

Retroviral and Lentiviral Vectors for Long-Term Gene Correction: Clinical Challenges in Vector and Trial Design

Session I: Session I. Overview of Human Gene Transfer Trials Involving Retroviral/Lentiviral Vector Transduction of Human Stem Cells

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Review of Gene Therapy for X-linked Severe Combined Immunodeficiency and for Chronic Granulomatous Disease

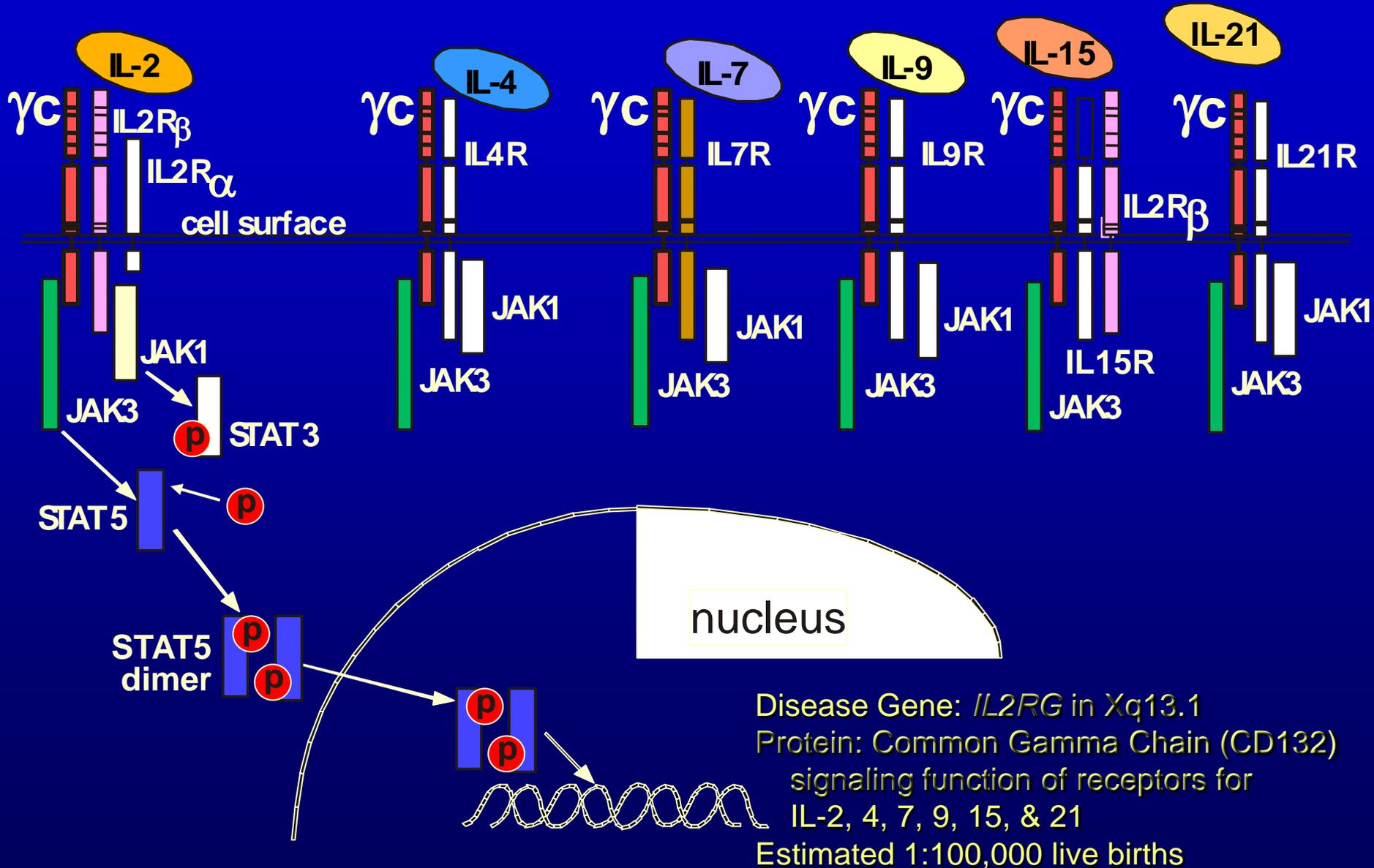


X-linked Severe Combined Immunodeficiency

SCID- X1 (XSCID)

- **Profound lack of T-cell, B-cell and NK cell immunity; T cells and NK cells absent or very low numbers, B-cells present but do not mature to produce immunoglobulins**
- **Recurrent opportunistic infections in early infancy**
- **Severe deficiency is fatal in infancy without treatment providing some immune reconstitution**
- **Partial function mutation (variant) XSCID patients may survive infancy but can have recurrent infections, autoimmunity and growth failure**
- **SCID-X1 (XSCID) is the most common type of SCID**

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



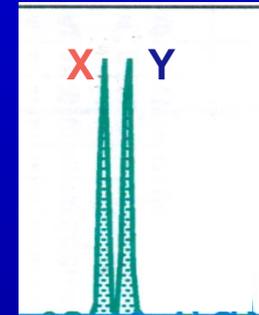
Treatment for X-linked Severe Combined Immunodeficiency SCID- X1 (XSCID)

- **Newly diagnosed XSCID in infancy most often is a medical emergency requiring urgent measures to control infection and provide a hematopoietic stem cell transplant to improve or restore immunity.**
- **HLA-matched sibling transplant is the treatment of choice (95% 5 yr survival and good restoration of immunity), but most infants lack a matched sibling.**
- **Haplo-identical T-cell depleted bone marrow transplant from a parent can be life-saving with 55-75% survival with restored T cell immunity (>90% if provided in the first 3.5 months of birth).**

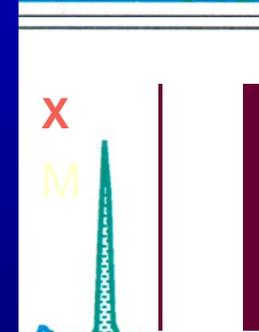
Limitations of T-Depleted Parent Derived Haplo-Identical BMT for XSCID

- Graft vs host disease may occur in up to 30%
- EBV lymphoproliferative disease may occur in 2-5%
- Persistent immune defects
 - >50% lack any or adequate donor B cell engraftment requiring lifelong IVIG
 - there may be progressive decreases in donor T cells and loss of donor T cell diversity over the first decade of life
 - NK defect is not repaired

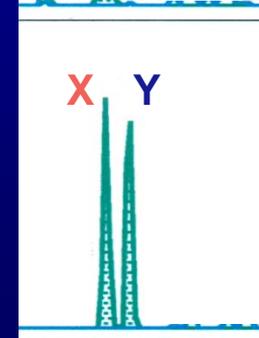
Example of graft from mother



CD14/15
granulocytes,
Monocytes
(only patient)



CD3^M
T cells
(all donor)



CD19
B cells
(few from donor)

Treatment for X-linked Severe Combined Immunodeficiency SCID- X1 (XSCID)

- **Murine retrovirus vector ex vivo gene transfer into autologous marrow CD34⁺ hematopoietic stem cells of infants and very young children with XSCID restores T cell and B cell immunity (Hacein-Bey-Abina S et al. 2002 N Engl J Med 346:1185; Gaspar HB et al. 2004 Lancet. 364:2181).** To date a total of 20 infants have been treated (10 in the Paris protocol; 10 in the London protocol).
- **Vector insertional mutagenesis mediated T cell leukemia is a significant risk of this treatment (Hacein-Bey-Abina S, et al. 2008 J Clin Invest 118:3132; Howe SJ et al. 2008 J Clin Invest 118:3143).** 5 of the 20 developed T cell lymphoproliferative disorder.

Gene Transfer in XSCID Infants:

Paris, MFG-Ampho 1999-2002							London, MFG-GALV 2002-2008					
Pt #	Age	Gamma c ⁺ CD34+ Cells x 10 ⁶ /kg	BM T	Ins. sites	Other chrom. Abnorm.	Status	Pt #	Age	Tot. Cells x 10 ⁶	Ins. sites	Other chrom. Abnorm.	Status
P1	11	3				AW	P1	10	180			AW
P2	8	5				AW	P2	10	180			AW-IVIG
P3	8	5	BMT			AW	P3	4	78			AW
P4	1	18	BMT	LMO2	CDKN2A del	Died	P4	36	115			AW
P5	3	20		LMO2	NOTCH mutation	Chemo; AW	P5	10	200			AW
P6	6	1				AW; IVIG	P6	10	200			AW
P7	11	4		CCND2	CDKN2A del	Chemo; AW; IVIG	P7	6	84			AW
P8	6	22				AW	P8	13	207	LMO2	NOTCH 1 mutation, CDKN2A del, TCRb/STIL- TAL1 translocation	AW-IVIG
P9 Austr		1.3	BMT			AW; BMT	P9	7	160			AW-IVIG
P10	9	11		LMO2, BMI1	NOTCH mutation	Chemo; AW; IVIG	P10	12	60			AW-IVIG

Age = months at time of treatment

Red = Leukemia

AW = Alive, well

Highlights of gene transfer outcome in the 20 infants and young children with XSCID

- **13 achieved event-free substantial immune correction of which 4 require IVIG.**
- **4 who developed T-cell leukemia achieved remission and continue to have gene transfer mediated immune correction of which 3 require IVIG**
- **1 who developed T-cell leukemia required BMT, but died of the leukemia (overall mortality 5%)**
- **2 did not achieve significant benefit from gene transfer and subsequently had successful BMT**
- **17/20 (85%) continue to benefit from gene transfer**

Conclusions from gene transfer outcome in the 20 infants and young children with XSCID

- **For infants and young children older than 3.5 months of age, the overall long term outcome of gene transfer compares favorably relative to the long term outcome of haplo-identical transplant even taking into account the risk of insertional mutagenesis mediated leukemia.**
- **Use of improved vectors that reduce the risk of insertional mutagenesis mediated leukemia should be studied in this patient population.**

Gene transfer in Older children with XSCID

NIH MFGS-GALV-gc (2004-6)					London/Paris MFG-Ampho-gc (2004-5)			
Pt #	Age Yrs	Total Cells/kg $\times 10^6$	T cell marking by PCR	Status	Pt #	Age Yrs	Total Cells/kg $\times 10^6$	T cell marking by PCR
1	11	74	2-6%	Successful BMT at 3 yrs post	1	20	2.8	<1% marking
2	10	76	>90%	Partial clinical benefit	2	15	35	Trace marking initially; none after 6 mo
3	14	64	7-8%	Chronic lung dis, short stature;				

Age = years at time of treatment

Older Children with XSCID are more resistant to engraftment or benefit from gene transfer

- Of 5 older patients with XSCID (age 10-20 years) treated with gene transfer only one (the youngest) achieved substantial and sustained gene marking (Thrasher AJ, et al. 2005 Blood 105:4255; Chinen J, et al. 2007 Blood 110:67).
- The one patient with substantial gene marking and improvement in measures of immune function did not demonstrate significant increase in overall T cell numbers. Despite some clinical benefit he continues to have infections, failure to grow, and nutritional deficiencies.
- Significant sustained benefit from gene transfer treatment in older children may require improved vectors and marrow conditioning.

New clinical trials for XSCID

- **Open for enrollment: Gene transfer for XSCID using a self-inactivating (SIN) gammaretrovirus vector for subjects in good condition who are >3.5 mo of age with no matched sibling or matched unrelated donor; or subjects of any age with therapy resistant infection (Sites: London-UCL-GOSH; Paris-Hôpital Necker; USA-Boston-Cincinnati-Los Angeles).**
- **In late regulatory review: Gene transfer for XSCID using a SIN, insulated lentivector for subjects between 3.5 and 12 mo of age with no matched sibling or available matched unrelated donor (Site: Memphis-St. Jude CRH); For subjects 2-20 years with no available matched sibling or available matched unrelated donor; subjects will be conditioned with busulfan 6 mg/kg (Site: Bethesda-NIH) .**

Chronic Granulomatous Disease

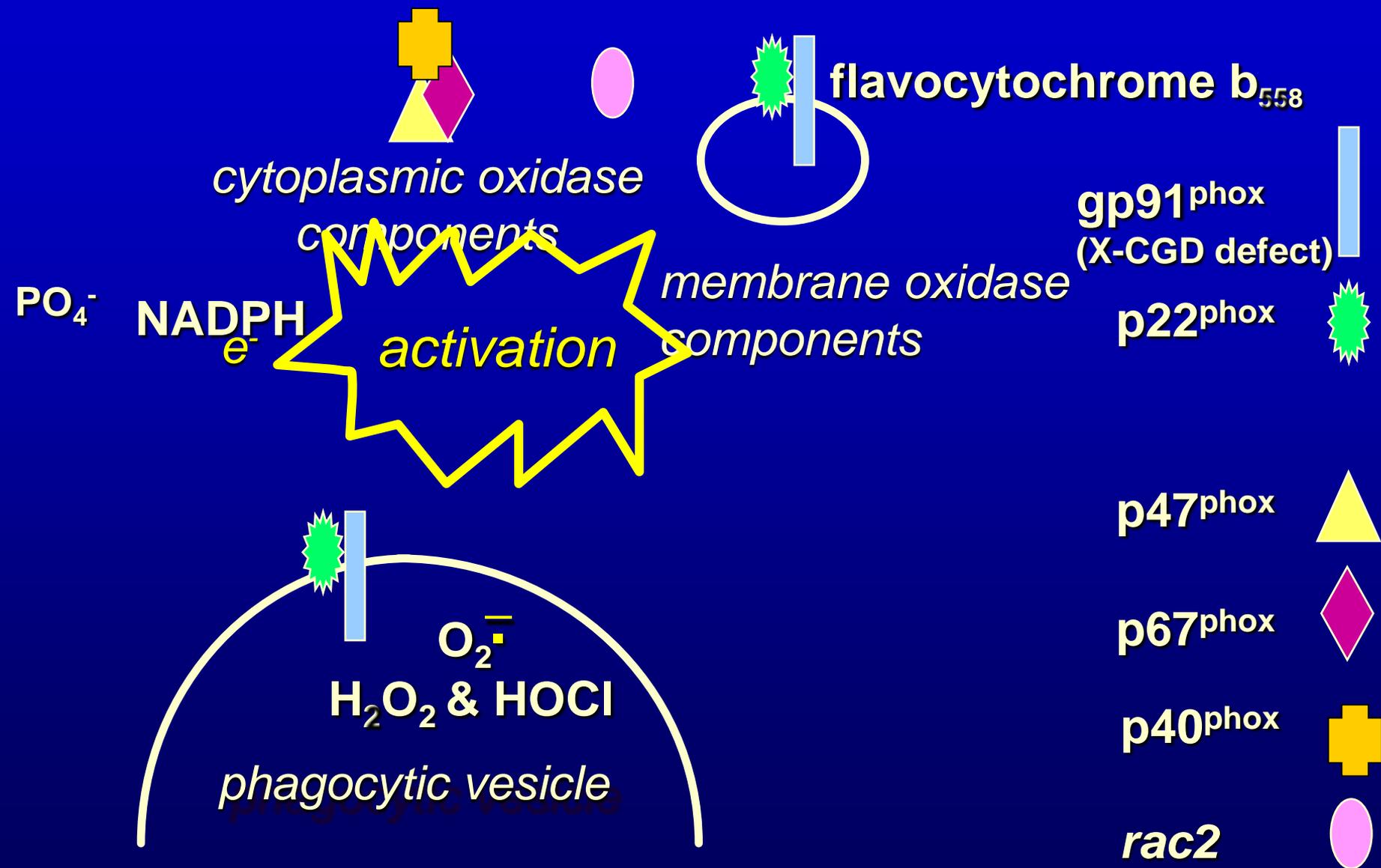
Phagocytes fail to produce microbicidal oxidants.

Patients suffer from recurrent infections and excessive granulomatous inflammation.

Mutations in phagocyte oxidase subunits cause CGD

Type	% affected	Chromosome
X-linked:		
gp91 ^{phox}	~70%	X
Autosomal recessive:		
p47 ^{phox}	~25%	7
p67 ^{phox}	~2.5%	1
p22 ^{phox}	~2.5%	16
p40 ^{phox}	1 patient reported	22

Phagocyte NADPH Oxidase



Chronic Granulomatous Disease

Standard of Care / Prognosis

- Infection prophylaxis with daily co-trimazole (Bactrim/Septra), daily itraconazole, and three times weekly interferon gamma.
- Yearly mortality for the X-linked CGD (X-CGD) is about 1-3% and is directly related to amount of residual superoxide production.
- Consensus is emerging that patients with oxidase negative CGD, history of recurrent infections, and an available HLA-matched sibling donor should be considered for transplant.
- Matched unrelated donor transplants are considered experimental for CGD but 30% of patients lack even a matched unrelated donor.

Early Clinical Trials of Gene Transfer for Chronic Granulomatous Disease with No Conditioning Achieved Only Transient Low Level Gene Marking/Correction

- **1995: NIH Study- Amphotropic MFGS-p47^{phox} vector ex vivo transduction of autologous CD34⁺ PBSC; 5/5 patients with p47^{phox} deficient autosomal recessive CGD demonstrated transient low level gene marking with appearance of oxidase normal neutrophils in the peripheral blood for several months.**
- **1998: NIH Study- Amphotropic MFGS-gp91^{phox} vector ex vivo transduction of autologous CD34⁺ PBSC; 3/5 patients with X-CGD demonstrated transient low level gene marking with appearance of oxidase normal neutrophils in the peripheral blood for several months.**
- **1998: Indiana University Study- GALV MSCV-gp91^{phox} IRES neo-resistance vector ex vivo transduction of autologous CD34⁺ PBSC; 2/2 patients with X-CGD demonstrated transient low level gene marking with appearance of oxidase normal neutrophils in the peripheral blood for several months.**

Outcome of X-CGD Gene Transfer Clinical Trials to Date Using Marrow Conditioning

Site	# Pts	Conditioning	gp91 ^{phox} Vector	% Trans	CD34 ⁺ x 10 ⁶ /kg	>1% at >1 yr	Genotoxicity
Frankfurt	2	Busulfan 8mg/kg	GALV SF71	40-45	9-11	>15%	Evi1/MDS1 MDS/Monos. 7
Zurich	1	Busulfan 8.8mg/kg	GALV SF71	32	6	>20%	Clonal myeloproliferation
London	1	Melphalan	Ampho MFGS	5-20	0.2-10	no	None
	3	140/mg/m ²	GALV SF71			no	None
NIH	3	Busulfan 10mg/kg	Ampho MFGS	25-73	19-71	1 pt at 1% - 3yrs	None
Seoul*	2	Busulfan 6.4mg/kg	GALV MT	11-29	5-6	no	None

Seoul group also used Fludarabine; Some patients from all studies had clinical benefit in that there was improvement in an infection not responding to conventional therapy or a reduction in infection frequency.

Modified from Table 1 of: Grez M, Reichenbach J, Schwäble J, Seger R, Dinauer MC, Thrasher AJ. Gene Therapy of Chronic Granulomatous Disease: The Engraftment Dilemma. Mol Ther. 2010 Nov 2. [Epub ahead of print]

Results of Clinical Trials of Gene Transfer for X-linked CGD using Bone Marrow Conditioning

Ott MG, et al. 2006 Nat Med 12:401.

Bianchi M, et al. 2009 Blood 114:2619

Stein S, et al. 2010 Nat Med. 16:198.

Kang EM, et al. 2010 Blood 115:783.

Highlights of gene transfer outcome in patients with X-CGD who receive conditioning

- **Busulfan conditioning resulted in significant levels oxidase positive neutrophils in the first month or two (3-24%), and this likely contributed to clinical benefit in the form of control of infection not responding to conventional therapy.**
- **At one year or longer, gene marking significantly greater than 10% persisted only in SF71-gp91^{phox} treated patients that later developed myelodysplasia or myeloproliferative disorder. Of the two patients developing Evi1/MDS1 insertional mutagenesis mediated MDS and monosomy 7, one died and the other was successfully transplanted**
- **One patient in the NIH study continues to have 0.8-1.1% oxidase normal gene-marked circulating neutrophils at 4 years post gene transfer treatment and no evidence for any genotoxicity.**

Conclusions from gene transfer outcome in patients with X-CGD who receive conditioning

- **Busulfan conditioning can enhance engraftment and persistence of gene marking, but additional maneuvers may be necessary to achieve safe, permanent, high levels of gene correction for X-CGD.**
- **The SFFV derived SF71 vector appeared to have a particular predilection for causing Evi1/MDS1 mediated genotoxicity.**
- **While no genotoxicity has been observed in CGD trials using the MLV derived MFGS or MT vectors, that class of vector has caused genotoxicity in gene therapy studies of other immune deficiencies. Thus, it would be prudent to develop vectors and approaches that reduce the risk of genotoxicity in X-CGD.**

