4-5 days of granulocyte colony stimulating factor (G-CSF) 10 µg/kg/day and stem cells are collected by apheresis technique on a cell separator machine. Peripheral blood stem cells can be cryopreserved for later infusion or given to the patient on the same day. Peripheral blood stem cells have more T cells than bone marrow and consequently there is an increased risk of chronic GVHD. Peripheral blood stem cells speedily engraft compared to other stem cell sources. However it is technically difficult to collect peripheral stem cells from small children
- (iii) *Umbilical cord blood*: Collection of Umbilical cord blood (UCB) is performed at the time of delivery, usually after delivery of the placenta. It must be performed after cleaning the cord, using aseptic technique a needle is inserted into the umbilical cord and blood is withdrawn. Manipulating the placenta to increase the yield is contraindicated as it may lead to contamination with maternal blood.

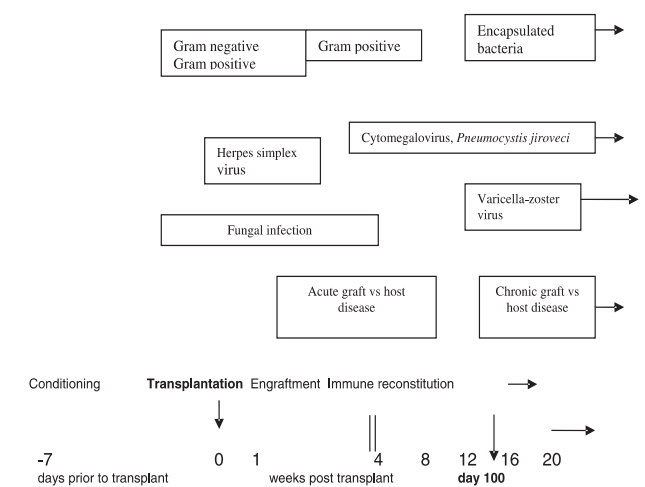
**c) Infusion of stem cells**

The mononuclear cell count and CD34(+) count in the peripheral blood determines the timing of collection. After collection flow cytometric enumeration of cells is performed. Although the minimum number required for engraftment is considered to be  $1 \times 10^6$  cells per kilogram of body weight, the preferred number is  $2-2.5 \times 10^6$  cells/kg or more. The stem cell infusion is given like a blood transfusion with monitoring and hydration. The CD34 and stem cells home to the bone marrow. The engraftment of hematopoietic cells occurs within 8-20 days depending on stem cell source and dose (table 1).

**COMPLICATIONS**

Hematopoietic stem cell transplantation (HSCT) related complications are classified into early and late depending on their timing. Early complications occur in the first 100 days of transplant. Usually post 100 days the patient if stable, is discharged from the hospital and continues on immuno-suppression for six months to one year (table 3).

**Table 3** Timeline of hematopoietic stem cell transplantation and common complications and infections post transplantation



**Early complications**

- a) *Acute graft versus host disease (aGVHD)* is a common complication of allogeneic transplantation and occurs within the first 100 days after the procedure. It is an immune response of donor T lymphocytes against host cells. The skin, gastrointestinal tract, and liver are the target organs involved<sup>8</sup>. Severe acute (grade IV) graft versus host disease is a life threatening disease. Every protocol usually uses cyclosporine and methotrexate for prophylaxis, for treatment of graft versus host disease corticosteroids, tacrolimus, mycophenolate mofetil (MMF) and several newer monoclonal antibodies have been used. The severity of GVHD is inversely related to the risk of relapse and strategies to reduce GVHD may increase relapse rates. New strategies are being developed to separate these effects and to decrease the incidence and severity of GVHD without increasing the risk of relapse.
- b) *Mucositis* is one of the most common adverse effects of transplantation, it is due to the conditioning regimen and varies in degree with drugs used and prior host related factors. This may be mild or severe enough to prevent oral intake, compromise nutrition and cause pain and bleeding. It is usually managed

symptomatically with narcotics and topical anesthetics.

- c) *Hemorrhagic cystitis* is a disorder that manifests as dysuria and hematuria. Hematuria may be microscopic or may be gross. This disorder usually occurs during the immediate post transplant period but may occur later. Cyclophosphamide and other drugs have been implicated and bladder protectant (MESNA) and hyperhydration are routinely used to reduce this complication. Late onset hemorrhage is associated with infections such as adenovirus or BK virus.
- d) *Sinusoidal obstructive syndrome (SOS)* is a potentially fatal syndrome of tender hepatomegaly, direct hyperbilirubinemia, ascites, and weight gain. SOS is caused by damage to the sinusoidal endothelium, which results in sinusoidal obstruction. This entity occurs within the first 20 days of hematopoietic stem cell transplantation, and preexisting liver disease and genetic polymorphisms which alter drug metabolism may increase its risk. SOS has an overall mortality rate of as much as 50%. No standard effective therapy is currently available. Defibrotide is an investigational agent which has shown favorable response rates.
- e) *Transplantation related infections* Life-threatening bacterial, fungal, and viral infections are common in patients undergoing hematopoietic stem cell transplantation. The patients are at risk due to prolonged neutropenia, use of immunosuppressants e.g. cyclosporine, steroids etc. and immunodeficiency associated with GVHD. Bacterial sepsis occurs early in the course of transplantation, whereas viral infections by cytomegalovirus and other viruses usually occur after engraftment. Fungal infections may occur after 7-10 days of onset of neutropenia and the patient is at risk till engraftment. Early recognition and prompt treatment are essential, prophylaxis for common infections is routinely given till immunosuppression is ongoing, this becomes prolonged in patients being treated for chronic graft versus host disease.
- f) *Graft rejection* is a serious complication, this occurs more commonly in transplantation for non-hematological disorders and multi-transfused allo-immunized patients. Chimerism monitoring is required to monitor for stable engraftment and identify graft rejection. Short tandem repeats/variable number tandem repeats (STRs/VNTRs) are the best tool for chimerism monitoring.

### **Late Effects**

- a) *Chronic graft versus host disease* is characterized by an auto-immune phenomenon like that seen in scleroderma, Sjögren syndrome and lupus. This results from thymic injury during conditioning, resulting in loss of negative selection of autoreactive T cells, and the alloreactivity of mature post thymic donor T lymphocytes. Immunosuppression with drugs like corticosteroids, tacrolimus, and mycophenolate mofetil are the mainstay of treatment.
- b) *Endocrine effects* in children such as impaired growth and hypothyroidism may be found.
- c) *Pulmonary effects* include restrictive and chronic obstructive lung disease. Conditioning regimens, infections, and GVHD are important risk factors. Bronchiolitis obliterans is a specific form of obstructive lung disease seen in hematopoietic stem cell transplant recipients and this has a fatality rate of 50%.
- d) *Neurocognitive effects*-Lower intelligence quotient (IQ) scores, fatigue, memory problems and even developmental delay have

been reported, these occur frequently in children who have received cranial radiation either as part of their oncological therapy or as part of their hematopoietic stem cell transplantation conditioning.

- e) *Immune effects*-Host immunity is suppressed for months to years after hematopoietic stem cell transplantation. This is due to severe neutopenia and lymphopenia due to the myeloablative conditioning, acute GVHD that further suppresses host immunity, and the use of immuno-suppressants to prevent or treat GVHD. These immune effects should be considered because these patients are prone to serious infections long after the completion of the stem cell transplant. There is consensus that re-vaccination is required to boost vaccine-acquired immunity. The repeat vaccination is required approximately one year after allogeneic stem cell transplantation, if there is no active chronic GVHD and the patient is off immunosuppressive therapy for at least 6 months. Live vaccines should never be given prior to one year post cessation of immuno-suppressants. Early vaccinations may not result in an appropriate immune response.

### **BONE MARROW TRANSPLANT SCENARIO IN INDIA**

The initial HSCT were performed in India in Tata Hospital, Mumbai and Christian Medical College (CMC) Vellore, with this initial success, several other centers around the country now have facilities for performing hematopoietic stem cell transplantation. Indian data for all pediatric transplants is unavailable but a total of approximately 1540 HSCT have been performed in adult and pediatric patients. The maximal allogeneic transplant experience is at CMC Vellore<sup>9</sup>.

At our center, Department of Hematology AIIMS we have performed allogeneic transplants for a variety of conditions, predominantly for aplastic anemia, leukemia, thalassemia major and myelodysplastic syndrome in adults and children. The HSCT have been performed safely with excellent outcomes. We did not experience any 30-day mortality and 100-day mortality was 2.5%. We have ascertained the safety of performing allogeneic HSCT in single. non-high efficiency particulate air (HEPA) filter rooms<sup>10,11</sup>. We have performed 20 pediatric HLA matched sibling transplants -Aplastic anemia 10, Thalassemia major 6, E Beta Thalassemia 1, relapsed Acute lymphoblastic leukemia 2, Chronic myeloid leukemia-1. Median age 9.5 years (1-17 years), male: female 16:4. Median time till neutrophil engraftment was 10 days (range 8-21). Day 100 mortality was nil, Kaplan Meier survival 90% with follow up ranging 4-54 months (unpublished data). Deaths occurred in 3 patients-child with aplastic anemia transplanted with fungal sinusitis died of chronic graft versus host disease and fungal sepsis at ten months, another with aplastic anemia suffered graft rejection and meningitis, the child of relapsed acute lymphoblastic leukemia suffered a second relapse one year later. Our infection risks are similar to those reported from other centers<sup>12</sup>.

### **CONCLUSION**

A major impediment to hematopoietic stem cell transplantation (HSCT) is the availability of a genetically matched donor. Smaller family size limits the options of sibling donors and because the Indian population has the presence of novel HLA alleles and unique haplotypes (HLA-A\*0211, B\*2707, A\*26-B\*08-DRB1\*03), many Indians who have these rare HLA alleles will find difficulty finding an unrelated optimally matched donor from foreign bone marrow registries. These limitations can be overcome by developing unrelated

volunteer marrow donor registries in India<sup>13</sup>. Also by improving technology and establishing the facilities required to perform matched and unrelated transplants we will be able to increase the number of children who can benefit from this potentially life saving procedure<sup>9,14</sup>.

## 2. STEM CELL THERAPY

Stem cell biology is currently one of the most exciting areas of biochemical research having the potential to provide therapeutic treatment for developmental and degenerative disorders. Advances in stem cell biology and the discovery of pluripotent stem cells have made the prospect of cell therapy and tissue regeneration a clinical reality. Cell therapies hold great promise to repair, restore, replace or regenerate affected organs<sup>15</sup>.

Although **embryonic stem cells** isolated from the inner cell mass (ICM) of the blastocyst or fetal gonadal tissue are the 'ultimate' stem cell, ethical issues and the potential danger of teratoma formation have limited the development of these cells as therapeutic options. However, hematopoietic adult stem cells have been used therapeutically for many years in malignant hematological diseases and are currently the best characterized stem cells. Therefore these adult stem cells are now at the forefront of therapeutic research and currently the most widely used cells in clinical trials. Below are details of promising clinical work being done presently at our center and other leading hospitals in India.

### **Congenital malformations**

Stem cells with the potential to transform into healthy cells and repair damaged cells may prove beneficial in various congenital malformations. Stem cells are being tried in selected congenital malformations for which the present treatment options are either limited or not available when the irreversible changes have already taken place at an early stage. These include extra hepatic biliary atresia (EHBA) and spina bifida with neurological deficiency.

Current work at AIIMS is being done in the field of EHBA, this is a condition in which outflow biliary channels from the liver are congenitally blocked, thus predisposing to cirrhotic liver and hepatocellular failure. The goal of stem cell therapy is to repair damaged tissue that has lost the property to heal itself. This can be accomplished by transplanting stem cells into the damaged area and directing them to grow new and healthy tissue. Autologous bone marrow stem cells are a type of adult stem cells, a multipotent unit still capable of differentiating into specialized cells. In this study, histopathology could be done at 6 months after the stem cell infusion, in the three patients who were alive with adequate follow-up. A comparative improvement in fibrosis was seen in all patients. Stem cells were used in spina bifida with neurological deficiency with an aim to repair the damaged neurons and improve the existing neurological deficits. Recovery of central nervous system disorders is hindered by the limited ability to regenerate lost cells, replace damaged myelin, and re-establish functional neural connections. Both hematopoietic stem cells and marrow stromal cells have been shown to have the potential to restore the injured spinal cord and promote functional recovery in mice<sup>16,17</sup>. This positive effect was most pronounced using mesenchymal stem cells. Progressive complete functional motor recovery with evident nervous tissue regeneration has been achieved following administration of bone marrow stromal cells in traumatic central spinal cord cavities of adult rats with chronic paraplegia due to a previous injury to the spinal cord. In our experience with favorable responses in 50% of cases, it is hoped that stem cells may prove

beneficial to improve the neurological deficits associated with cases of spina bifida. To conclude, stem cell therapy may prove beneficial in cases of liver cirrhosis due to congenital anomalies by reversing or delaying the onset of end-stage liver disease and decreasing the need for liver transplantation and immuno-suppression. The initial use of stem cells in meningocele has shown promising results. However, long term evaluation with randomized controlled trials is essential to draw conclusions. Major concerns remain: what prompts stem cells to assume specific functions and which factors would dictate them to stop multiplication once the aim is achieved.

### **Pediatric leukodystrophies**

This group comprises of diseases that manifest in childhood with deficiencies in myelin production or maintenance; these may be due to hereditary defects in one or more genes critical to the initiation of myelination, as in Pelizaeus-Merzbacher Disease, or to enzymatic deficiencies with aberrant substrate accumulation-related dysfunction, as in the lysosomal storage disorders. In the light of the wide range of disorders to which congenital hypomyelination and/or postnatal demyelination may contribute, and the relative homogeneity of central oligodendrocytes and their progenitors, the pediatric leukodystrophies are attractive targets for cell-based therapeutic strategies. As a result, glial progenitor cells (GPCs), which can give rise to new myelinogenic oligodendrocytes, have become the focus of great interest as potential therapeutic vectors for the restoration of myelin to the hypomyelinated or dysmyelinated childhood central nervous system. In addition, by distributing themselves throughout the deficient host neuraxis after perinatal allograft, and giving rise to astrocytes as well as oligodendrocytes, the glial progenitors appear to have great potential for rectifying enzymatic deficiencies<sup>18</sup>.

### **Multicystic dysplastic kidneys**

The incidence of embryological development disorders of the kidney and urinary tract varies from 0.3–0.8% in live born infants. Embryologically, when the ureteric bud makes contact with the metanephrogenic mesenchyme, a series of inductive signals are exchanged. As the uretric bud grows and branches, its tips contact fresh stem cells and induce them into the nephrogenic pathway. There is therefore a gradient of developmental age in a fetal kidney with the outermost cortex composed of stem cells that are not yet committed to differentiation, and the inner most region contains maturing nephrons and supporting stromal cells. Thus multicystic kidney disease (MCKD) results from incomplete development of the kidney. Recruitment of stem cells to the kidney to elicit repair and the function of dedifferentiation of resident renal cells has been studied and recognized. Stem cells whether recruited to the kidney from a distant organ or delivered to the kidney after *ex vivo* expansion of an isolated stem cell population, may contribute to repair via the production of specific cytokines, chemokines or growth factors, transdifferentiation into specific renal cell types or by cell fusion. Reports have suggested that bone marrow derived stem cells in the kidney can transdifferentiate into tubular epithelial cells, mesangial cells, glomerular endothelial cells and even podocytes. However, the lineage of bone marrow derived cells that appear in the kidney in response to damage is unclear, and their ability to elicit transdifferentiation is controversial because the possibility of cell fusion has not been eliminated. Some authors have concluded that though recruitment of bone marrow derived cells does occur, the repair of renal tissue is predominantly elicited via proliferation of endogenous renal cells<sup>19,21</sup>.

### Ocular surface disorders

The ocular surface comprises of the cornea, the conjunctiva and the limbus which is the transitional zone between the two. The cornea is the transparent tissue which consists of epithelium, stroma and endothelium. The main function of the epithelium is to provide a smooth and transparent surface.

Corneal limbus is known to be the source of corneal epithelial stem cells that are important as a regenerative source for epithelial cells. Limbal stem cells may become partially or totally depleted, resulting in varying degrees of stem cell deficiency with resulting abnormalities in the corneal surface. Limbal stem cell deficiency (LSCD) of any cause may lead to poor corneal epithelialization, persistent epithelial defects, corneal vascularization, corneal scarring, and so-called conjunctivalization of the cornea. These problems lead to decreased vision, ocular discomfort, pain and an unstable ocular surface.

Though there has been a significant improvement in the management of LSCD over the last 10-15 years, with penetrating keratoplasty [PK] and the use of artificial tears, patients with LSCD continue to have a poor prognosis. Now with improved microsurgical techniques and an understanding of the role of limbal stem cells and improvement in immunosuppressive therapies the treatment and the outcome of LSCD has improved. Partial stem cell deficiency can be managed by removing abnormal epithelium along with the transplantation of human amniotic membrane, to resurface cells derived from the remaining intact limbal epithelium. The same can also be combined with amniotic membrane transplantation (AMT).

In case of total stem-cell deficiency, autologous limbus from the opposite normal eye or homologous limbus from living related or cadaveric donors can be transplanted (directly or after *in vitro* expansion) to the affected eye. Transplanted limbal cells will require long-term systemic immunosuppression. At AIIMS we have performed over 35 cases in the pediatric age group and LVPEI, Hyderabad has conducted more than 600 cases in both adults and children. Significant improvement in visual acuity and ocular surface stability has been noticed in patients who have received cultured limbal stem cells along with amniotic membrane<sup>22,23</sup>.

### Muscular Dystrophy

Muscular dystrophy (MD) refers to a group of genetic, hereditary muscle diseases that cause progressive muscle weakness. Muscular dystrophies are characterized by progressive skeletal muscle weakness, defects in muscle proteins and the death of muscle cells and tissue. The best known type of MD is Duchenne muscular dystrophy (DMD). Duchenne muscular dystrophy is inherited in an X-linked recessive pattern, it is caused by mutation of the gene for the dystrophin protein and characterized by an elevated serum creatinine kinase and progressive muscle weakness starting in childhood and progressing to respiratory muscle failure and death. There is no treatment for this disorder.

Stem cell therapy has been tried for this fatal condition, one approach has been transplantation of muscle precursor cells (myoblasts) which can produce dystrophin protein. However, clinical trials have revealed that the transplanted human myoblasts are rapidly lost. Another very promising approach is to genetically modify the stem cells and to upregulate the dystrophin protein. This strategy introduces U7 RNA to induce exon 51 skipping. Skipping this exon places the mRNA back in frame so that a truncated, but functional, dystrophin protein missing only exon 51 is transcribed.

*Additional research is needed to be performed to substantially enhance*

*our understanding of the mechanisms underlying this effect and may lead to the improvement of gene and cell therapy strategies for DMD.*

The promising developments represented by these new approaches maintain the hope for DMD patients and their families for future autologous stem cell therapies<sup>24</sup>.

### CONCLUSION

Stem cells are being evaluated for many pediatric conditions such as osteogenesis imperfecta, Hirschsprung's disease, dilated cardiomyopathy and acute brain injury<sup>25</sup>. However considerable practical hurdles must be overcome prior to the broad application of stem cell therapies, including the issues related to the sourcing of material, safety, storage, tracking and standardization.

### REFERENCES

1. **Wagner JE, Barker JN, DeFor TE, et al.** Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood*. 2002 Sep 1;100(5):1611-8.
2. **Barker JN, Krepski TP, DeFor TE, et al.** Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow. *Biol Blood Marrow Transplant*. 2002;8(5):257-60.
3. **Lubin BH, Shearer WT.** Cord blood banking for potential future transplantation. *Pediatrics*. 2007 Jan;119(1):165-70.
4. **Sawani P, Sather H, Ozkaynak F, et al.** Allogeneic bone marrow transplantation in first remission for children with ultra-high-risk features of acute lymphoblastic leukemia: A children's oncology group study report. *Biol Blood Marrow Transplant*. Feb 2007;13(2):218-27.
5. **Lange BJ, Smith FO, Feusner J, et al.** Outcomes in CCG-2961, a children's oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. *Blood*. Feb 1 2008;111(3):1044-53.
6. **Stark B, Jeison M, Gabay LG, et al.** Classical and molecular cytogenetic abnormalities and outcome of childhood acute myeloid leukaemia: report from a referral centre in Israel. *Br J Haematol*. Aug 2004;126(3):320-37.
7. **Nguyen K, Devidas M, Cheng SC, et al.** Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. *Leukemia*. 2008 Dec;22(12):2142-50.
8. **Couriel D, Caldera H, Champlin R, Komanduri K.** Acute graft-versus-host disease: pathophysiology, clinical manifestations, and management. *Cancer*. Nov 1 2004;101(9):1936-46.
9. **Chandy M.** Stem cell transplantation in India. *Bone Marrow Transplant*. 2008 Aug; 42 Suppl 1:S81-S84.
10. **Kumar R, Naithani R, Mishra P, et al.** Allogeneic hematopoietic SCT performed in non-HEPA filter rooms: initial experience from a single center in India. *Bone Marrow Transplant*. 2009 Jan;43(2):115-9.
11. **Kumar R, Prem S, Mahapatra M, et al.** Fludarabine, cyclophosphamide and horse antithymocyte globulin conditioning regimen for allogeneic peripheral blood stem cell transplantation performed in non-HEPA filter rooms for multiply transfused patients with severe aplastic anemia. *Bone Marrow Transplant*. 2006 Apr;37(8):745-9.
12. **George B, Mathews V, Viswabandya A, Srivastava A, Chandy M.** Infections in children undergoing allogeneic bone marrow transplantation in India. *Pediatr Transplant*. 2006 Feb;10(1):48-54.
13. **Kanga U, Panigrahi A, Kumar S, Mehra NK.** Asian Indian donor marrow registry: All India Institute of Medical Sciences experience. *Transplant Proc*. 2007, 39 (3) :719-20.
14. **Talwar S, Khan F, Nityanand S, Agrawal S.** Chimerism monitoring following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2007, 39(9):529-35.
15. **Anderson DJ, Gage FH, Weissman IL.** Can stem cells cross lineage boundaries? *Nat Med* 2001,7:393-395
16. **Gupta DK, Sharma S, Venugopal P, Kumar L, Mohanty S, Dattagupta S.** Stem cells as a therapeutic modality in pediatric malformations. *Transplant Proc*. 2007, 39 (3) :700-2.
17. **Koshizuka S, Okada S, Okawa A, et al.** Transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. *J Neuropathol Exp Neurol* 2004, 63:64.
18. **Goldman SA, Schanz S, Windrem MS.** Stem cell-based strategies for treating pediatric disorders of myelin. *Hum Mol Genet* 2008,17:76-83
19. **Heikkinen ES, Herva R, Lanning P.** Multicystic kidney: A clinical and historical study of 13 patients. *Ann Chir Gynaecol* 1980,69 :15 -22.
20. **Imasawara T, Utsunomiya Y, Kawamura T, et al.** The potential of bone marrow derived cell to differentiate to glomerular mesangial cells. *J Am Soc Nephrol* 2001,12:1401- 1409.
21. **Ikarashi K, Li B, Suwa M, Kawamura K, et al.** Bone marrow cells contribute to regeneration of damage glomerular endothelial cells. *Kidney Int* . 2005, 67:1925 -1933.
22. **Sangwan VS, Matalia HP, Vemuganti GK, Fatima A, Iftekar G, Singh S, Nutheti R, Rao GN.** Clinical outcome of autologous cultivated limbal epithelium transplantation. *Indian J Ophthalmol*. 2006, 54(1):29-34.
23. **Fatima A, Sangwan VS, Iftekar G, Reddy P, Matalia H, Balasubramanian D, Vemuganti GK.** Technique of cultivating limbal derived corneal epithelium on human amniotic membrane for clinical transplantation. *J Postgrad Med*. 2006, 52(4):257-61
24. **Blau HM.** Cell therapies for muscular dystrophy. *N Engl J Med*. 2008, 59(13):1403-5.
25. **Carroll JE, Borlongan CV.** Adult stem cell therapy for acute brain injury in children. *CNS Neurol Disord Drug Targets*. 2008,7(4):361-9.