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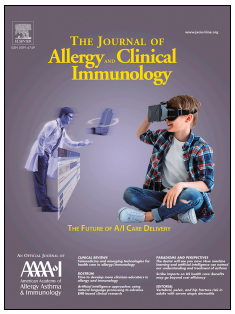

Gene therapy for X-linked severe combined immunodeficiency: historical outcomes and current status

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Gene therapy for X-linked severe combined immunodeficiency: historical outcomes and current status

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SCID-X1 background and outcomes of standard treatment

Severe combined immunodeficiency disorder (SCID) is a genetically heterogeneous group of disorders characterized by a profound absence of T lymphocyte function, resulting in lack of cellular and humoral immunity. The X-linked form (SCID-X1), accounts for 30-40% of cases, and is caused by defects in the common cytokine receptor γ chain (γc), encoded by the *IL2RG* gene. Originally identified as a component of the high affinity interleukin-2 receptor, γc is an essential component of the IL-4, -7, -9 -15, and -21 cytokine receptor complexes(1) (Figure 1A). The molecular defect in SCID-X1 results in the complete absence of T cell and natural killer cell development and a defect of terminal B cell maturation and function. The prognosis without treatment is uniformly fatal due to recurrent and opportunistic infection.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is curative for the disorder, however outcomes remain suboptimal for certain patients.(2) The Primary Immune Deficiency Treatment Consortium (PIDTC) published two retrospective studies of allo-HSCT outcomes, a study of 240 patients with typical SCID and a larger recent study of 662 patients with typical, leaky or Omenn SCID.(2) Both studies confirmed and refined the factors important for survival such as the presence of active infection, use of donors other than matched siblings, and age at transplant. The latter study for first time reported outcomes that are specific to certain genetic types of SCID, showing that patients with SCID-X1 have superior survival.

The lack of functional T and NK cells in SCID-X1 underlies the ability of many patients to develop a T cell graft without conditioning. Donor-derived HSC and progenitors (HSPC) seed the thymus, and can potentially sustain T cell generation for many years. However, without the use of myelosuppressive conditioning agents, cells other than T and NK cells remain largely of host origin. SCID-X1 patients who do not receive conditioning pre-transplant are therefore less likely to become independent of immunoglobulin substitution or respond to vaccination(2). Recent studies have clearly demonstrated that conditioning promotes more complete immune reconstitution in particular using the alkylating agent busulfan, which can be adjusted with pharmacokinetics to achieve a controlled drug exposure and limit toxicity.

The success of standard allo-HSCT remains limited due to the lack of matched donors for all patients, and importantly the complications of acute and chronic graft-versus-host disease (GVHD). Even in this young age group, the incidence of acute and chronic GVHD is approximately 20-25% and up to 16% respectively(2). Furthermore, use of mismatched donors, particularly haploidentical related donors was associated with greater risk of needing a second allo-HSCT(2). Transduction of autologous HSPC cells with an integrating retroviral vector that expresses a normal copy of the gene causing SCID-X1, would obviate the need to find a matched donor and eliminate the risk of GVHD and graft rejection.

Safety of gene therapy for SCID-X1 has improved with use of self-inactivating vectors

A summary of past and current trials of gene therapy for SCID-X1 is shown in Table 1. Seminal clinical trials were performed in Paris and London targeting high-risk patients in hopes of achieving robust immune reconstitution without the risk of GVHD intrinsic to allo-HSCT. CD34+ cells were purified from bone marrow and transduced with a vector expressing the *IL2RG* transgene (MFG-γc). Overall, 20 boys underwent gene therapy with rapid T cell reconstitution in 18 patients. Among the 17 survivors, all have been generally free of SCID-related infections.(3, 4)

The MFG-γc vector is a gammaretroviral (γRV) vector with intact long-term repeat regions (LTR) containing strong viral enhancers and promoters. These elements drove strong transgene expression and hence rapid T cell reconstitution, but also were associated with insertional oncogenesis. Of 20 boys treated, 6 (5 in Paris, 1 in London) developed clonal T cell proliferation phenotypically mimicking acute T cell lymphoblastic leukemia, each arising from clones bearing insertion sites near genes with lymphoid oncogenic potential (*LMO2*, *CCND2*, *BMI1*). The latency of leukemogenesis using the MFG-γc vector was long (2-15 years).(5)

One study directly tested the hypothesis that removing the strong viral elements from the LTR would deter overexpression of neighboring genes and reduce or eliminate insertional oncogenesis. The parent MFG-γc vector was modified to remove the strong viral elements from LTR, driving expression of the *IL2RG* transgene instead by a cellular promoter, elongation factor 1a short (EFS). This self-inactivating γRV vector (SIN-γc) was tested in parallel phase I/II gene therapy trials in United States, France and United Kingdom(6). T cell recovery in patients receiving adequate VCN was robust, and to date, none of the 14 patients in follow-up in the SIN-γc trial have developed leukemia (2.7-9.3 years, median 7.9 years). Interim analysis of 9 patients comparing insertion site pattern in the blood of patients receiving the MFG-γc vector to those receiving the SIN-γc vector demonstrated a strong reduction in clustering near *LMO2* and *CCND2*.(6) The global distribution of insertion sites between the two vectors is indistinguishable with regard to transcription start sites, gene density and epigenetic marks.

Safety is expected to be further improved with the use of self-inactivating lentiviral (SIN-LV) vectors, which have by nature a different distribution of insertion sites and do not favor transcription start sites. Three trials of gene transfer for SCID-X1 using SIN-LV, one in older patients who have failed allogeneic HSCT (NCT 01306019),(7) and two in newly diagnosed infants in US (NCT015132888)(8) and internationally (NCT03311503) have not reported any oncogenesis to date, albeit with short follow-up.

Conditioning improves humoral function after gene therapy for SCID-X1

In trials without chemotherapy conditioning, gene marking was extremely low or absent in B cells and in granulocytes,(3, 4, 6) and in general these patients have failed to mount vaccine specific immunoglobulin. In the absence of conditioning, allo-HSCT and gene therapy promote T cell development due to the ability of the infused HSC and committed progenitors to engraft and persist in the empty thymus of patients with SCID (Figure 1B). However, HSC remain deficient in IL2RG and the progeny of those HSC are therefore unresponsive to γc-dependent cytokines, such as IL-21, which are critical for B cell maturation into antibody-producing plasma cells. Conditioning is required to replace HSC with cells expressing γc that can develop into IL-21-responsive B cells. Supporting this notion, a PIDTC study of 48 SCID-X1 or JAK3 SCID patients post-allo-HSCT showed that chemotherapy conditioning was associated with restoration of in vitro response of B cells to IL-21 and clinical B cell function.(9) Use of busulfan conditioning prior to gene therapy would be expected to promote engraftment of gene-marked HSC and therefore gene-marked B cells capable to responding to T cell help (Figure 1B). The last patient enrolled on the SIN-γc trial received low busulfan conditioning and demonstrated recovery of gene marked B cells and humoral immune function (S-YP, unpublished results). All of the 3 currently open and enrolling SIN-LV trials use low dose busulfan.(7, 8) In a trial of 8 infants, 4/8 discontinued IVIG replacement therapy and 3 of these infants responded to vaccines.(8)

Future Directions

While the busulfan dose used in current trials is low, approximately 1/3 of the myeloablative dose, exposing these young infants to alkylating agents is not without consequences. In addition

to potential long-term toxicities such as impairment of linear growth, infertility, and school function problems, myelodysplastic changes may occur in the marrow and has been reported after autologous gene therapy for other disorders. Using nontoxic conditioning, for example using depleting antibodies that target HSPC, before gene therapy would further improve the safety profile of this treatment. Finally, all of the current trials use integrating vectors as a “gene addition” approach, which predisposes not only to oncogenesis, but also fails to completely recapitulate natural gene expression patterns. Gene editing on the other hand, could be used to correct a mutation *in situ*, or insert a working copy of the gene into the natural locus, allowing endogenous control of expression. Based on encouraging pre-clinical proof of concept, gene editing for SCID-X1 may also soon find clinical application.(10) Licensure of these technologies is anticipated to change the standard treatment for SCID-X1.

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Figure Legends

Figure 1A: The role of γc in multiple cytokine receptor complexes and cell type specific functions.

Figure 1B: Immune reconstitution after gene therapy for SCID-X1. Without treatment, T cells fail to develop and B cells do not express IL2RG. Without conditioning, gene marked progenitors seed the thymus and give rise to gene-marked T cells. B cells remain unresponsive to γc-dependent cytokines, remain naïve (light blue), and fail to mature into antibody producing B cells. With conditioning, gene marked HSC continue to seed the thymus with progenitors, sustaining T cell production. Gene marked HSC also develop into gene marked B cells that express γc, respond to IL-21, mature into memory B cells (dark blue), and secrete antibody.

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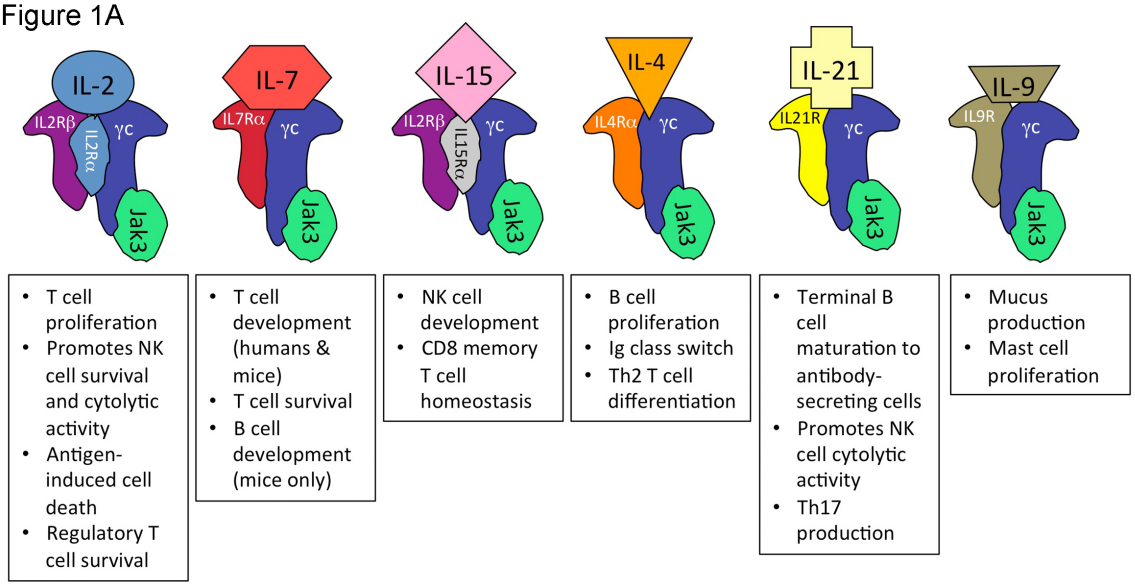
Table: Historical and current trials of gene therapy for SCID-X1

Vector	Year activated	ClinicalTrials.gov number	Sites	Conditioning regimen	Insertional oncogenesis	Target Population	Status
γRV	1999	n/a	Paris, France	none	Yes	Infants	Completed
	2001	n/a	London, UK	none	Yes	Infants	Completed
γRV	2001	NCT00028236	NIH, USA	Low dose busulfan	None reported	Post-transplant, > 18 months old	Completed
SIN-γRV	2010	NCT01129544	Boston, Los Angeles, Cincinnati, USA	none	None reported	Infants	Active not recruiting
	2010	NCT01175239	London, UK	none	None reported	Infants	Unknown
	2011	NCT01410019	Paris, France	none	None reported	Infants	Unknown
SIN-LV	2012	NCT01512888	St. Jude, UCSF, Seattle, USA	Low dose busulfan	None reported	Infants	Recruiting
	2017	NCT03315078	NIH, USA	Low dose busulfan	None reported	Post-transplant, > 2 years old	Recruiting
SIN-LV	2017	NCT03311503	Boston, Los Angeles, USA	Low dose busulfan	None reported	Infants	Recruiting
	2018	NCT03601286	London, UK	Low dose busulfan	None reported	Infants	Recruiting
SIN-LV	2017	NCT0321761	Shenzhen, China	Unknown	None reported	Infants	Recruiting
SIN-LV	2020	NCT04286815	Chongqing, China	Unknown	None reported	Infants	Recruiting

Figure 1A



- T cell proliferation
- Promotes NK cell survival and cytolytic activity
- Antigen-induced cell death
- Regulatory T cell survival

- T cell development (humans & mice)
- T cell survival
- B cell development (mice only)

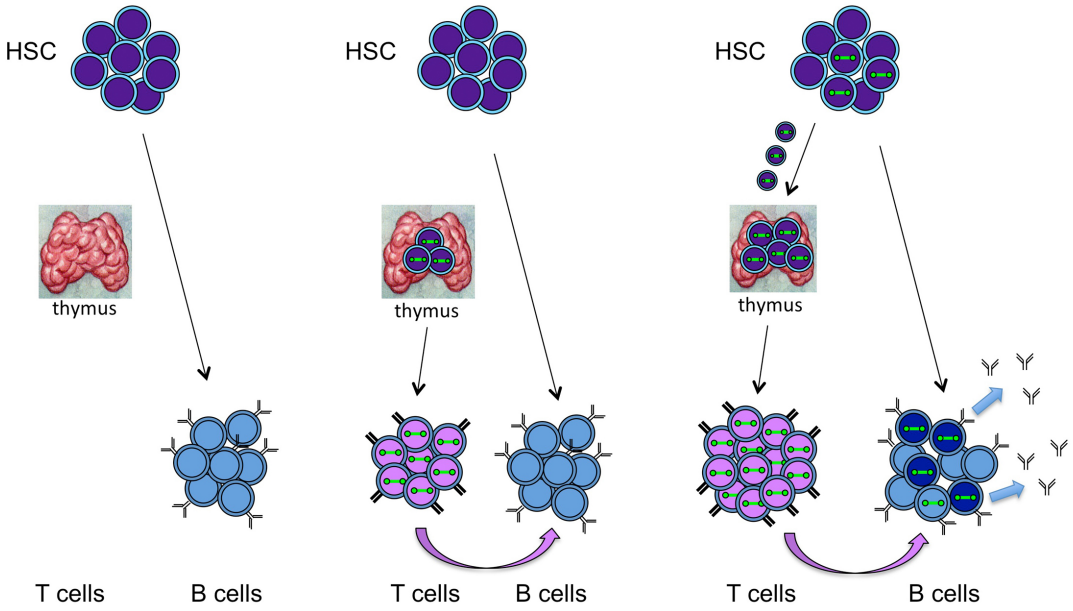
- NK cell development
- CD8 memory T cell homeostasis

- B cell proliferation
- Ig class switch
- Th2 T cell differentiation

- Terminal B cell maturation to antibody-secreting cells
- Promotes NK cell cytolytic activity
- Th17 production

- Mucus production
- Mast cell proliferation

Figure 1B



Prior to treatment
- T cells fail to develop
- No T cell help to B cells, B cells remain naïve (light blue)
- B cells γc deficient

Gene therapy w/o conditioning
- T cell development
- HSC and B cells no marking
- B cells γc deficient, fail to mature despite T cell help, remain naïve (light blue)

Gene therapy with conditioning
- T cell development
- HSC and B cells gene marked
- Marked B cells expressing γc respond to T cells making IL-21, mature into memory B cells (dark blue), and make antibody