



Bubble Boy Disease

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History of SCID



- ❖ Severe Combined Immunodeficiency Disease became widely known during the 1970s and 1980s, and was dubbed the "Bubble Boy Disease" because of the widely-publicized case of David Vetter, a boy with X-linked SCID, who lived for 12 years in a plastic, germ-free bubble, due to his extreme vulnerability to infectious diseases.
- ❖ It was between the years of 1968 and 1973 that doctors saw the beginning of this disease and were occasionally able to diagnosis a case of SCID; however, if there was no Human Leukocyte Antigens-matched sibling, no cure was available.



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Basic Description



- ❖ Severe Combined Immunodeficiency Disease, or “Bubble Boy Disease”, is a primary immune deficiency, in which both the humoral and cell-mediated branches of acquired immunity fail to function; B and T lymphocytes are crippled, due to a defect in one of several possible genes, including chromosomes 5, 10, and 19.
- ❖ SCID, is a severe form of heritable immunodeficiency and of course leads to the acquiring of many other diseases—including opportunistic ones such as cholera, meningitis, ear infections, recurrent *Pneumocystis jirovecii* pneumonia, and profuse oral candidiasis, which are often fatal to the defenseless patients.

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❖ The defining characteristic for SCID is a **severe defect in T cell production and function, with defects in B-lymphocytes as a primary or secondary problem and, in some genetic types, in natural killer cell production as well.** Research indicates SCID may be caused by a cytogenetic dysfunction of the embryonic stem cells in differentiating B-lymphocytes and T-lymphocytes. The affected individual consequently has a very small thymus and little or no protection against infection.

❖ However, the many different types of SCID have different causes, most of which are related to **mutations on chromosomes and enzymes that are key players in both innate and acquired immunity.**

Incidence



- ❖ Most cases of SCID are due to mutations in the gene encoding the common gamma chain (γc), a protein that is shared by the receptors for interleukins and inherited via sex-linked genes.
- ❖ These interleukins and their receptors are involved in the development and differentiation of T and B-lymphocytes, the major players of acquired immunity.
- ❖ The result is a near complete failure of the immune system to develop and function, with low or absent T-lymphocytes and NK cells, one of the most important players of innate immunity, and non-functional B-lymphocytes.
- ❖ The most common form of SCID is X-linked SCID, which directly deals with the gamma chain. The common gamma chain is encoded by the gene IL-2 receptor gamma, or IL-2R γ , which is located on the X-chromosome.

Other Types



- ❖ Another type of SCID deals with *Janus kinase-3 (JAK3)*, a tyrosine kinase, which is an enzyme that mediates transduction from cytokine receptors downstream of the γc signal and interacts with members of the signal transduction and activators of transcription family.
- ❖ Yet another type is *V (D) J recombination SCID*, which affects the process by which segments of a B-lymphocyte or T-lymphocyte's DNA, are rearranged to create a new T-cell receptor or B-cell receptor, and in B lymphocytes, also a template for antibodies.
- ❖ *Adenosine Deaminase Deficiency*, the second most common form of SCID, is due to a defect on chromosome twenty, which codes for the enzyme. Without this enzyme, there is an accumulation of deoxyadenosine, which causes an increase in S-adenosylhomocysteine; both substances are toxic to immature lymphocytes, which thus fail to mature.

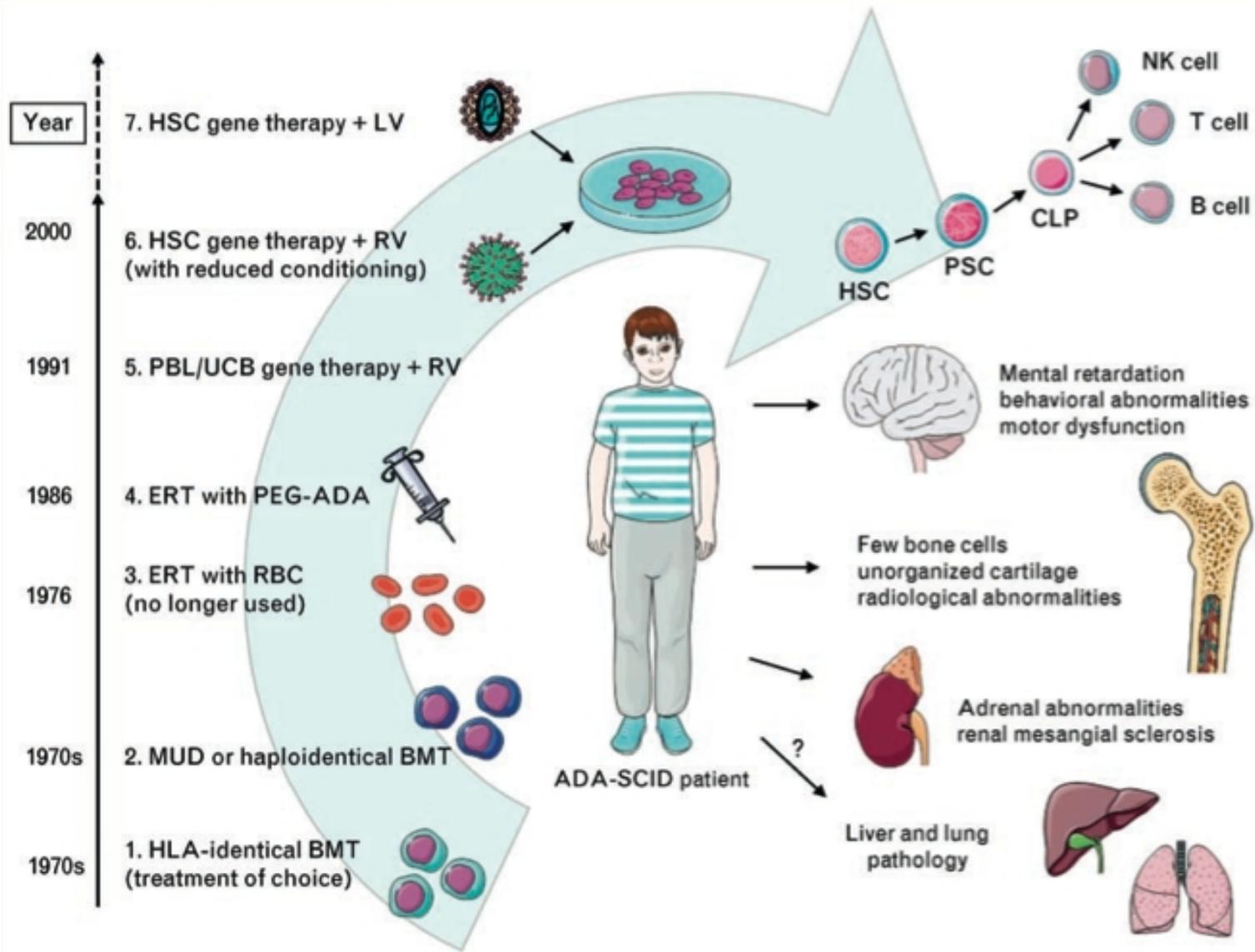


<u>Some of The Known Forms of SCID:</u>	<u>Gene</u>	<u>Lymphocyte Phenotype</u>
X-linked SCID (gamma chain gene mutations)	IL2RG	T(-) B(+) NK(-)
Autosomal Recessive SCID		
Jak3 gene mutations	JAK3	T(-) B(+) NK(-)
ADA gene mutations	ADA	T(-) B(-) NK(-)
IL-7R alpha-Chain mutations	IL7R alpha	T(-) B(+) NK(+)
CD3 delta or epsilon mutations	CD3 delta or epsilon	T(-) B(+) NK(+)
RAG1/RAG2 mutations	RAG1/RAG2	T(-) B(-) NK(+)
Artemis gene mutations	ARTEMIS	T(-) B(-) NK(+)
CD45 gene mutations	CD45	T(-) B(+) NK(+)

Treatment



- ❖ The most common treatment for SCID is bone marrow transplantation. Histocompatibility bone marrow transplantation done in the first three months of life has a high success rate, but there is always the risk for a graft-versus-host reaction.
- ❖ More recently, gene therapy has proved useful. Transduction of the missing gene to hematopoietic stem cells, mainly bone-marrow, using viral or bacterial vectors in ADA SCID and X-linked SCID.



Therapeutic options for ADA-SCID patients comprise bone marrow transplantation, enzyme replacement or gene therapy. Treatment of choice remains bone marrow transplantation from an HLA-identical sibling donor, whereas transplants from alternative donors are associated with high morbidity and mortality. Enzyme replacement therapy is no longer used and has been substituted by the development of pegylated bovine ADA.

Bone Marrow



Transplant

- ❖ Shortly after the discovery of HLA in 1968, immune function was transferred to an infant with SCID by transplantation of bone marrow from his HLA- identical sister.
- ❖ Over the ensuing decade, however, lethal graft-versus-host disease (GVHD) was found to be a major problem when marrow from HLA-mismatched donors was used.
- ❖ Because the defect in infants with SCID leads to a complete absence of T cell function, they cannot reject allografts. Therefore, successful marrow transplantation in SCID does not require pretransplant chemotherapeutic conditioning. Prophylaxis for GVHD is also not necessary after transplantation of HLA-identical or even rigorously T cell-depleted HLA haploidentical marrow.
- ❖ These circumstances provide a unique opportunity to employ bone marrow transplant as therapy.

Duke Study on BM Transplant



❖ In a study carried out in Duke University, a physician had performed hematopoietic stem cell transplantation in 132 consecutive infants with SCID over 21.3 years, and 102 of them survived. The outcome in all but 30 of these transplants had been previously reported.

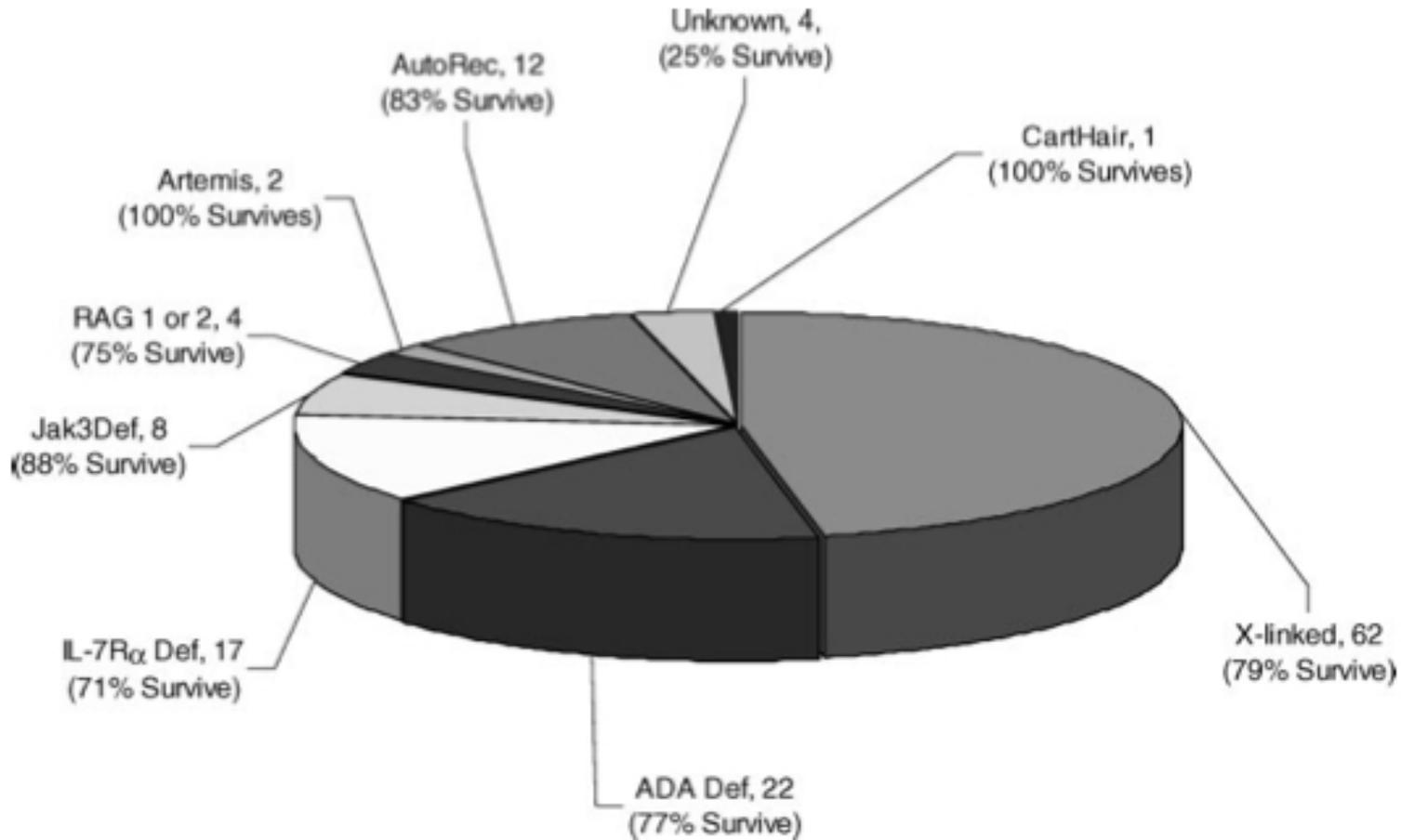
❖ The 132 patients ranged in age from newborn to 21 months at diagnosis. All fulfilled the criteria of the World Health Organization for diagnosis of SCID.

❖ The largest number of patients—62 boys from 50 families or 47%—had X-linked SCID; 8 patients from 8 families had Jak3 related SCID; 17 patients from 16 families had IL-7R α related SCID; 22 infants from 19 families had ADA related SCID; 15 patients from 15 families had autosomal recessive inheritance but unknown mutations; and 4 boys with no family history had SCID of unknown type.

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- ❖ Immunologic monitoring was done whenever feasible every 3 weeks until T cell function was established (usually at 3–4 months post-transplantation), then every 3 months for the next 9 months, every 6 months for the next 2 years, then once a year thereafter.
- ❖ All 117 HLA-haploidentical and 7 of the 15 HLA-identical transplants from related donors were T cell depleted.
 - ❖ Thirty-three patients received from one to three additional T cell–depleted marrow transplants, from either the original donor or another haploidentical relative.
- ❖ None of the marrow recipients received any pretransplant conditioning or post-transplant prophylaxis against GVHD.



Of the 132 SCID patients, **102 (77%) are alive**. None show any evidence of susceptibility to opportunistic infections and most are in good general health. The follow-up ranges from 2 months to 21.3 years after transplantation. Of these 102 patients, **96 have survived 1 or more years after transplantation**, **68 have been alive 5 or more years**, and **37 for 10 or more years**.

Graft vs. Host Disease (GVHD)



- ❖ In another study, GVHD was observed however in 40/117 patients given T cell– depleted haploidentical parental marrow, in 6/15 given unfractionated HLA-identical marrow, and in 4/5 given placental blood.
 - ❖ In 35/49 cases, this complication occurred when there was persistence of transplacentally transferred maternal T cells.
- ❖ Most of the GVHD after administration of T cell–depleted marrow was mild (Grade I or II) and required no treatment (70). Ten patients from the entire group had Grade III GVHD involving the skin, gastrointestinal tract, and/or marrow.
- ❖ One neonate with autosomal recessive SCID who received a 3-antigen mismatched transplant developed Grade 4 GVHD with autoimmune hemolytic anemia, bone marrow suppression, diarrhea, and cholestatic liver disease.

Table 3 Survival following HSCT for SCID based on stem cell source and conditioning regimen

	<i>Year</i>	<i>Reference</i>	<i>MRD</i>	<i>Haplo</i>	<i>Haplo</i>	<i>MUD</i>	<i>MUD</i>	<i>Cord</i>
<i>Conditioning</i>			None	None	MA	MA	RI	MA
Dror <i>et al.</i>	1993	3	—	67% (12)	50% (12)	—	—	—
Buckley <i>et al.</i>	1999	5	100% (12)	78% (77)	—	—	—	66% (3)
Bertrand <i>et al.</i>	1999	4	—	46% (50)	54% (129)	—	—	—
Dalal <i>et al.</i>	2000	15	—	—	—	67% (9)	—	—
Knutsen and Wall	2000	16	—	—	—	—	—	88% (8)
Antoine <i>et al.</i>	2003	6	81% (104)	—	—	63% (28)	—	—
Rao <i>et al.</i>	2005	17	—	—	—	71% (7)	83% (6)	—
Bhattacharya <i>et al.</i>	2005	18	—	—	—	—	—	80% (10) ^a
Grunebaum <i>et al.</i>	2006	14	92% (13)	—	53% (40)	81% (41)	—	—

Stem cell source: MRD (matched related donor) vs haplo (haplocompatible family donor) vs MUD (matched unrelated donor) vs Cord (unrelated cord blood stem cells). Conditioning: none vs MA (myeloablative) vs RI (reduced intensity). Percentage indicates overall survival (absolute number of patients).^aSome patients received no conditioning.

In the future, the success rates for T cell-depleted haplocompatible-related stem cells for treatment of SCID and other primary immunodeficiencies will continue to improve as new strategies are discovered to enhance immune reconstitution, such as adoptive immunotherapy utilizing alloreactive-depleted donor T cells, and to reduce short- and long-term side effects by the effective use of reduced toxicity regimens and possibly regimens that do not require alkylating agents or radiation.

Gene Therapy (ADA)



- ❖ The cumulative experience of early gene therapy trials pointed out a need to improve gene transfer efficiency and favor the engraftment of long-lasting gene corrected HSC.
- ❖ In 2000, an improved transduction protocol of bone marrow CD34⁺ cells was developed with the use of non-myeloablative chemotherapeutic conditioning to 'make space' in the bone marrow.
- ❖ Ten patients who lacked an HLA-identical sibling donor were enrolled in three phase I/II clinical protocols.

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❖ Gene therapy resulted in sustained engraftment of transduced CD34⁺ bone marrow progenitor cells, with multilineage differentiation, increase of lymphocyte counts and improvement of cellular and humoral responses.

❖ Pegylated bovine ADA (PEG-ADA) replacement therapy was discontinued to take advantage of the selective growth advantage for gene corrected over defective cells and to evaluate the efficacy of gene therapy alone.

❖ At present, all treated patients are alive and in good clinical conditions, with the exception of one patient who requires steroid treatment due to recurrent episodes of autoimmunity, previously observed while on PEG-ADA

A retrovirus that has been rendered harmless is used as a vector in this procedure, which exploits the ability of a retrovirus to insert a DNA transcript of its RNA genome into the chromosomal DNA of its host cell. If the foreign gene carried by the retroviral vector is expressed, the cell and its descendants will possess the gene product, and the patient may be cured. Cells that reproduce throughout life, such as bone marrow cells, are ideal candidates for gene therapy. If the treatment is successful, the patient's bone marrow cells will begin producing the missing protein, and the patient will be cured.

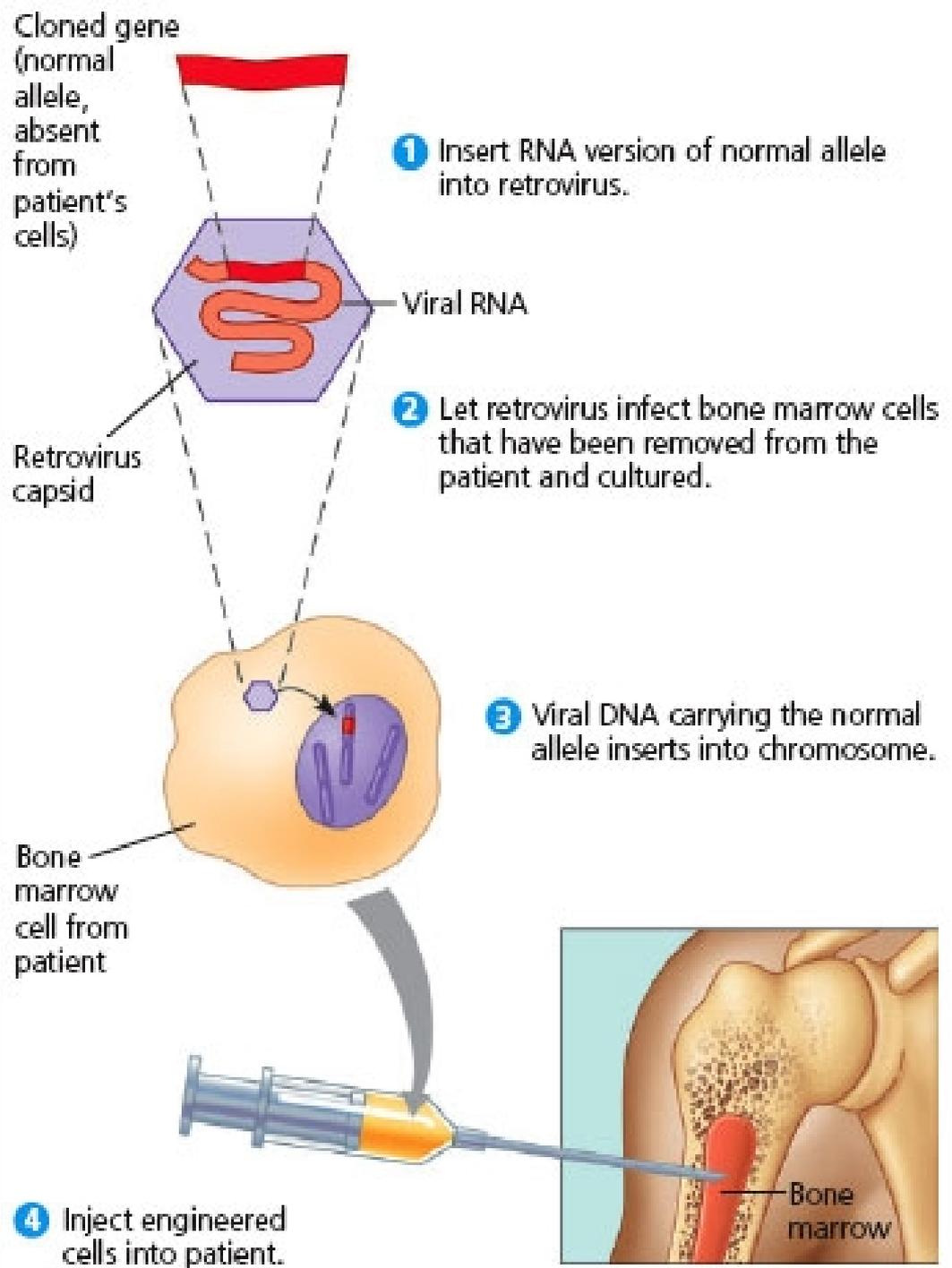


Table 1
Summary of clinical trials of GT for ADA deficiency

Center	No. of Patients	Follow-Up (Years)	Off Enzyme	Survival	DFS
Milan	10	1.8–8.0	8/10	100%	80%
London	6	1.0–6.0	3/6	100%	50%
CHLA-NIH	6	0.5–3	3/6	100%	50%
UCLA-NIH	3	0.1–0.5	3/3	100%	n.e.
Total	25	0.1–8.0	17/25	100%	67%

Abbreviations: DFS, alive without bone marrow transplantation or PEG-ADA restart; n.e., not evaluable; UCLA, University of California, Los Angeles.

Problems?



- ❖ Integrating vectors carry an inherent risk of insertional mutagenesis associated with retroviral vector insertions in sensitive genomic sites.
- ❖ By inserting into such regions, the strong enhancer elements found in the viral LTR can lead to aberrant gene expression, causing clonal expansion or leukemic proliferation, as occurred in the case of SCID-X1.
- ❖ Such complications or other adverse events have not been seen with ADA gene therapy, indicating that ADA-SCID gene therapy has a favorable risk/benefit profile.
- ❖ Unique risk factors may have contributed to the different outcomes of the SCID-X1 trials, such as vector constructs or promoters, inappropriate expression of transgenes involved in cell signaling, cooperation between transgene and cellular oncogenes, or disease background associated with high rate of transformation.

Gene Therapy (X-Linked)



- ❖ From 1999–2002, 11 patients with X-linked SCID were administered autologous bone marrow cells into which a normal γ c cDNA had been successfully transduced by retroviral gene transfer.
 - ❖ In 9 of the 11 patients, molecular studies demonstrated normal transgene expression in circulating T and NK cells by approximately 30–40 days after gene therapy, but it was minimal in B cells.
 - ❖ 2 of the patients did not express the transgene and were given allogenic bone-marrow transplants.
 - ❖ The 9 infants with transgene expression developed normal T cell function at between 90 and 120 days after the treatments, similar to the kinetics after T cell–depleted allografts.
 - ❖ The efficacy of gene therapy in conferring immune function in those infants with X-linked SCID seemed to be far superior to that of allogenic marrow stem-cell transplantation.

Problems?



- ❖ However, serious adverse events occurred in the fourth and fifth patients treated by the French group. Both children developed leukemic-like processes, with expanded clonal populations of T cells.
- ❖ The clones carrying the inserted γ c cDNA, and the leukemias were likely induced by the retroviral gene therapy by a process called insertional oncogenesis, like that seen in the case of SCID X-Linked.
- ❖ The positions of insertion in both children are in or near a gene on chromosome 11 called *LMO-2*. The product of *LMO-2* is crucial for normal hematopoiesis and serves a regulatory function. However, *LMO-2* is also an oncogene that is aberrantly expressed in acute lymphoblastic leukemia of childhood.

Table 1

Clinical trials of hematopoietic stem cell gene therapy for ADA-SCID conducted in the last decade

Study	No of Patients Treated	Retroviral Vector	Conditioning Regimen	PEG-ADA Discontinuation (Yes/No)
HSR-TIGET ^{26,34}	15	GIADAI	Busulfan (4 mg/kg)	Yes
GOSH ^{15,17}	5	SFFV-ADA-WPRE	Melphalan (140 mg/m ²)	Yes
CHLA-NIH 1 ^{33,35}	4	GCsap-M-ADA and MND-ADA	No	No
CHLA-NIH 2 ^{33,35}	6	GCsap-M-ADA and MND-ADA	Busulfan (75–90 mg/m ²)	Yes
Hokkaido ³⁶	2	GCsap-M-ADA	No	Yes

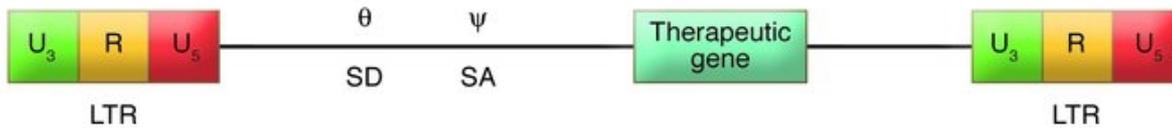
Abbreviations: HSR-TIGET, The San Raffaele Telethon Institute for Gene Therapy, Milano, Italy; MND, myeloproliferative sarcoma virus [MPSV] enhancer, negative control region deleted, dl587rev primer binding site substituted; SFFV, spleen focus-forming virus.

The use of self-inactivating human immunodeficiency virus (HIV)–derived **lentiviral vectors** may improve the safety and efficacy of gene transfer into HSCs. These vectors integrate efficiently into HSCs, allow stable and robust trans-gene expression, and significantly alleviate safety concerns associated with retro-viral vector integration. They can also be adapted to contain physiologic cellular promoters rather than viral promoters used for retroviral vectors.

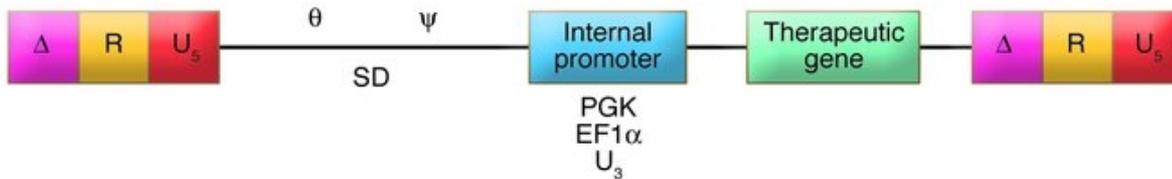
Vectors



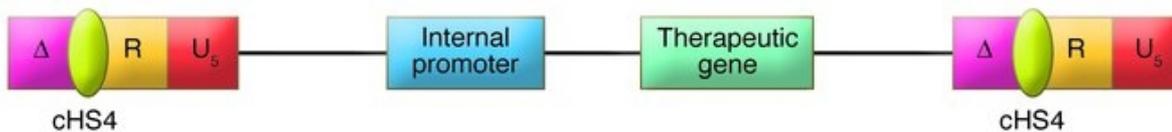
A Retroviral vector used for the SCID clinical trials



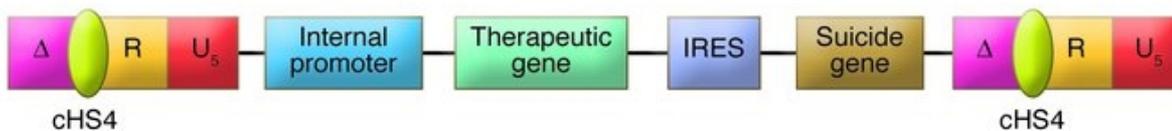
B Self-inactivated vectors



C Self-inactivated vector containing 2 x (250 bp) cHS4 insulators



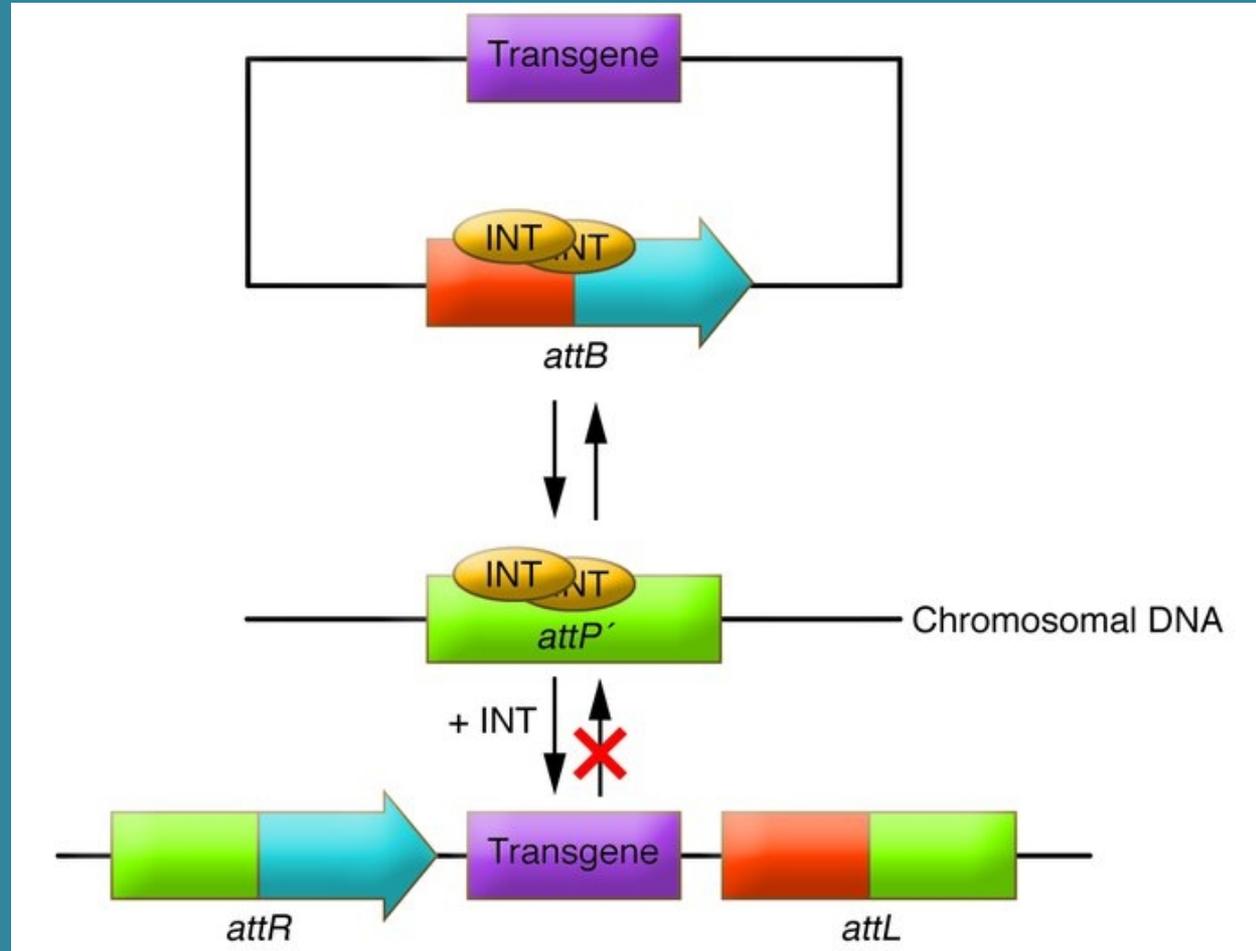
D Self-inactivated vector containing insulator and a suicide gene (TK)



(A) Transcription is driven by the enhancer-promoter activity of the U₃ region of the retroviral LTR. (B) Driven by the addition of an internal promoter. The U₃ region of the retroviral LTR has been almost completely deleted. (C) The provirus contains the cHS4 element (i.e., insulator) in order to protect the transcriptional cassette against position effects. (D) This provirus contains 2 cassettes: a) the therapeutic gene driven by a first internal promoter & b) a suicide gene that could allow the elimination of gene-corrected cells if an adverse event such as a monoclonal proliferation occurs.

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Major advances are expected from the development of new approaches that target specific genomic sites for integration or gene correction. The use of site-specific integrases (INs) could allow this goal to be attained. Bacteriophage IN phiC31 is able to perform site-specific integration via recombination between an attP recognition site encoded in the phage genome and an attB site in the bacterial chromosome.



Integration process mediated by the bacteriophage phiC31 (INT).



Sterile Bubble s

- ❖ Since treatment of SCID seeks to develop the immune system and to prevent infection, placing the infant with SCID in a completely sterile environment for a long time is a method of treatment that has prolonged the life of some affected individuals, but this option does not prove successful if the infant involved has already had recurring infections, nor does it make them healthier.

Versus



- ❖ The choice between HSCT or GT as a definitive procedure is again difficult.
- ❖ The choice is between the short-term risks associated with HSCT against the potential for long-term side effects associated with GT.
- ❖ MUD HSCT can present 67% survival outcome given the present data, but this involves only a small subset of individuals. These figures may change significantly even if only a handful of further transplants are under-taken.
- ❖ It is clear, however, that outcome following these procedures is very good, with almost complete T- and B-cell reconstitution, and presently no evidence of long-term side effects.

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- ❖ By contrast, GT has an excellent survival outcome and to date more than 20 children in 3 trials have survived the procedure without any significant side effects.
 - ❖ The drawbacks of GT are twofold. First, there is **the potential for insertional mutagenesis using the current vector technologies**, although importantly the follow-up does not show any evidence of clonal proliferation in treated individuals.
- ❖ The other issue is that of **immune recovery**; at present 67% have been able to discontinue treatment on a long-term basis and approximately 50% have been able to discontinue Ig replacement.
- ❖ Thus the choice as of now is largely determined by the parental and physician attitude toward the risks and benefits associated with the 2 different treatment options.

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The End.

