

# *US X-SCID Gene Therapy: Where do we go from here?*



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# Brief Review of the NIH Clinical Trial of Gene Therapy for XSCID in Older Children

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## 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)

**Examples of the problems affecting a subset of older children with X-SCID who have poor engraftment and / or waning immunity in the 10 years following their haploidentical T-cell depleted transplant in infancy:**

# ***Alopecia and rashes***



# ***Widespread Molluscum contagiosum***



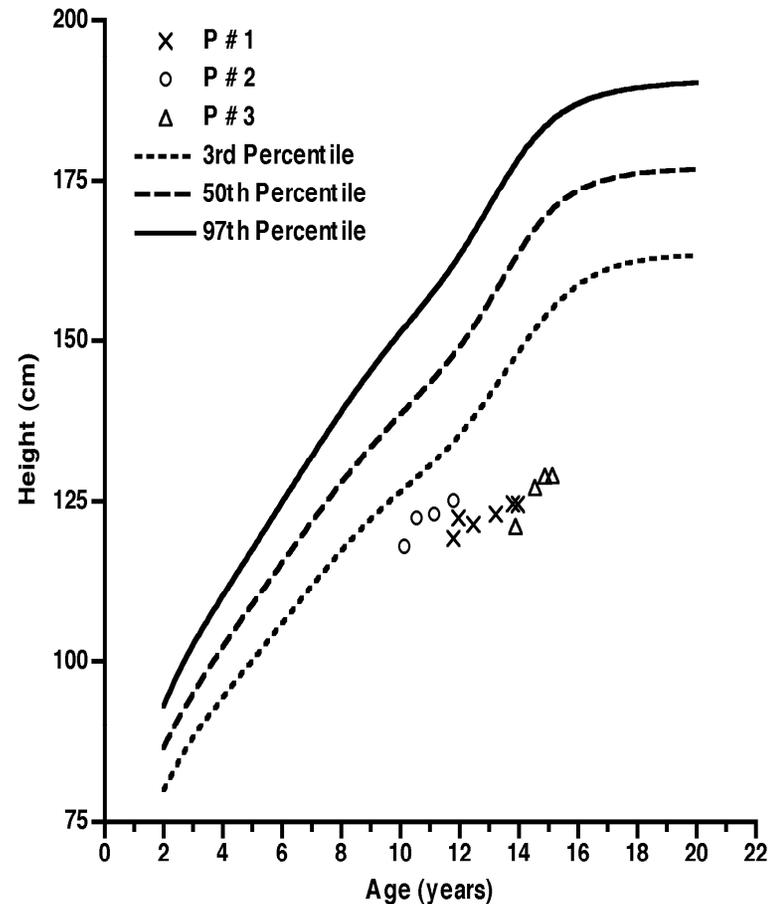
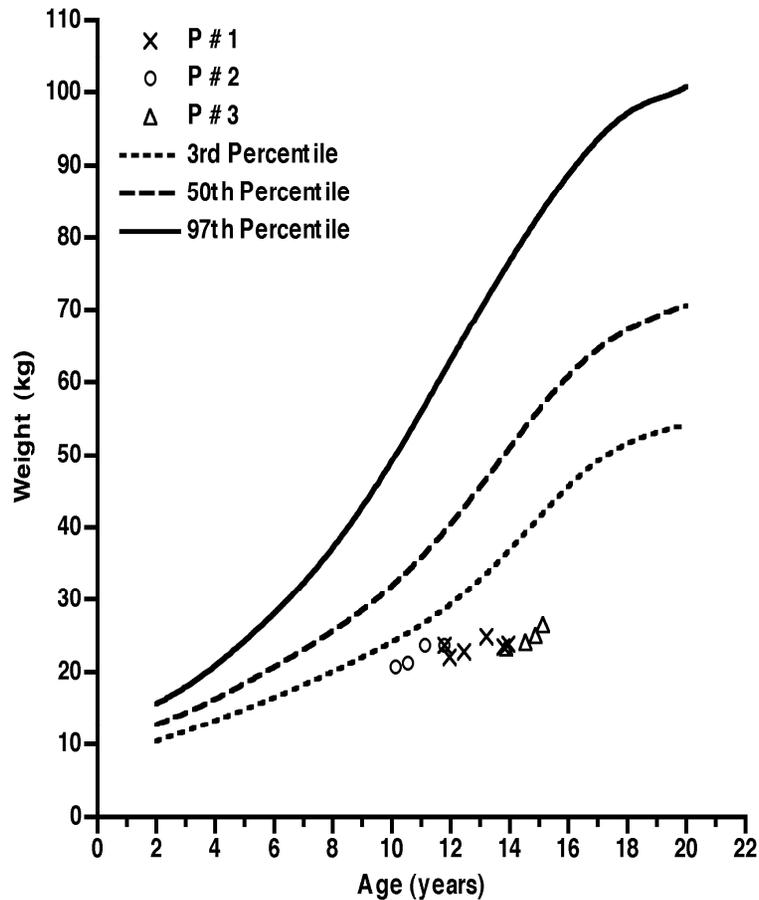
# Severe warts (HPV)



**Severe progressive bronchiectasis /  
recurrent pneumonias and/or  
bronchiolitis obliterans**



# Extreme growth delay which may or may not be associated with malabsorption / colitis / chronic diarrhea



# Three patients have been treated in the NIH protocol:

Patient #	Treatment Date	Age at Treatment
P1	January 2004	11 yo
P2	August 2004	10 yo
P3	July 2005	14 yo

**The results of these studies have been published:**

Chinen J, et al. Blood 110:67, 2007

# Brief recap of protocol:

Conditioning:

None

Vector:

Amphotropic MFGS-IL2Rgamma-chain( $\gamma$ c)

Ex vivo transduction target:

Mobilized peripheral blood CD34<sup>+</sup> stem cells

Transduction:

Daily x4 d; growth factors SCF/FLT3L/TPO/IL6/IL3

= 38-42% transduction rate

Infused: ~30 million  $\gamma$ c<sup>+</sup> CD34<sup>+</sup> per kg in all three patients

# Brief Summary of Outcome To Present:

P1: Long term gene marking of 2% of T cells  
No change in immune cell numbers or function  
No evidence for long term clinical benefit  
Continued high rate of infections and other severe problems  
August 2007 elective unrelated donor cord blood transplant

P2: Long term gene marking of >90% of T cells  
Modest improvement in T cell function and numbers  
Increase in CD4 RA T cells and appearance of TRECS  
Normalization of V-beta spectratyping (improved TCR diversity)  
Significant clinical improvement: no major infections

P3: Long term gene marking of 6% of T cells  
Similar pattern as P1; no improvement in immune parameters  
No evidence for long term clinical benefit  
Continued high rate of pulmonary infections

# Brief Summary of Outcome To Present:

Long term safety studies:

P1 no longer has gene marking post cord blood transplant

P2 and P3 continue to demonstrate polyclonality with no dominant insert clone(s) post gene therapy.

We previously reported 260 distinct insertion sites (24 from P1, 219 from P2 and 17 from P3). None were found 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). Further insert site analyses of P2 and P3 remain consistent with this previously reported pattern.

## **Treatment alternative comment:**

**P1, failing to achieve clinical benefit from gene therapy, and continuing to suffer from frequent severe recurrent infections and other life-threatening problems electively underwent a sub-ablative conditioning regimen with unrelated cord blood transplant at NIH, and has achieved full donor engraftment without GVHD at 7 months. No appropriately matched adult donor was available in the NMDP registry.**

## **Treatment alternative comment:**

**MUD or cord blood transplant with sub-ablative conditioning is an option for the older X-SCID patient with poor or waning immunity post haploidentical transplant. However, despite the good outcome to date in our P1, it must be emphasized that this is a high risk alternative therapy.**

**In addition a significant number of patients may not even have an appropriately matched cord blood or adult donor in the NMDP registry.**

# **Proposed Modifications to the NIH X-SCID Gene Therapy Protocol**

## **Why modify this protocol for older children?**

**Age matters- Older children with XSCID treated with gene therapy do not demonstrate the vigorous in vivo expansion of gene marked T cells observed in XSCID infants.**

**Possible factors- Is this poor thymic function, poor marrow engraftment, or blocking of expansion by presence of resident donor T cells or small numbers of patient non-functional T cells?**

# Proposed Modifications to the NIH X-SCID Gene Therapy Protocol

## Conditioning:

Marrow conditioning- Busulfan 4 mg/kg

Lymphocyte conditioning- Fludarabine 80 mg/m<sup>2</sup>

Prevent mucositis (possibly augment thymic stromal function)- Keratinocyte growth factor

# Conditioning schedule:

Day -10	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2	Day +3
KGF 60mg/kg/d	KGF 60mg/kg/d	KGF 60mg/kg/d								Infuse gene corrected CD34+ cells	KGF 60mg/kg/d	KGF 60mg/kg/d	KGF 60mg/kg/d
			Fludar 20mg/ m <sup>2</sup> /day	Fludar 20mg/ m <sup>2</sup> /day	Fludar 20mg/ m <sup>2</sup> /day	Fludar 20mg/ m <sup>2</sup> /day							
							Busulf 2mg/kg /day	Busulf 2mg/kg /day					

# Next Generation Gene Therapy for XSCID

- 1. Develop vectors that reduce targeting of transcriptional start sites (lentivectors).**
- 2. Use self-inactivating vectors; incorporate insulators; use mammalian promoters.**
- 3. Extensively test new X-SCID vectors for oncogenic potential using informative mouse model systems.**
- 4. Test the efficacy and safety of new vectors in a large animal model such as the X-SCID dog that closely models human X-SCID.**

# *Gene Therapy For XSCID*

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  - Ken Hines
  - Elizabeth Read (now in SF)
  - Charles Carter (deceased)
- **Duke University**
  - Rebecca Buckley

**OBA staff requested that I end my discussion by briefly reviewing the adverse events in two patients recently noted by investigators in the Frankfurt, Germany study of gene therapy for X-linked chronic granulomatous disease. Only information presented in public forum by that research team will be noted.**

**These events may or may not be relevant to the discussion of X-SCID.**

## ***Recent Trial of Gene Therapy for X-CGD by Ott et al***

Ott et al Nat Med 12:401, 2006 demonstrated high level of gene transfer in circulating neutrophils of X-CGD in two adult patients treated with ex vivo gene therapy using non-myeloablative busulfan (8 mg/kg) conditioning, and using SF71-gp91<sup>phox</sup>, a strong-promoter-driven spleen focus forming gamma retrovirus vector.

Each patient received  $3-6 \times 10^6$  / kg CD34<sup>+</sup> PBSC at 38-40% ex vivo marking transduction efficiency.

Initially marking of circulating neutrophils was 15-20%, but unexpectedly, marking rapidly increased selectively in the neutrophil lineage up to 35-60%.

Average oxidase activity per gene-corrected circulating neutrophil was initially 14% and 30% of normal control neutrophils, respectively, in each of the two patients, but progressively decreased over time.

There was a profound in vivo clonal outgrowth of those myeloid clones where vector had inserted in and activated MDS1-EVI1, PRDM16 or SETBP1.

>80% of vector inserts were in these three genes with one or two clones dominating myelopoiesis.

Subsequently, one patient died of infection at two years, having lost oxidase activity, despite high level gene marking. The second patient continues to have high level gene marking, but also lost oxidase activity (**Grez et al. Blood Cells Mol Dis 40:268, 2008**).

At the **4th Conference on Stem Cell Gene Therapy, Thessaloniki, Halkidiki, Greece, September 13-17, 2007**, Dr. Grez also reported that that the dominant gene marked clones with inserts in MDS1-EVI1 in each patient exhibited monosomy 7. The safety of the SFFV based vector used in this study has been called into question, though other unknown factors may be responsible for these events.

