

## \* CHAPTER 40

# HSCT for children with severe combined immunodeficiencies (SCID)

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## 1. Introduction

Severe combined immunodeficiency (SCID) disorders result from genetically determined blocks in the T-lymphocyte differentiation programme. Overall, the incidence is estimated to be 1 in 75,000 births. There is considerable genetic heterogeneity as eleven different conditions all resulting in a SCID have been fully characterised (Table 1).

Mechanisms	Mutated gene	Inheritance	Affected cells
Premature cell death	ADA	AR	T, B, NK
Defective cytokine-dependent survival signalling	$\gamma$ c	X-L	T, NK
	JAK3	AR	T, NK
	IL7RA	AR	T
Defective V(D)J rearrangement	RAG1 or RAG2	AR	T, B
	Artemis	AR	T, B
Defective pre-TCR and TCR signalling	CD3 $\delta$ , $\zeta$ , $\epsilon$	AR	T
	CD45	AR	T

AR: autosomal recessive; X-L: X-linked

Four main mechanisms have been described:

- a) Premature cell death caused by the accumulation of purine metabolites, as seen in adenosine deaminase (ADA) deficiency.
- b) Defective cytokine-dependent survival signaling in T-cell precursors (and sometimes NK cell precursors). This mechanism accounts for more than 50% of cases of SCID. Deficiency in expression or function of the gamma common ( $\gamma$ c) cytokine receptor subunit shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 causes the X-linked form of SCID (SCID-X1), characterised by the complete absence of both T and NK lymphocytes (1). Deficiency in JAK3, which is normally associated with the cytoplasmic region of  $\gamma$ c, results in an identical phenotype.
- c) Defective V(D)J rearrangements of the TCR and B-cell receptor genes. In our experience, this group accounts for 30% of SCID cases. Deficiency in either RAG1 or RAG2 (the lymphoid-specific recombination initiating elements) or artemis (a factor involved in the non-homologous end-joining repair pathway) leads to defective V(D)J rearrangements and thereby thymocyte and pre-B-cell death.
- d) Defective pre-TCR and TCR signalling. Pure T-cell deficiencies are caused by defects in either a CD3 subunit (such as CD3  $\delta$ ,  $\epsilon$ , or  $\zeta$ ) (2, 3) or the CD45 tyrosine phosphatase, key proteins involved in pre-TCR and/or TCR signalling at the positive selection stage.

Some researchers include other T-cell immunodeficiencies in the SCID group, such as ZAP-70 deficiency, CD3 $\gamma$  deficiency, human leukocyte antigen (HLA) class II expression deficiency, purine nucleoside phosphorylase deficiency, ligase IV or Cernunnos deficiencies, and Omenn's syndrome. However, since these conditions are characterised by the presence of mature (though functionally defective) T cells, they raise very distinct issues as far as therapy is concerned (see below) and so are not considered in this review.

## 2. Clinical manifestations

The clinical presentation of the different SCID conditions is fairly uniform and is characterised by the early onset of infections (usually in the respiratory tract and the gut). Common opportunistic organisms such as *Pneumocystis carinii* and *Aspergillus* and intracellular organisms such as *Cytomegalovirus* can cause recurrent infections and a failure to thrive. Live vaccines, such as Bacille Calmette-Guérin (BCG), can also cause life-threatening infections. The persistence and recurrence of infections in SCID patients rapidly leads to growth impairment and malnutrition. Non-infectious clinical manifestations consist mainly of graft-versus host disease (GvHD) caused by the patients' inability to reject allogeneic cells. The two possible sources of allogeneic cells are maternal lymphocytes and transfusion. The severity of these clinical manifestations makes SCID a medical emergency that, in the absence of treatment, leads to death within the first year of life.

## 3. Diagnosis

Diagnosis of SCID is easy when there is a family history of early death from infections and the symptoms described above. In most cases, clinical examination together with very simple tests can confirm a suspicion, i.e. lack of palpable lymph nodes, especially in the inguinal area, with absence of visible tonsils, absence of a thymic shadow on the chest X-ray film and lymphocytopenia. The latter is of great value in young children since normal absolute lymphocyte counts are high (around  $6 \times 10^9/L$ ).

## 4. Allo-HSCT for SCID patients

SCID is a paediatric emergency that needs to be treated as soon as possible once the diagnosis is confirmed. The treatment of choice is an allo-HSCT, which will provide the missing progenitor of T cells and allows a survival rate of more than 90% when carried out shortly after birth (4–6). Unfortunately, an early diagnosis is not always made and the survival rate is very variable, depending on a number of prognostic factors such as the clinical state at the time of diagnosis, in particular

the presence of a lung infection. In the presence of an HLA genotypical donor (20% of SCID patients), HSCT can be performed without any conditioning regimen and its course is characterised by the remarkable rarity of acute and chronic GvHD in the absence of any prophylaxis and by the rapid development of T-cell function post-transplant. HLA genotypically identical donor includes also one antigen (A or B) mismatched donor; in this last case, cyclosporin A will be used in the post-transplant period to prevent the occurrence of acute GvHD.

The overall survival rate of SCID patients after HLA-genotypical sibling transplant is good, and with recent advances in the management of severe infection management, now reaches over 90% (4).

In the absence of a HLA genotypical sibling, HSCT can be performed with:

- a phenotypically identical family donor (in the case of consanguinity) or a phenotypical cord blood
- a matched unrelated donor (MUD)
- HLA partially identical (haplo-identical) family donor. It should be noted that the time required to the search for a MUD is compatible only in the presence of a patient free of severe infections, in good clinical condition, waiting in a protective environment.

In all these cases, the use of a conditioning regimen is recommended. This usually consists of busulfan (day -10 to day -7) (oral: 4 mg/kg/day x 4 or IV 3.2 mg/kg/day x 4) and cyclophosphamide (day -5 to day -2) (50 mg/kg/day x 4). Anti-thymoglobulin (ATG) will be added if a MUD is available; in this case, the GvHD prophylaxis consists in cyclosporin A. If an HLA partially identical family donor is used, the HSC harvest will be *ex vivo* manipulated to eliminate the mature T- and B-cells contaminating the graft; usually this is obtained by means a CD34+ selection. Cyclosporin A is not indicated except in the case of primary GvHD from maternal-foetal transfusion or Omenn syndrome. In the latter setting therapy/prophylaxis of GvHD is usually needed and should be continued for three months. Ideally, HSCT graft should contain  $5 \times 10^6$  CD34/kg of body weight and less than  $1 \times 10^4$  T-cells/kg, thus strongly limiting the risk of acute severe GvHD. The presence of a severe infection necessitates modification or complete omission of the conditioning regimen. A full conditioning regimen increases the chance of obtaining HSC engraftment and sustained T and B-cell reconstitution but because these regimens have considerable toxicity, research is needed to find less toxic myeloablative regimens.

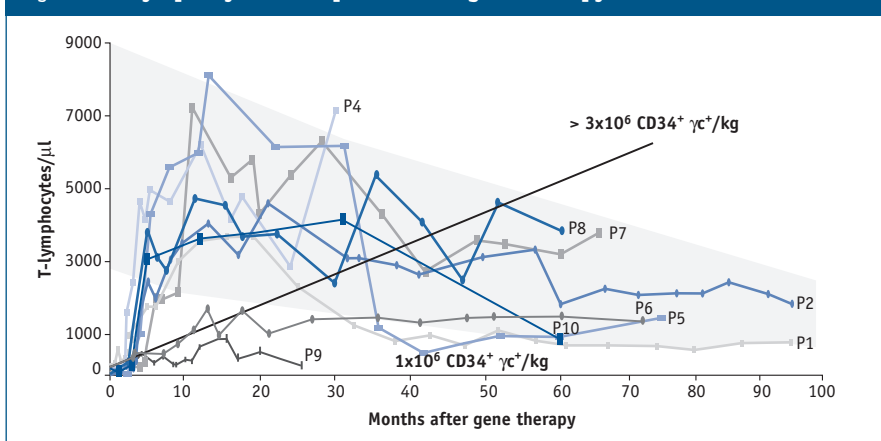
Patients are placed in a protective environment and usually receive prophylactic antimicrobial medication to eliminate the intestinal microflora, and IV Ig therapy weekly for three months after HSCT and then every three weeks up to the detection of B-cell function. Around 40% of all transplanted SCID forms require long-life Ig replacement because of absence of B-cell engraftment.

The survival rate of patients treated by an haploidentical T-cell-depleted HSCT is less good than that reported for an HLA genoidentical donor, and ranges from 50 to 78% (4, 5). Again, results have improved over time (from 35% survival in patients transplanted before 1985 in Europe to 75% in those treated from 1996 to 1999) (4). Better prevention of GvHD and treatment of infections probably account for the improved survival rates. Three parameters play a role in determining overall survival rate: GvHD, graft rejection and kinetics of T-cell development, the latter being the major parameter affecting the different outcome after HLA partially identical HSCT. In the light of these partially unsatisfying clinical results, gene therapy protocols have been initiated in Europe to try to improve the clinical results for SCID patients lacking a genoidentical sibling donor.

### 5. Gene therapy trials for SCID

The first clinical trial for SCID-X1 was initiated in 1999. The protocol has been described elsewhere (7) and is quite simple in its principle. Patients with no HLA-identical siblings were eligible, and no myeloablative treatment was given. Among the 10 treated patients with typical SCID-X1, T-cell development occurred in nine (7–9). A correlation between quality of T-cell reconstitution and number of transduced CD34+ cells infused was detected (Figure 1).

**Figure 1: T-lymphocyte development after gene therapy for SCID-X1**



P1 →10: each curve represents T-cell counts for a given patient. In P6 and 10, who received  $1 \times 10^6 \gamma\text{-chain} (\gamma\text{c})+\text{CD34}^+$  cells/kg T-cell development was suboptimal (below the line) as compared to the other patients who received  $> 3 \times 10^6 \gamma\text{c}+\text{CD34}^+$  cells/kg

Suboptimal T-cell development occurred in two patients who received  $1 \times 10^6$  CD34<sup>+</sup>  $\gamma$ c<sup>+</sup>/kg, whereas the T-cell pool fully developed in those who received  $>3 \times 10^6$  CD34<sup>+</sup>  $\gamma$ c<sup>+</sup>/kg, providing a minimal threshold of total number of cells to inject. These results have since been confirmed in 10 additional patients treated by Thrasher et al. (10) in London with a similar protocol.

Until now, correction of immunodeficiency, which also includes in part B-cell immunity, is good enough so that patients cope normally with infections, including those caused by VZV, and live normally without therapy.

Efficacy of gene therapy also has now been reported in the treatment of 10 patients with ADA deficiency using similar methodology (11). Usage of a low-dose myeloablative therapy (busulfan, 4 mg/kg) may have increased the observed rate of myeloid cell transduction, thereby potentially improving long-term efficacy. These results open the door to an extension of gene therapy to other SCID conditions, as the selective growth advantage concept should apply, albeit with a variable intensity in different forms of SCID.

There are some limitations to gene therapy of SCID. Failure to correct immunodeficiency occurred in a child with SCID-X1 who had an enlarged spleen caused by a disseminated BCG infection (8). It appeared from analysis of the spleen upon removal that transduced cells were probably trapped in the spleen, thus impairing T-cell differentiation in the thymus. Therefore, such (rare) patients might require splenectomy prior to be eligible for gene therapy.

Gene therapy in older SCID-X1 patients, either patients with an incomplete phenotype or a patient in whom HSCT at least partially failed, did not succeed. This failure is likely the consequence of the premature loss of thymic function in SCID patients in the absence of functional T-cell precursor cells.

Four patients from our SCID-X1 gene therapy trial developed clonal T-cell proliferation, as described in detail elsewhere (9). In two of these clonal proliferations, the primary cause was insertion of the provirus within the LMO-2 locus, leading to aberrant expression of LMO-2 in mature T cells and thereby uncontrolled proliferation. It was found that there is a higher risk of this serious adverse effect occurring in the context of SCID-X1, as also suggested in a murine model of leukaemogenesis.

Further application of gene therapy in SCID nevertheless should try to use potentially safer vectors, including self-inactivated long terminal repeats and perhaps insulators and a rescue "suicide" gene.

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### Note

*While writing this Chapter, one case of leukaemia-like lymphoproliferative disease occurred in the English trial.*

## References

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## Mutiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

### 1. The common characteristic of the different forms of SCID is:

- a) Absence of circulating white blood cells .....
- b) Absence of T- and B-cells .....
- c) Absence of T- and NK cells .....
- d) Absence of mature T-cells .....
- e) Leukopenia .....

**2. SCID forms are primary immunodeficiencies and the short-term prognosis without treatment is:**

- a) A few days .....
- b) A few weeks .....
- c) Some months .....
- d) 1 year .....
- e) A few years .....

**3. The search for a MUD is indicated in the presence of:**

- a) Severe lung infection .....
- b) Generalised BCG infection .....
- c) CMV infection .....
- d) Adenovirus infection .....
- e) No opportunistic infections .....

**4. The outcome of an haploidentical HSCT is heavily influenced by:**

- a) The frequency of rejection .....
- b) The occurrence of GvHD .....
- c) The occurrence of VOD .....
- d) A long lasting immunodeficiency .....
- e) The frequency of pulmonary acute hypertension .....

**5. The major risk factor linked to gene therapy is:**

- a) Failure .....
- b) Partial immunological reconstitution .....
- c) Age of patients .....
- d) Insertional mutagenesis .....
- e) Splenomegaly .....