NIH Protocol 02-I-0057

Ex Vivo Retroviral Gene Transfer For Treatment of X-Linked Severe Combined Immunodeficiency (XSCID)

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Patient Characteristics
NIH Trial of Gene Therapy for XSCID

This is protocol is designed to be "salvage" therapy for patients without an HLA-matched sibling who continue to have clinically significant impairment of immune function despite previous treatment(s) with a haploidentical donor, lymphocyte-depleted bone marrow graft.

Patient Characteristics
NIH Trial of Gene Therapy for XSCID

Up to 6 patients with XSCID

Previous history of haploidentical donor bone marrow transplant

2 to 20 years of age

Low or absent donor T-lymphocyte engraftment

Poor or absent lymphocyte mitogen and antigen responses

IVIG dependent

Recurrent bacterial and viral infections

Clinical evidence of severe chronic disease:

- Growth failure
- Diarrhea, malabsorption
- Progressive lung disease
- Eczyma and hair loss

Two patients have been enrolled in this protocol and their clinical status before gene therapy is noted below

- Growth failure (<<3% Ht & Wt)
- Chronic diarrhea
- Recurrent pneumonias and other infections
- Eczema and alopecia
- Skin infections
- IVIG dependent
- Frequent school absences

Patient (age)	Previous BMTs	T cells (cells/ul)	NK (cells/ul)	B cells (cells/ul)	IgA (mg/ml)	PHA	Candida
1 (12y)	4	96	9	263	<10	POOR	POOR
2 (10y)	2	135	0	435	<10	POOR	POOR

Protocol Characteristics
NIH Trial of Gene Therapy for XSCID

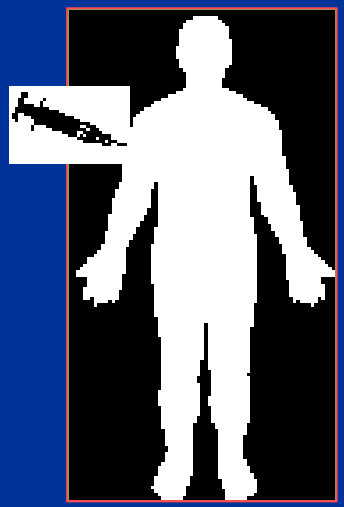
Ex Vivo Transduced Cell Product: Patient CD34+ hematopoietic stem/progenitor cells obtained by G-CSF mobilization, apheresis and immunomagnetic bead purification

Vector: MFGS-IL2RG (MuLV derived), GALV envelope pseudotyped

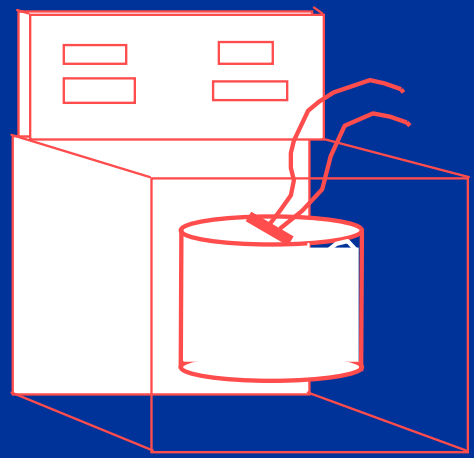
Culture conditions: Serum free medium with SCF, TPO, FLT3L 50 ng/ml, IL6 25 ng/ml and IL3 10 ng/ml in fibronectin fragment coated gas permeable flexible plastic bags

Transduction Conditions: 18 hours pre-stimulation followed by 7 hour transductions each day for four days at MOI of about 2-3

Patient CD34+ Cell Mobilization, Collection, Selection



G-CSF Mobilizes Hematopoietic Stem Cells to Peripheral Blood

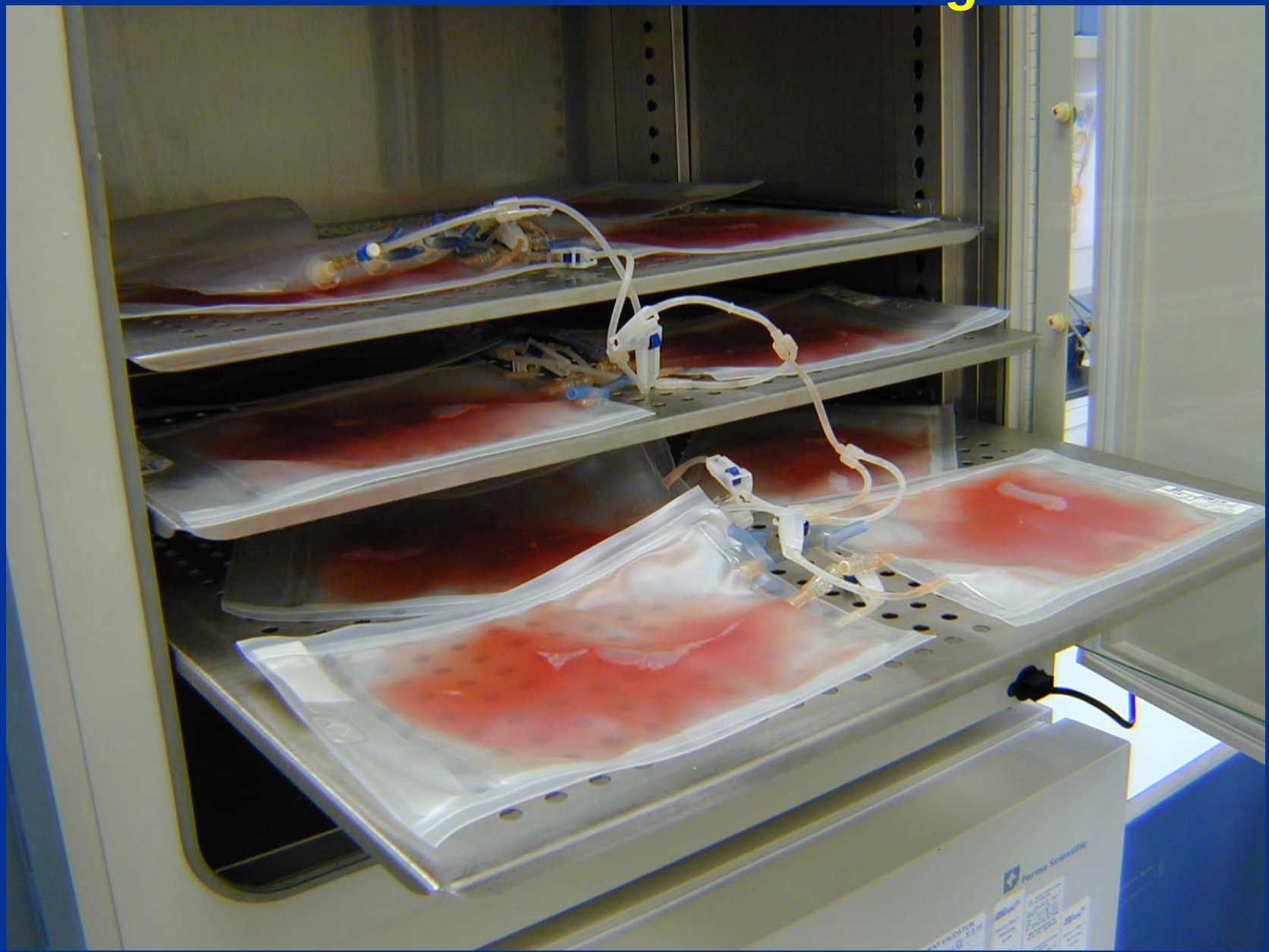


Collect by Apheresis

Immunomagnetic Bead Purification of CD34+ Cells Using Isolex 300i

About 5 x 10^8 CD34+ cells were obtained for ex vivo transduction from both Patients 1 and 2, and 100% of CD34+ cells were of host origin

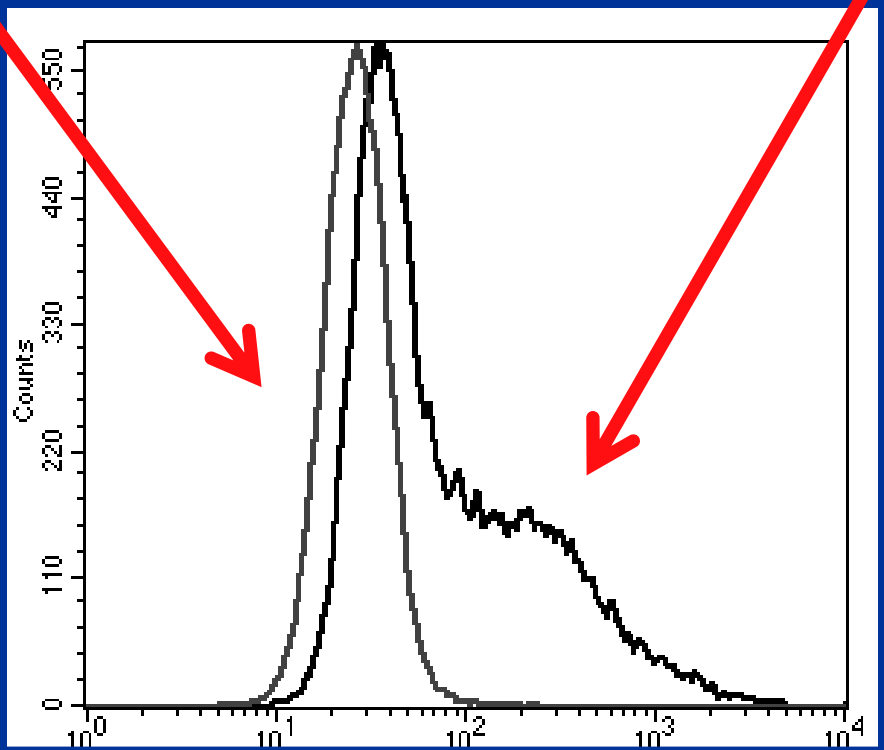
Transduction x4 days in Retronectin Coated Gas Permeable Flexible Plastic Bags



Flow Cytometry Analysis of IL2R γC Chain Transgene Expression on the Ex Vivo Transduced CD34+ Cells from Patient 1

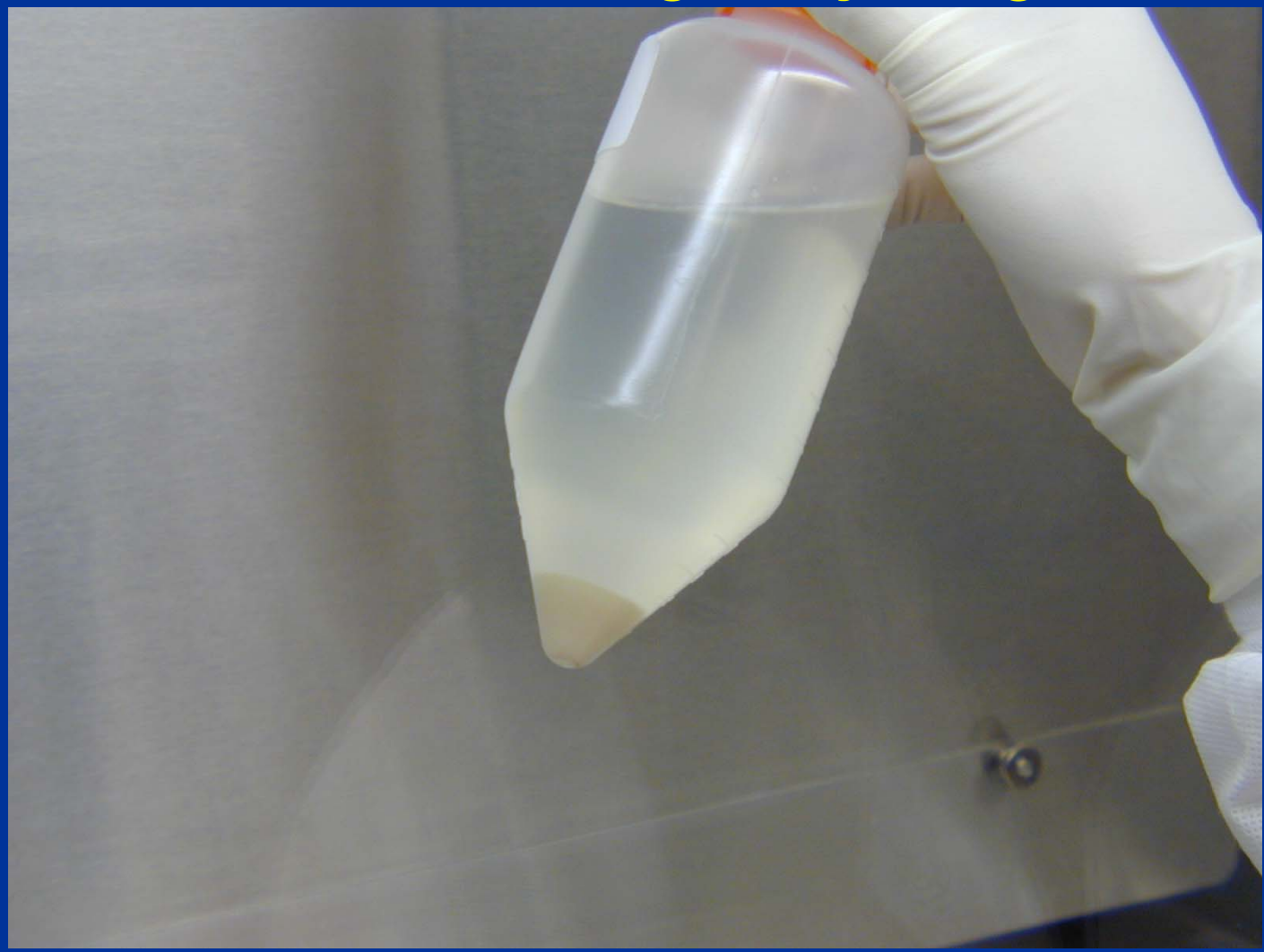
Non-Transduced CD34 Cells

Transduced CD34 Cells (40% expressing γc chain)



γc chain

3.5 fold proliferation; 80% of cells still CD34+ at end of culture; 40% transduction efficiency; both Patient 1 and 2 infused IV with about 32 x 10^6 transduced CD34+ cells / kg body weight

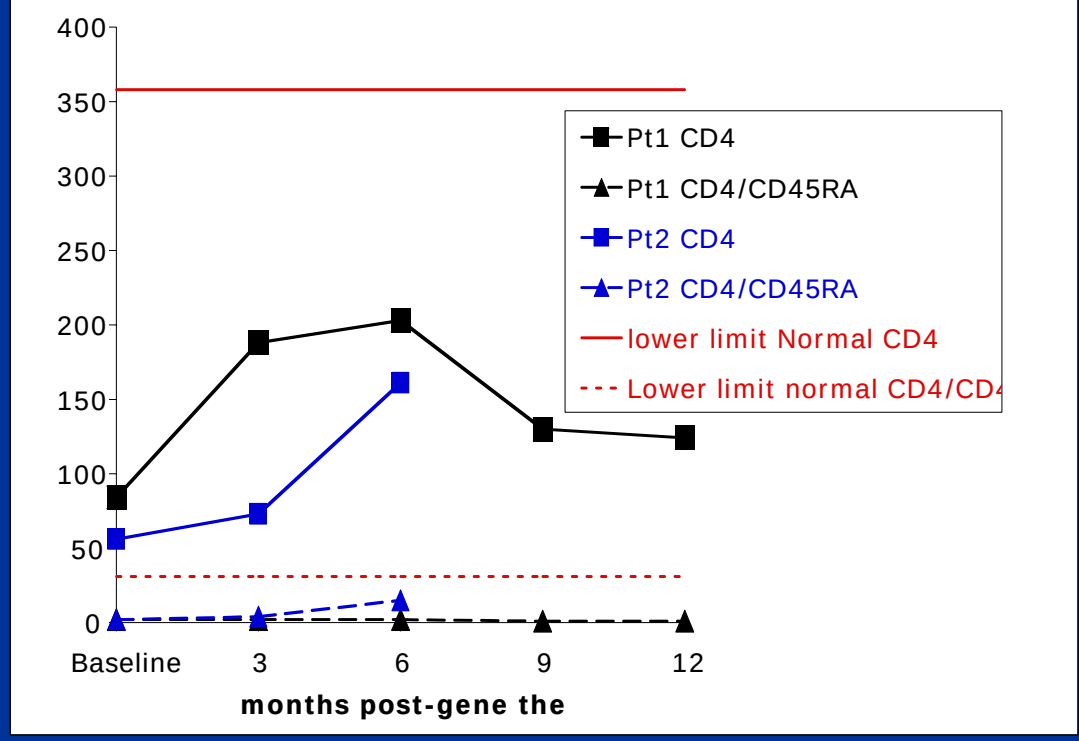


Vector Copies in Peripheral Blood Cell Lineages Expressed as Percentage Relative to a Single Copy Control Cell Line = 100%

Patient 1: vector copies/cell by real-time PCR			
Time post Gene Therapy	CD3 T Cells	CD19 B Cells	CD15 Granulocytes
6 months	4.3%	0.1%	0.1%
12 months	2.6%	0.4%	Undetectable

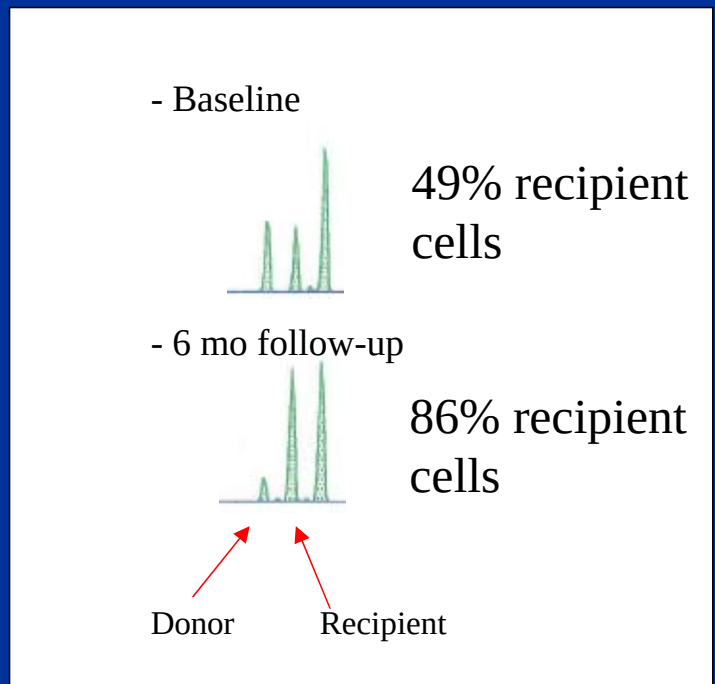
Patient 2: vector copies/cell by real-time PCR			
Time post Gene Therapy	CD3 T Cells	CD19 B Cells	CD15 Granulocytes
6 months	137.0%	44.4%	0.7%

CD4 T cells and CD4/CD45RA T cells

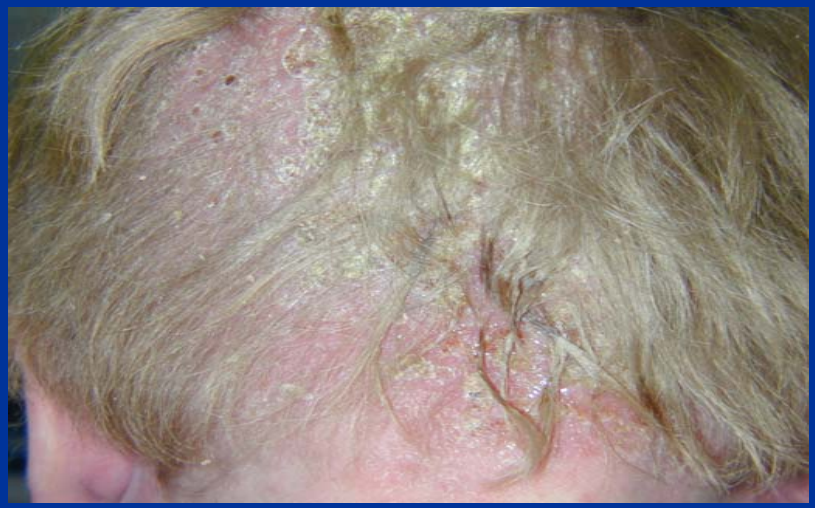


Patient 2:
At 6 months lymphocyte proliferation to Candida was newly detected

Chimerism in Patient 2



Patient 1: Improvement in eczema and alopecia



before gene therapy

6 months after gene therapy

Reports and Observations at 6 months

Patient 1

- Improved well-being, fewer missed school days
- Resolution of chronic abdominal pain
- Height: 2 cm growth in 6 months
- New cervical lymph node and tonsils
- Reduced chronic abdominal distension
- Improved eczema

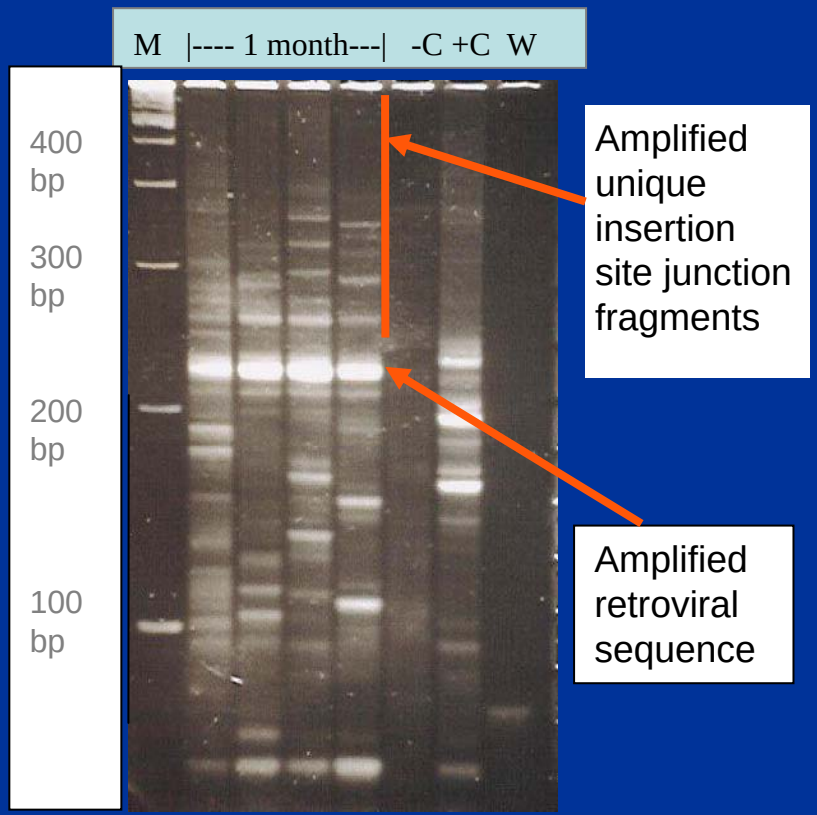
Patient 2

- Fewer headaches
- Reduced frequency of daily diarrhea
- Height: 3 cm growth in 6 months

  Weight: +0.5 kg

Safety Monitoring: Polyclonality of Insertion Sites

1. LAM-PCR: Polyclonal pattern



2. Insertion site sequencing

- 203 unique authentic insertion sites obtained by linker-PCR (Wu, 2003) from a sample of Pt 1 transduced CD34 cells, before infusion

- No insertions were found near *LMO2*

Conclusions

SAFETY:

No adverse effects to date in 2 pre-adolescents with XSCID who received autologous *IL2RG*-gene-corrected CD34 cells.

Post-infusion peripheral blood shows polyclonality of transduced cells.

RCR testing negative in product and in patient blood cells

- EFFICACY:

Multi-lineage marking of blood cells, at 6 months after gene transfer in both patients.

Lineage-specific vector provirus marking in T cells, B cells and NK cells (dependent on γc).

In Patient 2, at six months, we observed increase of CD4 T cells, increase of naïve T cells, and new substantial T-cell proliferative response to Candida.

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XSCID patients and their families