

Human Gene Transfer Protocol #0901-964:
*Lentiviral Gene Transfer for Treatment of Children
Older Than 1 Year of Age with X-SCID*

PIs:

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Protocol #0901-964: LVXSCID-OC

- 1. Prior results of clinical gene transfer treatment of older children with X-SCID.***
- 2. Rationale for use of busulfan conditioning.***

**Harry L. Malech M.D.
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Previous NIH Clinical Trial of Gene Transfer for XSCID (3 Patients treated 2004-5; Jennifer Puck and Harry Malech)

Chinen J, et al: Gene therapy improves immune function in preadolescents with X-linked severe combined immunodeficiency. Blood 2007 110:67

Salvage treatment for older children with X-SCID:

No HLA-matched sibling;

Previous haploidentical BMT as infants;

Did not achieve or sustain immune reconstitution;

Have chronic severe medical problems:

Recurrent infections

Severe growth failure

GI dysfunction: Diarrhea, malabsorption, colitis

Skin rashes, alopecia, other auto/alloimmune problems

Skin warts, molluscum

Mucocutaneous candidiasis

**Pulmonary dysfunction (e.g. bronchiectasis/bronchiolitis
obliterans/fibrosis/pulmonary hypertension)**

Brief review of previous protocol:

Conditioning: None

Vector: Amphotropic MFGS-IL2Rgamma-chain(γ c)
(gamma retrovirus vector encoding common gamma chain)

Ex vivo transduction target: Mobilized PB CD34⁺ stem cells

Transduction: Daily x4 d = 38-42% transduction rate

Infused: ~30 million γ c⁺ CD34⁺ per kg in all three patients

Three patients treated at NIH:

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

NIH XSCID gene therapy patients: Pre-treatment Immune status

Immunophenotype and Lymphocyte Proliferation before Gene Therapy

PT #	CD4 T cells/uL (NI:362-1275)	CD8 T cells/uL (NI:344-911)	CD56+ CD3- NK cells/uL (NI:87-505)	B cells/uL (NI:49-424)	TREC	PHA (cpm) (NI:>35,000)	Candida (cpm) (NI:>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

Pt#	Type of mutation	γ c function	Donor Engraftment
1	PolyA tail defect	Trace normal γ c	None in any lineage
2	Mis-sense	Protein +; Function -	51% mother; T cells only
3	Truncation	Protein -; Function -	98% father; T cells only

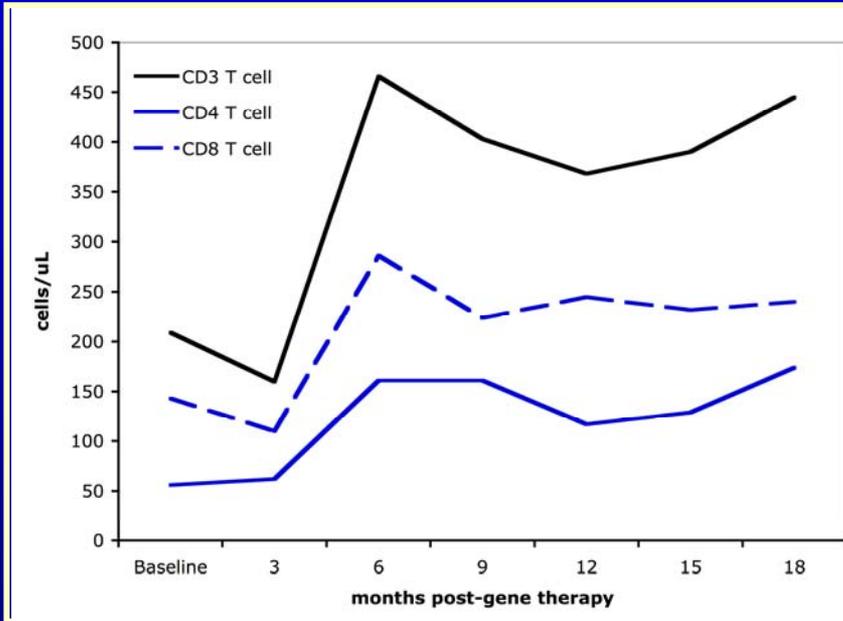
No donor CD34⁺ cells detected in any of the 3 patients.

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	0.06	0.01	0.001

These marking levels and patterns persisted for 3.5, 4.5 and 3.5 years in P1, P2 and P3 respectively. P1 underwent a successful cord blood transplant at 3.5 years post gene therapy.

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

**Patient 2 has acquired:
Normal lymphocyte
proliferation to Candida
and mitogens.**

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

- CD4/CD45RA T cells increased significantly from 1-2 to 14-20/uL
- NK cells increased from 0-2 to 17-29
- TRECs increased to almost normal levels; Vβ spectrotypes normalized
- All improvements have persisted

Experience of other investigators treating older X-SCID patients with gene transfer

Thrasher AJ, et al: Failure of SCID-X1 gene therapy in older patients. Blood 2005 105:4255.

P1: 20 yo; truncation mutation; previous haplo transplant paternal donor; <1% marking after gene transfer.

P2: 15 yo; mis-sense mutation with trace γ c; only trace marking after gene transfer (undetectable by 6 months).

Combined experience from Chinen et al and Thrasher et al:

Of five post-haplo transplant older patients with X-SCID treated at 10-20 years of age with gene transfer, only one patient (the youngest) had substantial sustained gene marking (> 90% of autologous T cells) and evidence of clinical benefit. However, even that patient failed to achieve normal numbers of T cells and continues to require IVIG.

We may conclude that:

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** or **inadequate marrow engraftment?**

Is Poor Thymic Function the Main Barrier

Patient #2 in our study produced CD4⁺ CD45RA⁺ cells and acquired TRECs following gene transfer.

Patient #1 in our study at age 15 (3 yrs post gene transfer) received a matched unrelated donor cord blood transplant with busulfan/radiation/ATG conditioning. At 1.5 years post transplant he is fully engrafted with improved immune function, including production of IgG.

We conclude that older X-SCID patients do retain sufficient thymic function to achieve clinical benefit, if normally functioning T cell precursors are provided in sufficient numbers by gene transfer or allogeneic donor transplantation. Marrow conditioning prior to gene transfer may help enhance production of T cell precursors.

Previous experience with marrow conditioning to enhance outcome of gene transfer

Aiuti, A et al, Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency, N Eng J Med (2009) 360:447-458

Supplementary Discussion section commentary regarding the rationale for use of Busulfan 4 mg/kg total to achieve substantial enhancement of immune correction after gene transfer in ADA deficient SCID:

“Busulfan was included in our protocol because of its potent effects on primitive hematopoietic progenitors and the lack of organ toxicity at reduced doses in nonmyeloablative regimens for hematopoietic stem cell (HSC) transplant. Studies in non human primates indeed showed that low dose busulfan provides a strong competitive advantage for the survival and proliferation of transplanted HSC during suppression of endogenous hematopoiesis.”

Previous experience with marrow conditioning to enhance outcome of gene transfer

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1. Busulfan resulted in an average of 5% persistent gene marking of CD34+ cells in the bone marrow of patients and 3.5% marking of granulocytes.
2. Busulfan was well tolerated in most patients (7 patients had more than 1 day of granulocytes <500/ul; one patient required G-CSF treatment and one patient required platelet transfusions and infusion of reserve marrow; all patients fully recovered hematopoiesis).
3. Level of correction appeared to be related to both the number of infused gene corrected cells and to the period of neutropenia (i.e. the functional level of conditioning related myelosuppression).

Gene Transfer for X-linked Chronic Granulomatous Disease: The NIH Experience without and with Busulfan

1. 1998-2000 study with no conditioning: In 3 of 5 adult patients with any detectable gene marking, initial marking peaked at 0.1% and did not persist.
2. 2006-present study using busulfan 10mg/kg: In 3 of 3 adult patients treated, marking peaked at 24%, 5.1% and 4.1% respectively and has persisted in 2 of 3 patients. The first patient still has >1% gene corrected granulocytes at more than 2 years after treatment.

The only difference is the use of busulfan conditioning.

Busulfan was well tolerated and hematopoiesis fully recovered. However this dosing in all three patients resulted in neutropenia and thrombocytopenia requiring both G-CSF administration and platelet transfusions.

We Propose a Conditioning Regimen to Enhance Engraftment of Gene Corrected Hematopoietic Stem Cells in the Older Children X-SCID Gene Transfer Protocol

Marrow conditioning- Busulfan 6 mg/kg total (more than the 4 mg/kg used in the Aiuti et al study and less than the 10 mg/kg used in our X-CGD study)

Prevent mucositis (and possibly augment thymic stromal function) with Keratinocyte Growth Factor

Schedule for proposed conditioning:

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
			Busulfan 3mg/kg/d	Busulfan 3mg/kg/d					

