Haemopoietic stem cell transplantation for genetic disorders

C G Steward, A Jarisch

Stem cell transplantation (SCT) is used to cure or greatly ameliorate a wide variety of genetic diseases, ranging from inherent defects of haemopoietic cell production or function to metabolic diseases mostly affecting solid organs. It ranks as one of the most remarkable therapeutic advances of the past 40 years. Despite rapid technological improvements, however, there are still many short term risks and potential long term toxicities. As a consequence, the rapid emergence of alternative therapies (including new drugs, enzyme and gene therapies), necessitate constant re-evaluation of the risk/benefit ratio for each disease and hence the appropriateness of SCT. This review describes the major aspects of the transplant process, indications for transplantation, outcome statistics, and areas where alternative therapies are becoming available.

DONOR SELECTION AND CELL SOURCE

Major improvements have occurred in tissue typing (especially molecular matching by gene sequencing), cell collection/manipulation, and the development of worldwide donor registries searchable by computer. At the outset, SCT was mostly performed using donor bone marrow from siblings with an identical tissue type. The choice has now widened to include use of peripheral blood stem cells (PBSC) mobilised into the blood of the donor using granulocyte colony stimulating factor (G-CSF), marrow from matched or mismatched family members or unrelated donors and cord blood from siblings or unrelated babies. There is also increasing use of parents who are only half matched for their child’s tissue type (haploidentical), and recently even simultaneous use of cord blood units from two babies in larger patients. This means that transplantation can be performed for the vast majority of candidates, although risks remain substantially higher in the more mismatched transplants. In utero transplantation has a niche role in immunodeficiency diseases.1

CONDITIONING THERAPY

Successful SCT relies on the use of pre-transplant “conditioning therapy” to both eradicate the patient’s marrow (“myeloablation”: creating space for the incoming donor cells to engraft) and to suppress rejection reactions. In malignant disease the most popular conditioning regime has been a combination of cyclophosphamide and total body irradiation (TBI), although this carries many short and long term side effects. For example, late effects of TBI include endocrine and growth problems, infertility, impaired neuropsychological function, organ damage, and malignancy.2-4 In order to avoid some of these effects, busulfan has historically been substituted for TBI when transplanting genetic diseases. This is generally less effective at producing total marrow ablation and complete donor engraftment (termed complete donor “chimaerism”), but partial chimaerism is often sufficient to cure or massively improve genetic diseases. Unfortunately busulfan carries its own side effects, including veno-occlusive disease (VOD) of the liver, fits, and infertility. Other major side effects of SCT include serious bacterial, fungal, or viral infection, primary graft rejection or later secondary graft failure (where the percentage of donor cells falls progressively, often without perceptible change in

Abbreviations: GVHD, graft versus host disease; MSD, matched sibling donor; MUD, matched unrelated donor; PCR, polymerase chain reaction; SCID, severe combined immunodeficiency; SCT, stem cell transplantation; TCD, T-cell depletion
INFECTIOUS COMPLICATIONS
Infection remains one of the greatest challenges in SCT. Immunodeficient patients often go into transplantation with resident infections. In all patients, bacterial infection is common due to co-existent neutropenia and mucositis induced by the conditioning therapy, and the presence of indwelling central venous catheters. Fungal infection (especially with Candida and Aspergillus species) is potentially lethal, the latter occurring principally due to inhalation of airborne spores. Viral problems range from nosocomial infection with CMV, adenovirus, and post-transplant lymphoproliferative disease (PTLD) (due to Epstein Barr virus (EBV)).

The frequency of fungal and viral infection can be reduced by nursing in isolation cubicles with positive pressure ventilation and HEPA filtration. There have been major advances in prophylactic therapy including the use of aciclovir to prevent herpes infections, fluconazole and itraconazole to prevent fungal infections, ciprofloxacin to reduce Gram negative bacterial infections, and cotrimoxazole to combat Pneumocystis. Viral reactivation can be monitored accurately in blood by quantitative PCR analyses, allowing careful pre-emptive use of toxic antiviral drugs before viral disease becomes established. These assays have allowed radical reductions in the frequency of CMV disease by guiding early introduction of ganciclovir or foscarnet (although viral reactivation remains common). Similar approaches are now being used to assess EBV reactivation and introduction of the anti-B-cell antibody Rituximab to prevent cases of PTLD. There remain no reliably effective drugs against adenovirus or parainfluenza.

Technological advances include specific selection of antiviral T-cells which can attack virally infected cells but carry a low risk of producing GVHD,9 the use of new antifungal drugs (for example, caspofungin, voriconazole), and mobilisation of granulocytes from volunteer donors by G-CSF and dexamethasone.10 Future strategies may also rely on immunisation of donors against common viruses.

Table 1 Red cell and platelet disorders treated by SCT

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Indications</th>
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<tbody>
<tr>
<td>Red cell disorders</td>
<td></td>
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<tr>
<td>Thalassemia major</td>
<td>MSD: routine</td>
</tr>
<tr>
<td></td>
<td>MUD/cord donors: developmental</td>
</tr>
<tr>
<td></td>
<td>or pilot studies undertaken in</td>
</tr>
<tr>
<td></td>
<td>specialist units</td>
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<tr>
<td>Sickle cell disease</td>
<td>MSD: when severe complications</td>
</tr>
<tr>
<td>Congenital erythropoietic</td>
<td>MSD/MUD/cord</td>
</tr>
<tr>
<td>porphyria (CEP, Gunther’s</td>
<td></td>
</tr>
<tr>
<td>disease)</td>
<td></td>
</tr>
<tr>
<td>Congenital dyserythropoietic</td>
<td>Severe affected patients only</td>
</tr>
<tr>
<td>anaemia (CD4A) types I and II</td>
<td></td>
</tr>
<tr>
<td>Hereditary sideroblastic</td>
<td>Severe affected patients only</td>
</tr>
<tr>
<td>anaemia (CDA) types I and II</td>
<td></td>
</tr>
<tr>
<td>Pyruvate kinase deficiency</td>
<td>Severe affected patients only</td>
</tr>
<tr>
<td>Platelet disorders</td>
<td></td>
</tr>
<tr>
<td>Glanzmann’s thrombasthenia</td>
<td>Severe affected patients only</td>
</tr>
<tr>
<td></td>
<td>MSD, matched sibling donor; MUD, matched unrelated donor.</td>
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</tbody>
</table>
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N
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N
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**CLASSES OF DISEASE TREATED**

SCT acts in one of three ways in treating genetic diseases:

- By directly replacing diseased marrow or blood cells operating within the blood system
- By replacing phagocytic cells of the monocyte/macrophage lineage which operate in solid organs; for example, osteoclasts in osteopetrosis, macrophages and histiocytes in haemophagocytic conditions
- By acting as a source of indwelling enzyme therapy in metabolic diseases.

Genetic diseases treated by SCT are listed in detail in the tables and boxes. In the descriptive section which follows, these are grouped according to the above categories.

required varies depending on the disease treated. In some conditions, for example Fanconi anaemia and thalassaemia major, it is common to see mixed chimaerism in the early months after the transplant followed by spontaneous improvement of chimaerism to 100% donor. This implies a competitive advantage for the normal cells and may be an important clue as to whether gene therapy can be successful in these conditions in the future.

In many matched transplants for immunodeficiency, little or no conditioning therapy has been used since graft rejection is rare due to lack of T-cell function. As a consequence, many patients engraft exclusively with donor T-cells but these coexist with progenitor cells, myeloid cells, and B-cells largely of recipient origin. Defective B-cell function means that patients often need intravenous immunoglobulin infusions for many years after transplantation.

In metabolic diseases controversy remains as to (a) whether homozygous donors with normal enzyme levels produce superior outcome over carrier siblings/other relatives (who usually have 50% normal enzyme levels), and (b) whether mixed chimaerism is important since this lowers the proportion of donor cells and so reduces the amount of enzyme available.

**Box 1: Immunodeficiency diseases treated by SCT**

**Major severe combined immunodeficiency (SCID) syndromes**
- SCID without B- or T-cells
- SCID with B-cells
- ADA deficient SCID

**Non-SCID immunodeficiency diseases**
- T-cell immunodeficiencies
  - Omenn’s syndrome
  - Purine nucleoside phosphorylase deficiency
  - Major histocompatibility complex class II deficiency
- Wiskott-Aldrich syndrome
- CD40 ligand deficiency (X-linked hyper-IgM syndrome)
- Phagocyte disorders
  - Agranulocytosis
  - Leucocyte adhesion deficiency
  - Chronic granulomatous disease
- X-linked lymphoproliferative disease

**Box 2: Other haematological conditions treatable by SCT**

**Bone marrow failure/cytopenic conditions**
- Pancytopenia
  - Fanconi anaemia
  - Shwachman-Diamond syndrome
  - Dyskeratosis congenita
- Red cell aplasia
- Diamond-Blackfan anaemia
- Neutropenia
  - Severe congenital neutropenia
- Thrombocytopenia
  - Congenital amegakaryocytic thrombocytopenia

**Disorders of phagocytic cells operating in solid organs**
- Haemophagocytic conditions
  - Haemophagocytic lymphohistiocytosis
  - Chediak-Higashi syndrome
  - Griscelli syndrome type 2 (due to RAB27A mutations)
- Malignant infantile osteopetrosis

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**Table 2 Recommended indications for SCT in metabolic diseases (EBMT, 2004)**

<table>
<thead>
<tr>
<th>Disease</th>
<th>SCT</th>
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<tbody>
<tr>
<td>Adrenoleukodystrophy (ALD)</td>
<td>R</td>
</tr>
<tr>
<td>Aspartylglucosaminuria</td>
<td>D</td>
</tr>
<tr>
<td>Batten disease (neuronal ceroid lipofuscinosis, NCL)</td>
<td>D</td>
</tr>
<tr>
<td>Fucosidosis</td>
<td>D</td>
</tr>
<tr>
<td>Gaucher’s disease, types I and III</td>
<td>D</td>
</tr>
<tr>
<td>Globoid cell leukodystrophy (GLD)</td>
<td>D</td>
</tr>
<tr>
<td>Infantile GLD (Krabbe)</td>
<td>D</td>
</tr>
<tr>
<td>Later onset GLD</td>
<td>CRP</td>
</tr>
<tr>
<td>α-Mannosidosis</td>
<td>CRP</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy (MLD)</td>
<td>D</td>
</tr>
<tr>
<td>Late infant MLD (pre-symptomatic only)</td>
<td>D</td>
</tr>
<tr>
<td>Juvenile and adult onset MLD</td>
<td>CRP</td>
</tr>
<tr>
<td>Mucolipidosis, type II (cell disease)</td>
<td>D</td>
</tr>
<tr>
<td>Mucopoly saccharidosis</td>
<td>D</td>
</tr>
<tr>
<td>Type I: Hunter’s syndrome (not in Scheie or Hurler/Scheie)</td>
<td>R</td>
</tr>
<tr>
<td>MPS type IV: Moroteaux-Lamy syndrome (severe form)</td>
<td>CRP</td>
</tr>
<tr>
<td>MPS type VII: Sly syndrome</td>
<td>CRP</td>
</tr>
<tr>
<td>MPS types II, III, and IV: Hunter’s, Sanfilippo’s, and Morquio</td>
<td>C</td>
</tr>
<tr>
<td>GM1 and GM2 gangliosidosis</td>
<td>C</td>
</tr>
<tr>
<td>Niemann-Pick disease types A and C</td>
<td>C</td>
</tr>
</tbody>
</table>

R, routine in selected patients; CRP, to be undertaken in approved clinical research protocols; D, developmental or pilot studies undertaken in specialist units; C, contraindicated.

**Direct replacement of diseased blood/marrow cells**

**Red cell and platelet disorders**

**Haemoglobinopathies**

Thalassaemia major constitutes the single largest indication for SCT among the genetic diseases, with more than 2300 MSD transplants performed since 1981. In young children, for whom iron overload and HLA sensitisation have not become established, event free survival exceeds 90%. For older patients with significant iron overload, better pre-transplant preparation and modified conditioning regimes are improving survival, although risks of graft failure or toxicity remain high. Recurrent venesection is frequently used to reduce iron overload following transplant and may
result in reversal of early hepatic fibrosis. However, the development of oral iron chelators and the success of rigorous iron chelation are increasing life expectancy. As a result, alternative donor (non-sibling) transplants should only be performed in exceptional circumstances.

By contrast, SCT is relatively rarely employed in sickle cell anaemia, with only approximately 200 transplants having been performed worldwide. In Britain, current guidelines restrict the use of transplant to those with severe SS anaemia aged less than 16 years who have a MSD and severe sickle complications, for example, cerebrovascular accident and recurrent chest syndrome. In three series, event-free survival was 75–84% at 6–11 years post-SCT. Inexplicably, several children who have rejected grafts have subsequently developed increased fetal haemoglobin levels (22–33%) which have rendered them symptom free.

Other red cell and platelet diseases
Small numbers of patients have been transplanted for each of the red cell and platelet defects listed in table 1. SCT is not routine in any of these conditions and is reserved for patients with severe disease refractory to more conventional therapy.

White cell disorders
Severe combined immune deficiency (SCID)
Results for transplant in SCID are remarkable considering the high infective load of many patients going into transplant. The European Blood and Marrow Transplant Group (EBMT) report three year survival with sustained engraftment of 77% for all patients transplanted from 1968 to 1999 from an HLA identical related donor, and 54% after mismatched transplant. The poorest results are seen in patients with absent T- and B-cells but reasonable natural killer cell function (T-B-NK+). Within this data results have steadily improved due to better transplant regimens and supportive care; for some groups survival now exceeds 90%, especially where patients are transplanted before 6 months of age. There are also impressive results from haploidentical transplantation, with 78% long term survival in the largest series and no deaths due to GVHD (although this series lacked T-B-NK+ variants). Increasingly SCID is now classified on a genetic basis and it is becoming clear that some subtypes have better cure rates than others, which will in turn allow better stratification of therapy.

Other immunodeficiency diseases
SCT is also employed in a wide variety of other immunodeficiency diseases as detailed in box 1. Results tend to be less good than for SCID, probably because patients tend to present later when significantly infected, for example in Wiskott-Aldrich syndrome where results are best in those transplanted before 5 years. Selection of appropriate patients is often difficult. Transplantation would cure a majority of those affected if performed at an early age before onset of significant complications. However, because of the risk of early death with SCT, it is conventional to use more conservative treatments (for example, prophylactic antibiotics, intravenous immunoglobulin), withholding SCT until complications ensue. Unfortunately, this greatly increases the risk of SCT at that stage. An excellent example of this dilemma is provided by chronic granulomatous disease, where prophylactic approaches are improving, but many patients will eventually develop serious infective complications. At present, SCT in granulocyte disorders remains restricted to patients with significant complications, especially where matched sibling donors are not available.

Bone marrow failure syndromes
Although mechanistically very different, these conditions are grouped together since they cause isolated cytopenia(s) or pancytopenia. Many carry an excess risk of myelodysplasia, acute myeloid leukaemia, or aplasia. The paradigm condition is Fanconi anaemia where defective chromosome repair results in extreme toxicity with conventional conditioning regimens and acceptable transplant results have only been achieved by marked reduction in doses of conditioning therapy. Concerns remain about the long term risk of developing solid tumours, which is already increased in later life in Fanconi anaemia. As one example, patients who develop moderate/severe GVHD have a very high risk of developing squamous carcinoma of the mouth or pharynx. As a generalisation, all patients with bone marrow failure conditions show a high incidence of graft failure or complications. While RIC approaches may reduce toxicity, it is imperative that transplantation is only performed by physicians expert in these conditions or after wide consultation.

Replacing phagocytic cells in solid organs
This is the major mechanism of cure in osteopetrosis and haemophagocytic disorders. Osteopetrosis is caused by defective formation or function of osteoclasts. SCT results in gradual replacement of these cells of monocyte/macrophage lineage and normalises bone density within 12 months of transplant (fig 2). Haemophagocytic disorders occur when the mechanisms which control histiocytes are defective. Life threatening haemophagocytosis is often triggered by infections in children under the age of 2 years. Transplantation, even with the donor cells at levels as low as 10–20%, can reimplant immune control and hence cure the disease.

Metabolic diseases
This principally applies to lysosomal storage diseases (LSD). In these, enzymes are synthesised in lymphocytes, exported into the plasma and taken up into cells of the reticuloendothelial system or solid organs, where they are deposited in lysosomes. Replacing bone marrow provides lifelong enzyme therapy, but an important advantage over infused enzyme therapies is that donor cells penetrate the blood-brain barrier and differentiate into microglia, carrying enzyme into the CNS highly effectively. It should be stressed that this mechanism cannot be operative in X-linked adrenoleukodystrophy, where progressive CNS deterioration can be prevented if treated at a very early stage of the disease. Enzyme transfer cannot be responsible since the defective ABCD1 transporter protein is membrane bound and aggressive immunosuppression without transplant is ineffective.

The success of SCT in metabolic diseases is determined particularly by the degree of tissue damage present by the time of transplantation and the rate of progression of the disease. Children with the most severe variants, for example metachromatic leukodystrophy presenting in infancy and type 2 Gaucher’s disease, tend to do the least well. Where CNS disease occurs, stabilisation of deterioration will often take 12–18 months (perhaps even longer in more severely affected children with ALD). This places the emphasis on early diagnosis and urgent referral for transplantation.

There is a differential benefit between different organ systems. Thus, in Hurler syndrome, IQ can be stabilised and breathing difficulties, hepatosplenomegaly, facial appearance, corneal clouding, and cardiac deterioration greatly ameliorated, but dysostosis multiplex remains a major factor. In order to preserve maximum mobility, many orthopaedic operations are required.

Gaucher’s disease is a good example of a condition where pharmaceutical developments can overcome the need for transplantation. Children with severe forms of type 1 Gaucher’s disease respond extremely well to infused enzyme therapy. Only those who develop complications (for example, pulmonary) despite this treatment or patients with...
type 3 disease (Norbotian, chronic neuronopathic, with CNS abnormalities) stand to benefit from transplantation.

Two of the major mysteries in transplantation of LSD are that: (1) transplant rejection is comparatively common in MPS disorders, even from MSD; and (2) some lysosomal storage diseases do not respond at all, for example, Sanfilippo syndrome. The full list of conditions which may benefit and those for whom transplantation is still relatively experimental is given in table 2. It is still not known whether transplants from normal homozygous donors are better in the long term than those from heterozygous carrier donors, who would usually only have 50% of normal enzyme levels.

**NEW DIRECTIONS**

Despite major advances in the applicability and safety of SCT, substantial risks remain and many patients will be rendered infertile by their conditioning therapy. It is therefore critical to restrict SCT to patients with life threatening disease or complications in whom alternative therapies are not available. Important examples of the latter are the use of enzyme therapies for patients with less aggressive forms of MPS and Gaucher’s disease. Unfortunately these tend to be extremely expensive: the cost of just one year’s therapy for a larger child may equate to the entire cost of an SCT. It is to be hoped that gene therapy can eventually replace all of these modalities of therapy, especially if lentiviral vectors (based on immunodeficiency viruses) live up to the promise shown in animal models.

**REFERENCES**