Gene Therapy for X-linked Severe Combined Immune Deficiency in Preadolescents

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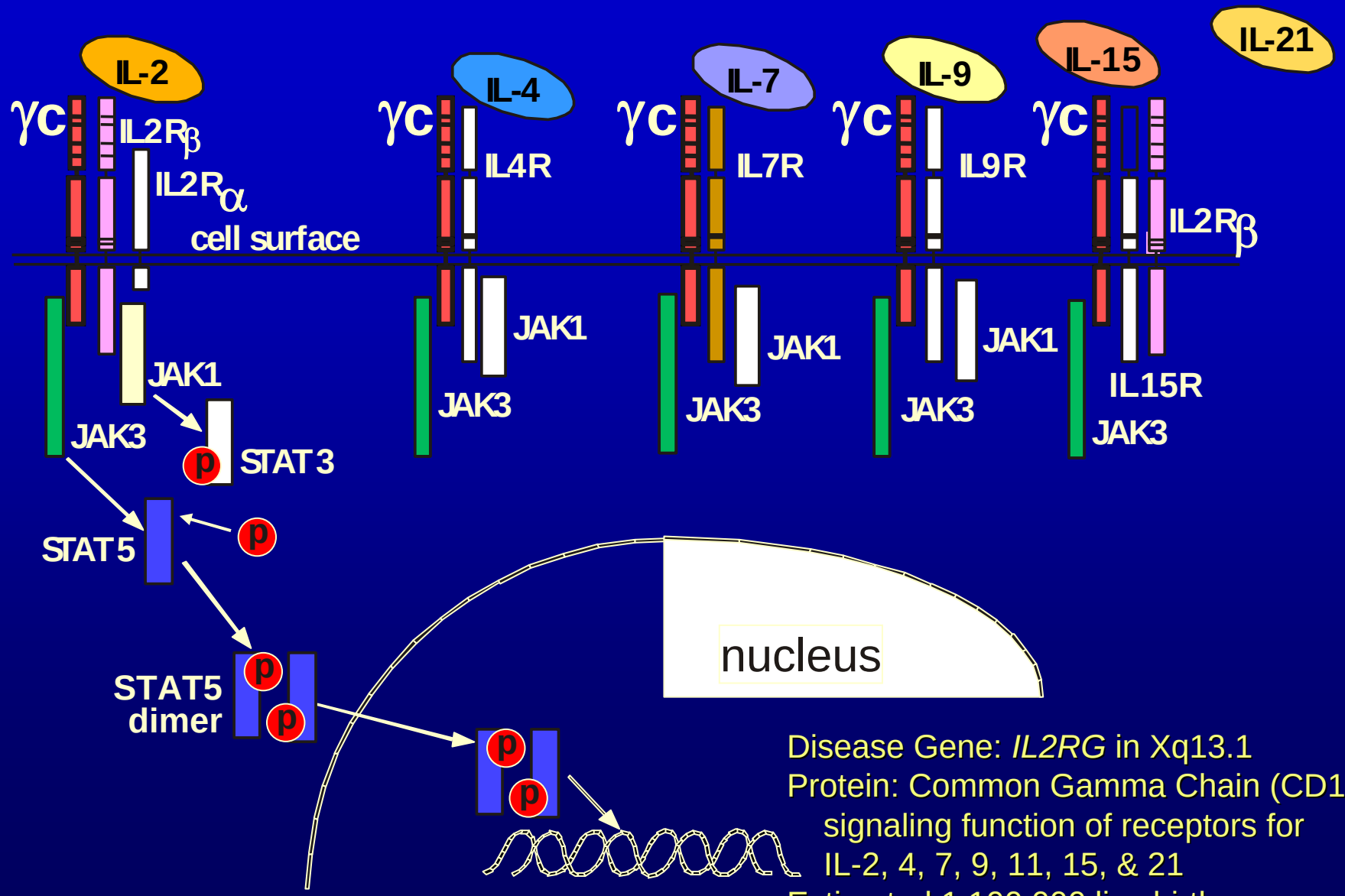
Severe Combined Immunodeficiency--SCID

- **Profound lack of T-cell and B-cell immunity**
- **Recurrent opportunistic infections beginning at 2-4 months of age**
- **Fatal in infancy without treatment providing some immune reconstitution**
- **Most common form is X-linked (SCID-X1)**

XSCID Gene *IL2RG*: Common Gamma Chain (γc) of Cytokine Receptors for Interleukin 2, 4, 7, 9, 15, & 21

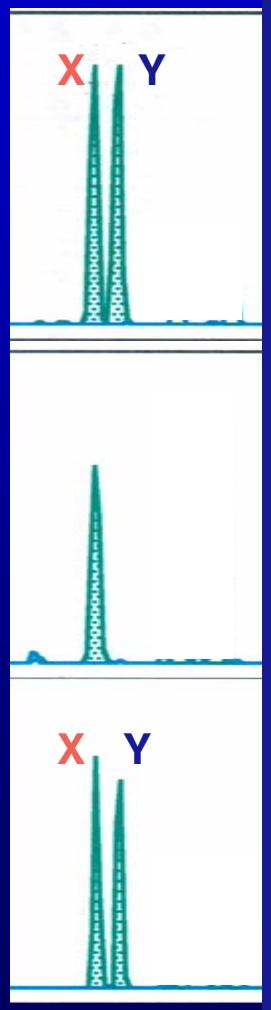


Disease Gene: *IL2RG* in Xq13.1
Protein: Common Gamma Chain (CD132)
signaling function of receptors for
IL-2, 4, 7, 9, 11, 15, & 21
Estimated 1:100,000 live births

Limitations of Parent Derived Haploidential Lymphocyte Depleted Bone Marrow Transplant for XSCID

- **Most patients lack an HLA-matched sibling and receive graft from a parent**
- **Graft vs host disease**
- **Persistent immune defects**
  - **Poor or lack of donor B cell engraftment where host B cells are not functional**
  - **Progressive decreases in donor T cells and loss of donor T cell diversity**
  - **NK defect not repaired**

Example of graft from mother



CD14/15 granulocytes, monocytes

CD3 T cells

CD19 B cells

Reports of Correction of XSCID Infants with Gene Therapy as the Primary Treatment in Infants Lacking a Matched Sibling Donor

Cavazzana-Calvo, Hacein-Bey, de Saint Basile et al. **Gene Therapy of Human Severe Combined Immunodeficiency (SCID)-X1 Disease.** Science 2000:669-72.

Hacein-Bey-Abina, Le Deist, Carlier et al. **Sustained Correction of X-Linked Severe Combined Immunodeficiency by ex Vivo Gene Therapy.** New Engl J Med 2002; 288:1185-92.

Gaspar HB, Parsley KL, Howe S, et al. **Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector.** Lancet 2004;364:2181-7.

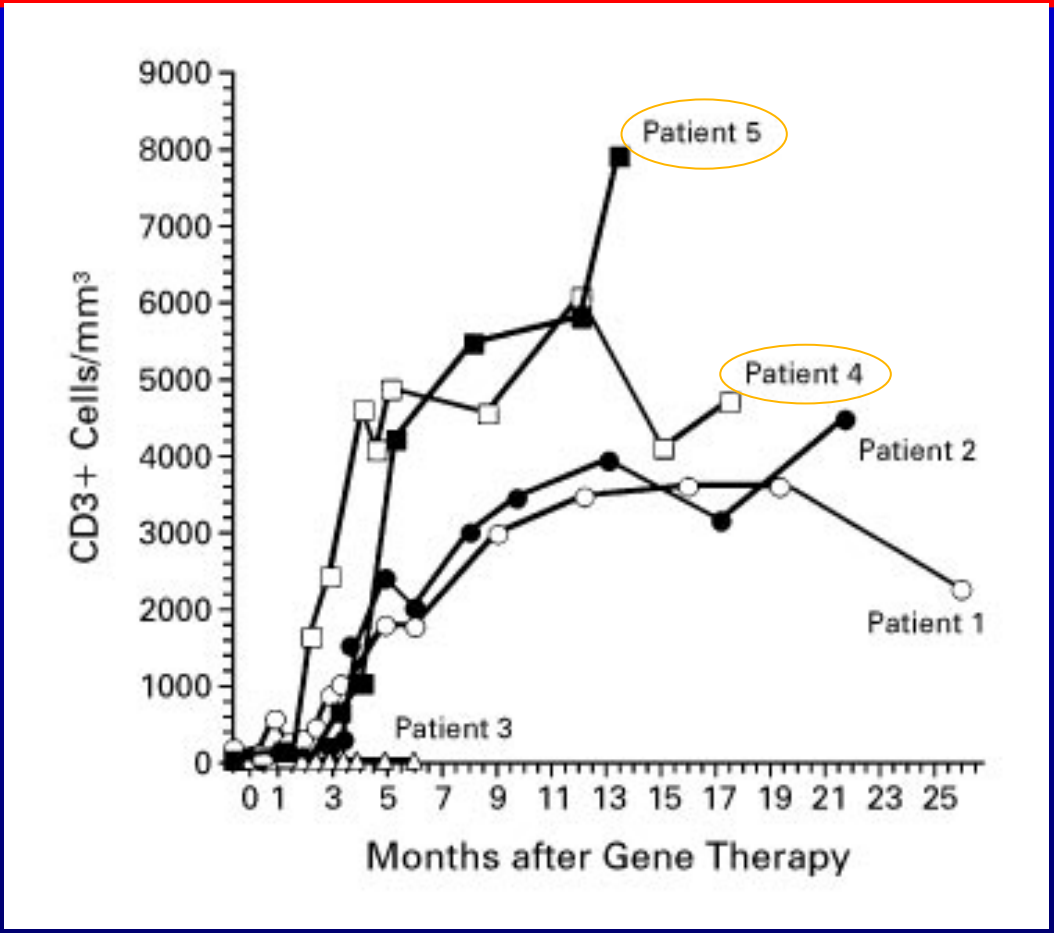
Gene Therapy for Infants with XSCID

European Trials: Gene therapy as primary treatment of XSCID infants who lack an HLA-matched related donor. 10 infants treated in France and 10 in England.

Advantages:

- Tolerated in severely ill patients
- Sustained immune reconstitution seen in 18 patients receiving >3 million corrected cells/kg
- No graft vs. host disease
- Some B cell correction observed

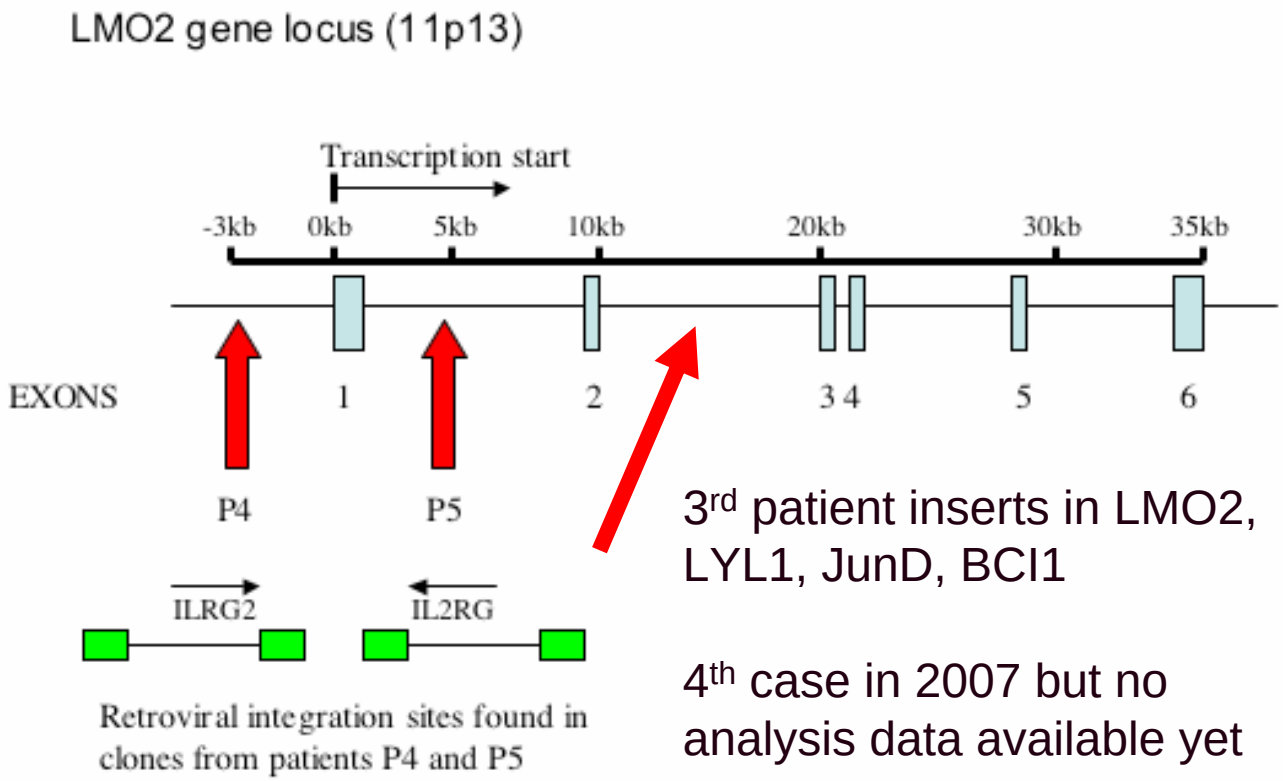
In 2002-3 Leukemia first seen after 3+ years in 2 XSCID patients among the first five



Hacien-Bey-Abina et al. NEJM, 2002

In late 2004 a 3rd patient and in 2/2007 a 4th patient of the 10 treated in France developed lymphocytic leukemia.

LMO2 Insertion Sites in 3 Cases of Leukemia after XSCID Gene Therapy (4th case recently)



3rd patient inserts in LMO2, LYL1, JunD, BCI1

4th case in 2007 but no analysis data available yet

NIH Clinical Trial of Gene Therapy for XSCID

Salvage Treatment Protocol for:

Older children-

Previously receiving one or more haploidentical T-lymphocyte depleted BMT from parent(s) as infants;

Did not achieve satisfactory immune reconstitution or subsequently have significant waning of immune function and loss of T cell diversity;

Have chronic severe medical problems that include:
- **Severe growth failure (<<3% height and weight)**
- **Diarrhea, malabsorption, colitis**
- **Skin rashes, alopecia, warts, molluscum**
- **Progressive lung disease (bronchiectasis/bronchiolitis obliterans/recurrent pneumonias)**

At the end of January 2004 the first patient (P1), an 11 years old, was treated in the XSCID Gene Therapy Trial initiated at NIH.

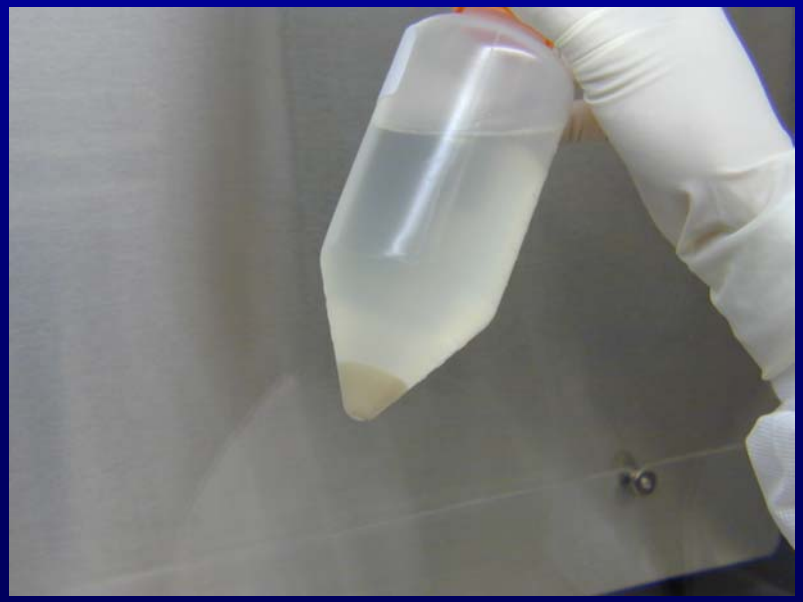
Patients 2 (P2) and 3 (P3) were 10 and 14 years old when treated in August 2004 and in July 2005, respectively.

Some Comparisons of XSCID Trials

Factor	France	England	USA (NIH)
Patients	Infants	Infants	Preadolescents
Therapy	Primary	Primary	Post haplo transpl.
CD34 source	Marrow	Marrow	Mobilized PBSC
Vector type	MFG	MFG	MFGS
Envelope	Amphotropic	GALV	GALV
Medium	X-VIVO 10	X-VIVO 10	X-VIVO 10
Protein suppl.	4% Fetal CS	1% Hum Ser Alb	1% Hum Ser Alb
Cytokines	SCF 300	300	50 ng/ml
	FLT3L 300	300	50 ng/ml
	IL3 60	20	5 ng/ml
	----	TPO 100	50 ng/ml
	----	----	IL6 25 ng/ml
Cell growth Over ~4 days	~ or >5x	~4x	~3.5x
Time to Full Reconstitution	~6 mo	>>6 mo	NA

Transduction Protocol

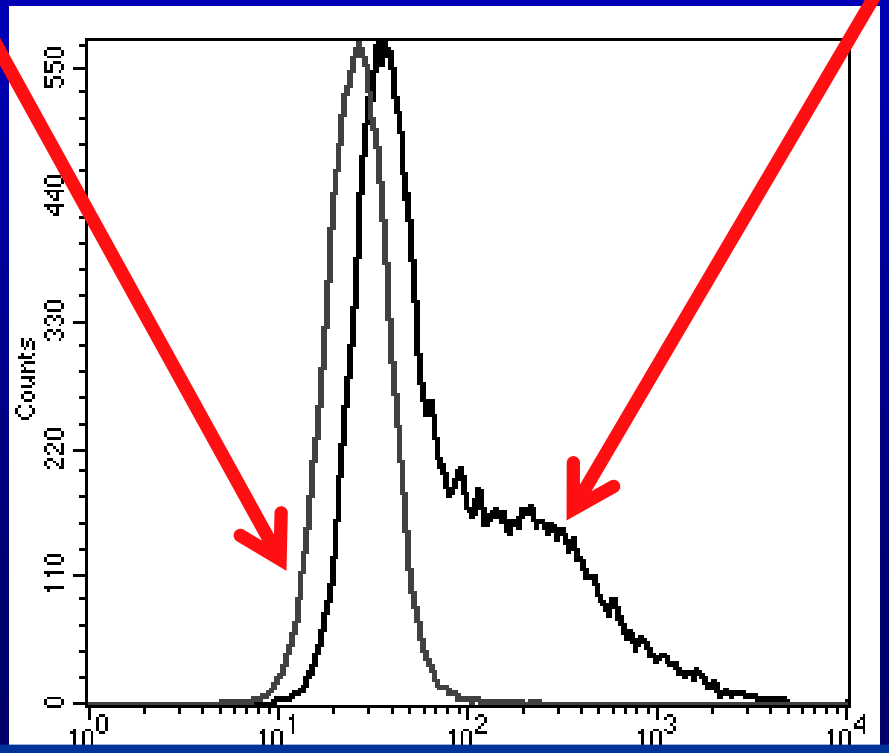
1. Thaw frozen CD34 cells
2. Incubate 24 hrs in X-VIVO media with SCF, Flt3-L, TPO, IL-6 and IL-3 in Fibronectin-coated plastic bags
3. Expose cells to viral vector-containing supernatants daily for four days
4. Wash, then infuse transduced cells



γc Chain Staining of Transduced Cells

Non-Transduced CD34$^+$ Cells

Transduced CD34$^+$ Cells (40% expressing γc chain)



γc chain

~30 million γc$^+$ CD34$^+$ cells/kg were infused into each of the three patients with XSCID; ex vivo transduction ranged from 39%-42%

Patient	P1	P2	P3
Early history			
IL2RG mutation	Poly-A addition site	R289X	M145 frame shift
Expression of γc protein at cell surface	Trace amount; functional	Normal amount; non-functional truncated	None
Age at first BMT (months)	6	11	6
Number of prior haploidentical parental BMTs	4	2	1
Adequate T-cell function for >2 years post BMT	No	Yes	Yes
Evaluation prior to gene therapy			
Age at gene therapy (years)	11	10	14
Weight and height percentile for age[a]	<<3%	<<3%	<<3%
Chronic conditions in addition to recurrent pneumonias and poor growth	Diarrhea, eczema, asthma, alopecia	Diarrhea, sinusitis, asthma, alopecia, malabsorption	Diarrhea, sinusitis, bronchiectasis, warts
BMT donor chimerism	No donor engraftment	T cells 49% donor; other lineages 100% host	T cells 100% donor; other lineages 100% host
$CD3^+$ T cells/μL (nl, 650-2108)	91	209	1,361
$CD4^+$ T cells/μL (nl, 362-1275)	84	56	168
$CD19^+$ B cells/μL (nl, 49-424)	263	435	14
$CD16/56^+$ $CD3^-$ NK cells/μL (nl, 87-505)	9	0	
Transduction of $CD34^+$ cells			
Initial $CD34^+$ cells/kg placed in culture[a]	2.77×10^7	2.33×10^7	1.79×10^7
Total cells/kg harvested (80-90% $CD34^+$)[b]	9.5×10^7	8.0×10^7	7.8×10^7
$CD34^+$ γc-transduced cells/kg, final product[c]	2.92×10^7	2.85×10^7	3.13×10^7

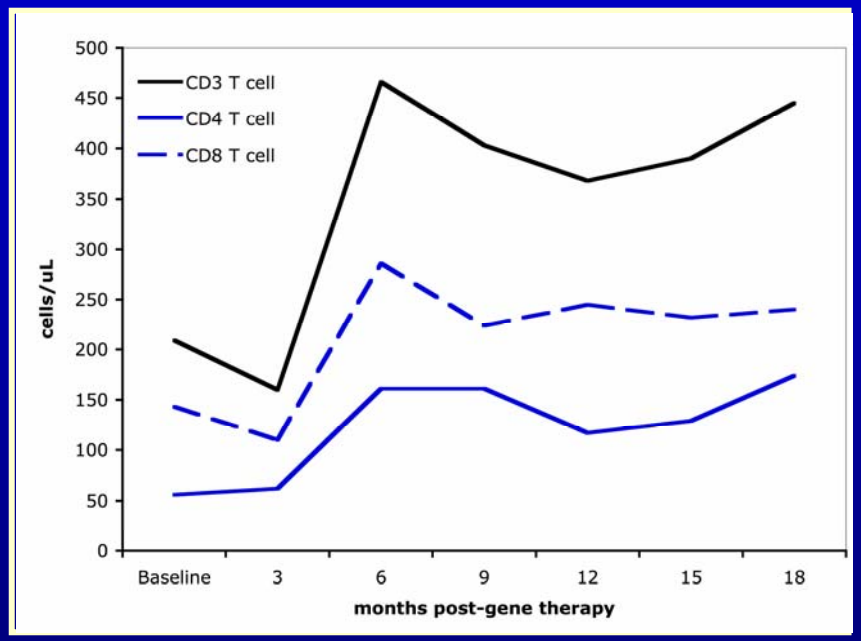
NIH XSCID gene therapy patients: Pre-treatment Immune status

Immunophenotype and Lymphocyte Proliferation before Gene Therapy							
PT #	CD4 T cells/uL (Nl:362-1275)	CD8 T cells/uL (Nl:344-911)	CD56+ CD3- NK cells/uL (Nl:87-505)	B cells/uL (Nl:49-424)	TREC	PHA (cpm) (Nl:>35,000)	Candida (cpm) (Nl:>8,000)
1	84	7	9	263	0	1,136	260
2	56	143	0	435	0	3,944	1,792
3	168	990	3	14	0	7,853	3,945

Vector copies per peripheral blood lineage cell by quantitative real-time DNA PCR.

Months Post Gene Therapy	P1			P2			P3		
	T cell	B cell	Myeloid	T cell	B cell	Myeloid	T cell	B cell	Myeloid
3	0.04[a]	ND[b]	ND	0.17[a]	ND	ND	0.28	0.10	0
6	0.005	0.001	0.001	1.37	0.44	0	0.24	0.10	0.001
9	0	0	0.016	0.93	0.08	0.07	0.25	0.05	0.001
12	0.03	0.004	0.001	1.11	0.06	0.04	0.22	0.03	0.011
18	0.05	0.06	0.004	1.04	0.04	0.04			
24	0.04	0.03	0						
27	0.06	0.003	0						

Subject P2 demonstrated the most substantial increase in peripheral blood lymphocytes; appearance of $CD4^+RA^+$ T lymphocytes and NK cells; and normalization of T cell receptor excision circles (TRECS)



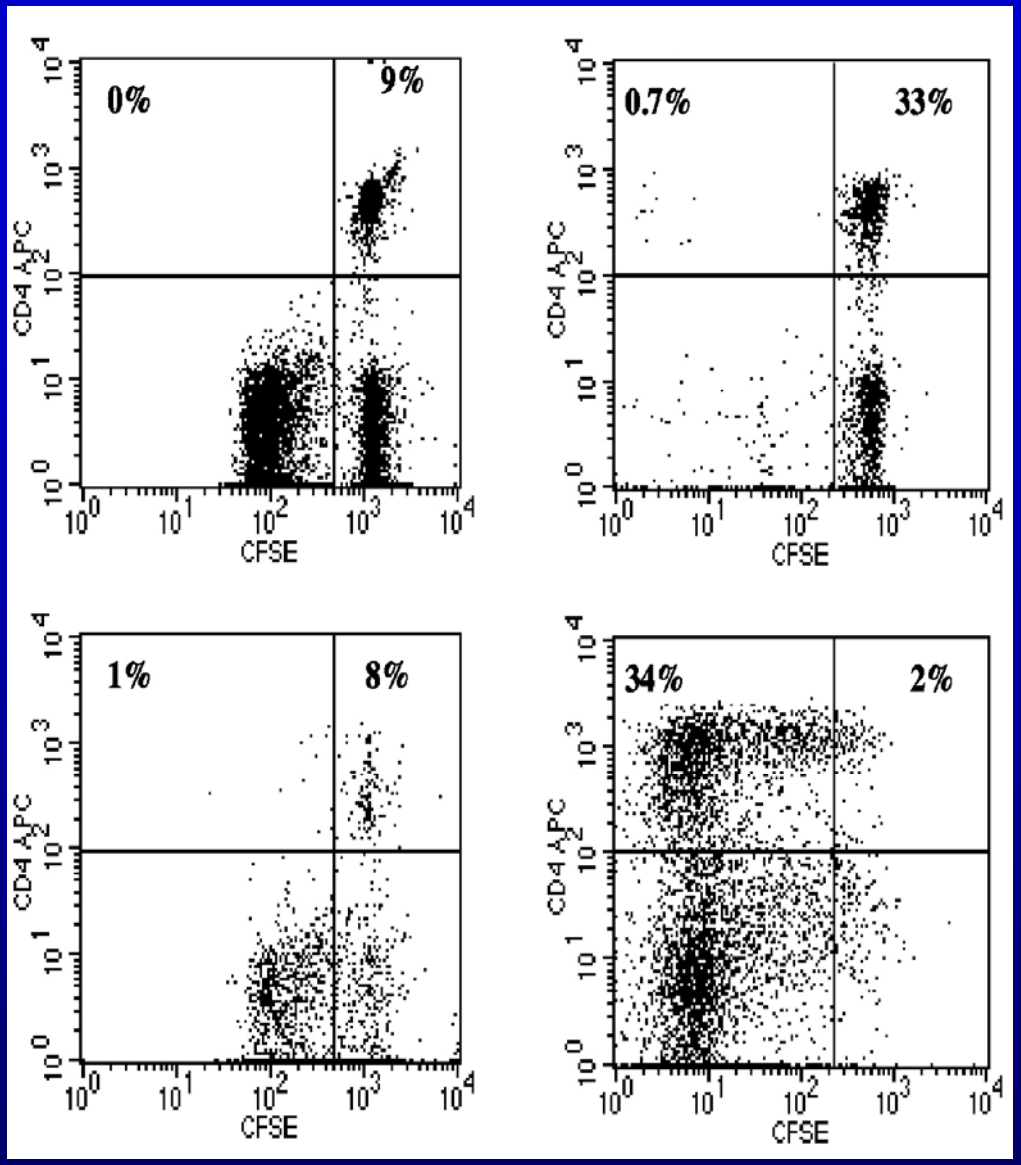
T cell subsets

	months post gene therapy						
	Baseline	3	6	9	12	15	18
CD4/CD45RA T cell/uL (normal:31-533)	2	4	**15**	**20**	**14**	**16**	**17**
CD8/CD45RA T cell/uL (normal:101-636)	19	**22**	**37**	**29**	**33**	**54**	**39**
NK cell/uL (normal:87-505)	2	**17**	**7**	**17**	**29**	**10**	**10**
TREC/ug DNA (PBMC)	<50	<50	<50	**3,910**	**2,560**	**Not done**	**2,835**

Patient 2:
At 6 months lymphocyte proliferation to Candida was newly detected in P2 and has persisted

- CD4/CD45RA T cells increased significantly from 1-2 to 14-20/ul
- NK cells increased from 0-2 to 17-29
- TRECs were undetectable before gene therapy and increased to almost normal levels for age

Gene therapy of P2 restores T cell proliferation in response to PHA mitogen stimulation as measured by dilution of CFSE fluorescence following cell division



Medium (no stimulation)

PHA stimulation

CD4

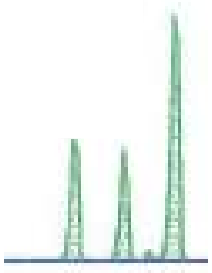
Intensity of CFSE Fluorescence

Pre-treatment

Post-treatment (12 months)

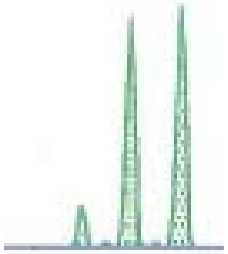
Percent of donor CD3 T Cell chimerism in P2 decreased

- Baseline



49% recipient cells

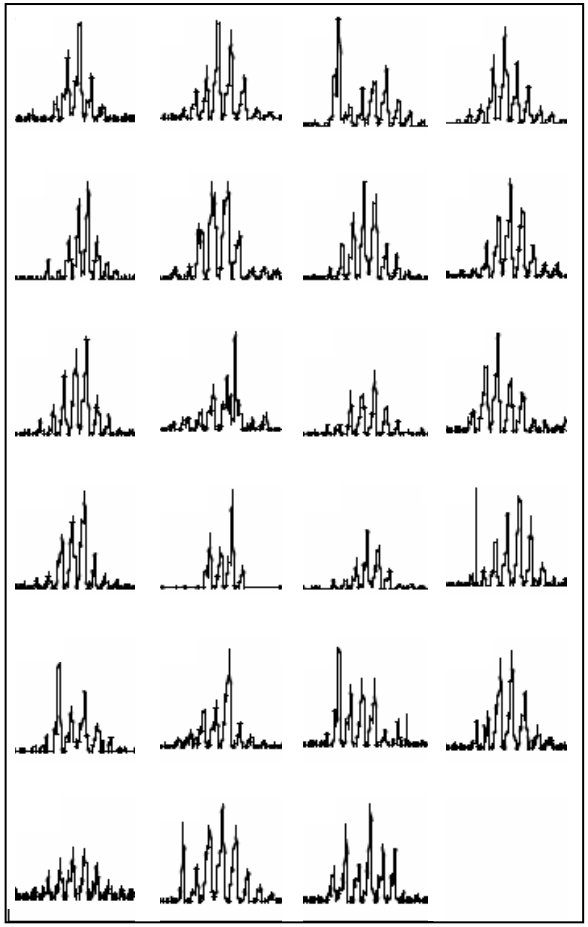
- 6 mo follow-up



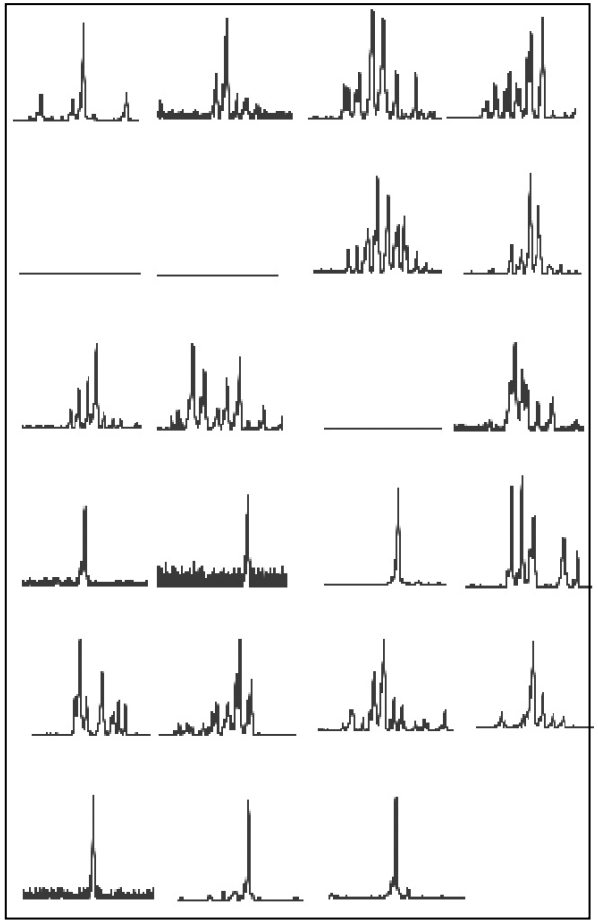
86% recipient cells

Donor Recipient

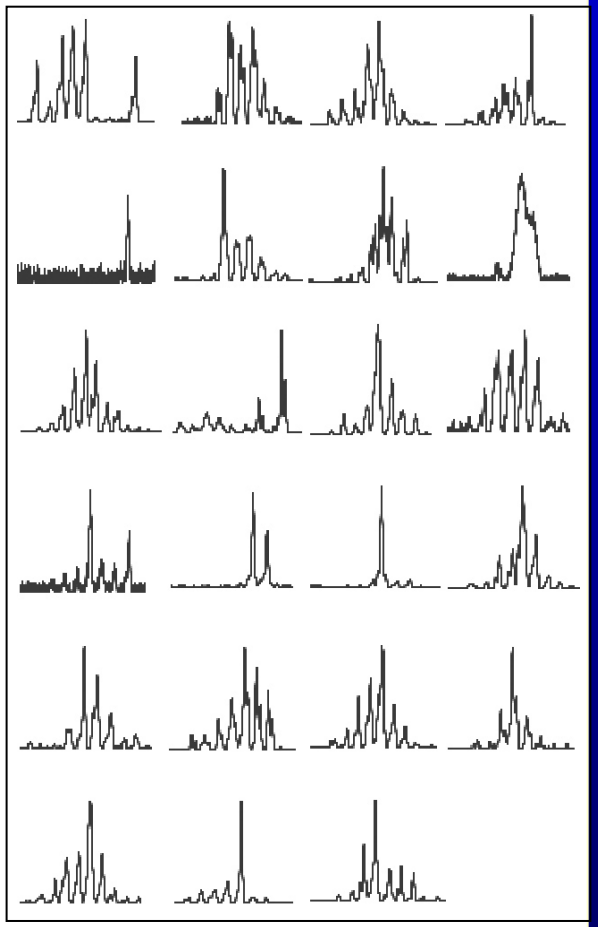
V-beta Spectrotyping (a measure of T Cell Receptor diversity) improved after gene therapy in Subject P2



Normal Control



P2 Pre-Gene Therapy



P2 12 months Post Gene Therapy

Clinical evaluation

PT 1

- Improved well-being, fewer missed school days;
- Had resolution of chronic abdominal pain
- New cervical lymph node and tonsils
- Reduced chronic abdominal distension
- Improved eczema
- But this improvement has not been sustained in all areas

PT 2

- Improved sense of well-being and fewer infections
- Accelerated growth
- Fewer headaches
- Significant reduction of diarrhea
- Resolution of chronic sinusitis

PT 3

- Initial improved well-being; but more recently has had exacerbation of bronchiectasis-related infections and bronchiolitis obliterans type of interstitial pneumonitis

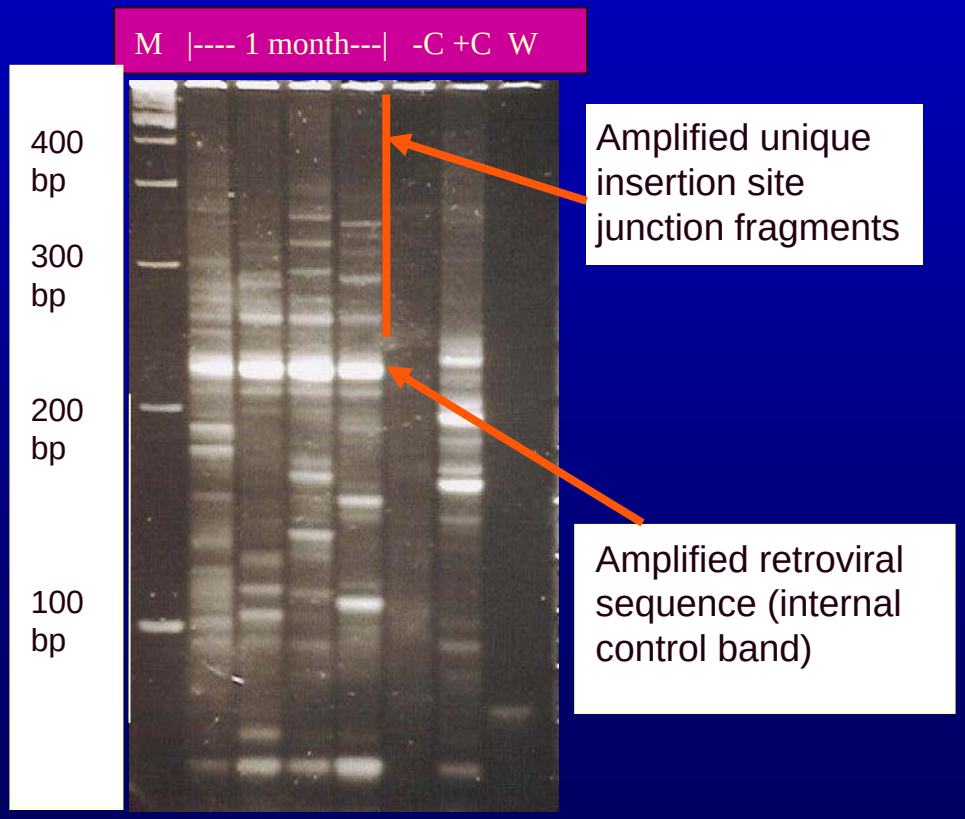
Patient 1: Improvement in eczema and alopecia

before gene therapy

6 months after gene therapy

Safety Monitoring: Polyclonality of Insertion Sites

Example of LAM-PCR: Polyclonal pattern



LAM-PCR and Linker Mediated-PCR:

Pre-infusion transduced CD34+ cells from P1-
203 insertion sites sequenced showing insertions near genes and near the 5' end of genes were more frequent than random and none near LMO2.

Post-treatment peripheral blood samples from all 3 patients-
260 distinct insertion sites (24 from P1, 219 from P2 and 17 from P3). None within 5 MB of *LMO2* or *ZNF217* and only one 58 Kb 5' to *CCND2* (genes suggested to be common integration sites with oncogenic potential).

Safety Monitoring Conclusions to Date: Polyclonality of Insertion Sites – No Sites of Concern

- Insertion site analysis using several methods to analyze gene marked in T lymphocytes from all three patients CD3 T cells demonstrates polyclonal marking without predominance of any particular insert site
- No insertions have been identified near *LMO2* or other sites of particular concern in our patient samples

Conclusions

SAFETY:

- **No adverse effects of the gene therapy to date in 3 pre-adolescents with XSCID who received autologous *IL2RG*-gene-corrected CD34 cells**

- **Analysis of retroviral insertion sites and TCR repertoire studies indicates polyclonality of transduced cells**

EFFICACY:

- **Vector provirus levels were highest in T cells; present in B cells and NK cells, lineages that are also dependent on γc expression; and lowest in myeloid cells**

- **T cell reconstitution, particularly CD4 in PT2 >> PT1, PT3; but did not reach normal numbers in any of the patients**

- **In PT2, we observed increase of CD4 T cells (though not to normal levels) and appearance of new naïve T cells, and new T-cell proliferative responses to Candida, as a result of gene therapy**

Future Gene Therapy for XSCID

1. Consider use of low dose stem cell conditioning (busulfan) and possibly even low dose lymphocyte conditioning (fludarabine) to enhance gene therapy correction in these older post-transplant patients with XSCID.
2. Continue to use cell growth conditions leading to modest expansion (<5x) before infusion.
3. Develop vectors that reduce targeting of transcriptional start sites (lentivectors).
4. Use self-inactivating vectors; incorporate insulators; possibly include suicide genes.

Gene Therapy For XSCID

- NIAID/LHD group
  - Suk See De Ravin
  - Elizabeth Kang
  - Gilda Linton
  - Narda Theobald
  - Nora Naumann (Germany)
  - Nana Kwatemaa
  - Effie Nomicos
  - Jean Ulrick
  - Tyra Estwick
  - Dianne Hilligoss
  - Julia Friend
  - Morvarid Moayeri
  - Elaine Shum
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  - Jennifer Puck (now UCSF)
  - Javier Chinen (now Baylor)
  - Joie Davis
- NIH Clinical Center DTM
  - Susan Leitman
  - Hanh Khuu
  - Ken Hines
  - Elizabeth Read (now in SF)
  - Charles Carter (deceased)
- Duke University
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