

Protocol #808-950: Gene Therapy for SCID-X1 Using a Self-Inactivating (SIN) Gammaretrovirus

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Presentation Content

- Results of allogeneic HCT in SCID-Luigi Notarangelo
- Gene therapy outcome - Adrian Thrasher
- Vector development to minimise toxicity – Christopher Baum
- SCID-X1 protocol and vector toxicity studies - Adrian Thrasher
- Further points

Goal of gene therapy for SCID-X1

- To reconstitute T and B cell immunity in patients with SCID-X1 effectively without toxicity
- The next few slides will review previous experience with gene therapy for correction of SCID-X1, and will analyze the factors that influence outcome after HCT. When possible, the HCT data are presented for patients with SCID-X1 or with B+ SCID (most of whom have SCID-X1)
- These data were used to define those patient populations that we propose could benefit from gene therapy

Overall, our approach was developed with the appreciation that current treatment with hematopoietic cell transplantation (HCT) gives excellent overall results only when an HLA-identical donor is available.....

Various HCT regimens from various donor sources result in different survival rates for SCID

<i>Donor</i>	<i>n</i>	<i>years surveyed</i>	<i>conditioning</i>	<i>source</i>	<i>patient population</i>	<i>survival</i>
sibling	73	1990 on	no	SCETIDE (Europe)	all SCID	86% (10 y)
matched UD	78	1990 on	yes	SCETIDE (Europe)	all SCID	72% (10 y)
mismatched related	262	1990 on	yes/no	SCETIDE (Europe)	all SCID	59% (10 y)
sibling	15	1983-2004	no	Duke (US)	all SCID	100%
mismatched related	117	1983-2004	no	Duke (US)	all SCID	74%

(EBMT Meeting, 2008)

(Buckley, *Annu Rev Immunol* 2004)

Factors which impact on outcome post HCT

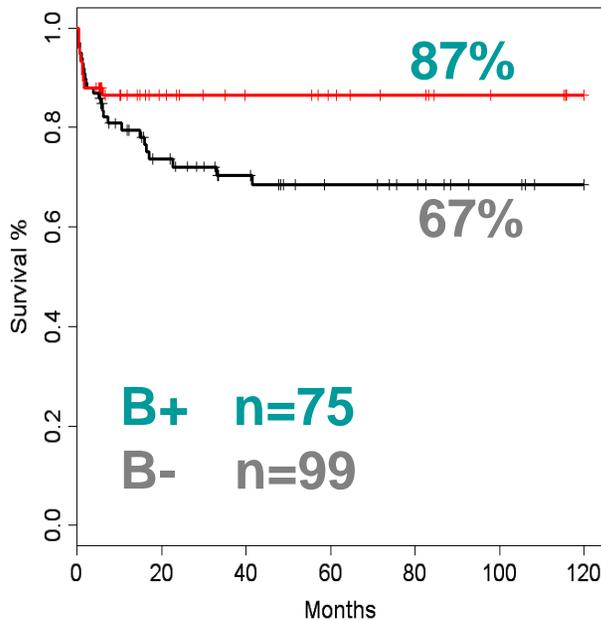
- Type of SCID
- Type of donor
- Infection at the time of transplant
- Conditioning - B cell reconstitution

Outcome post HCT

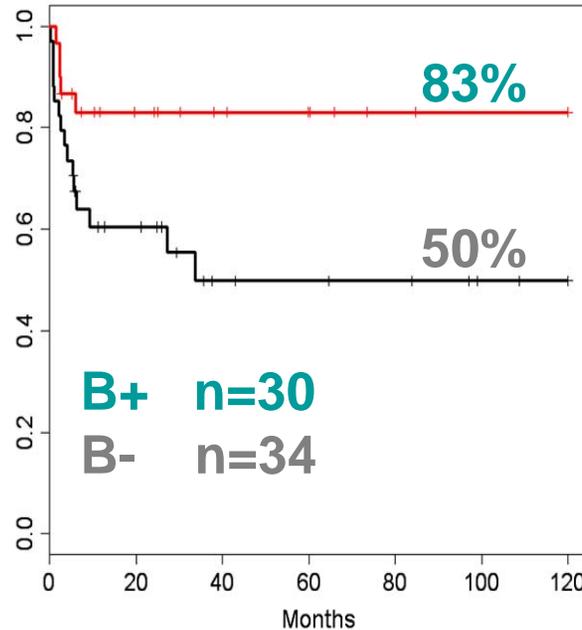
In the absence of nation-wide U.S.A. Registry data of outcome of hematopoietic cell transplantation for SCID, we will review the European data on 629 patients, 338 of whom had B⁺ SCID.

10-year survival rate after HCT for SCID (SCETIDE European Registry)

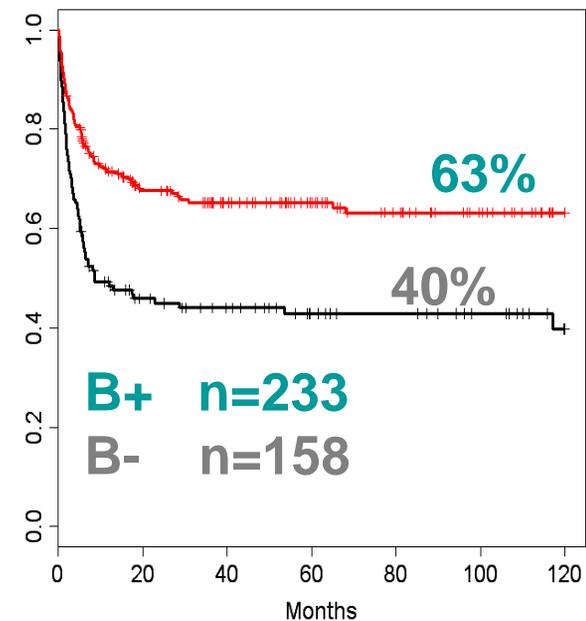
geno/pheno-identical



MUD



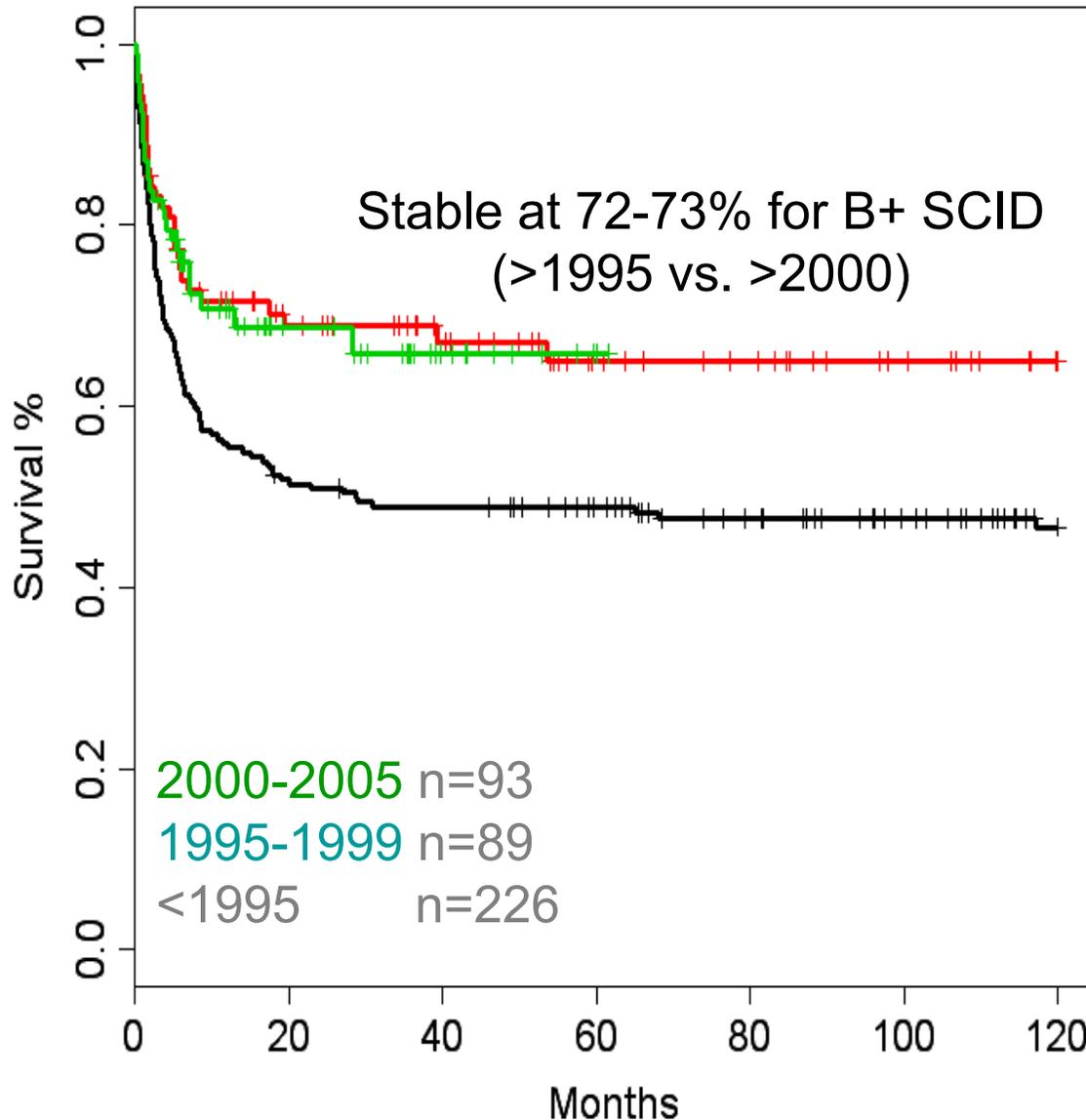
mismatched related



In the European series patients with B+ SCID have equivalent survival after sibling or matched unrelated donor HCT, poorer survival after mismatched related HCT

(EBMT Meeting, 2008)

Probability of survival in mmRel HCT for SCID according to period



5 years Survival rate

2000-2005 : 66%

1995-1999 : 65%

Before 1995 : 47%

p=0.005

(EBMT Meeting, 2008)

Pre-HCT infection impacts negatively on outcome after mismatched related HCT in SCIDX1/JAK3 deficiency (Paris and Brescia after 1991)

n=30

16 severely infected at HCT

{ 8 alive
8 deceased

14 without severe infections at HCT

{ 12 alive
2 deceased

outcome

20 alive (Follow up: 1-17 yrs)

13 well

7 chronic/severe infections

1 with severe colitis (nutritional support)

8 on IVIG

10 deceased (infection)

Unconditioned mismatched related HCT for SCID results in poor humoral immunity

- Many recipients of unconditioned HCT remain on IVIG
52% (65 out of 125) of all SCID, but
67% (38 out of 57) of those with SCID-X1

(Buckley, Annu Rev Immunol 2004)

- Long-term dependence on IVIG is associated with a significantly increased risk of infections after HCT for SCID

(Mazzolari et al., Immunol Res 2008)

Conclusions

In the European series, sibling matched HCT and closely matched unrelated donor HCT for B+ SCID results in 80% survival, mismatched related HCT in 60-70% survival

Only 50% of SCID-X1 patients who undergo haploidentical HCT when infected survive

Unconditioned mismatched related HCT often does not reconstitute B cell function

The first trials of gene therapy for SCID-X1 (PARIS and LONDON)



Harvest

CliniMacs CD34+ bone marrow

Pre-activation (40hours)

X-Vivo10 (serum free)

SCF 300ng/ml, FL 300ng/ml, TPO 100ng/ml,

IL-3 20ng/ml

Transduction (3 cycles over 72 hours)

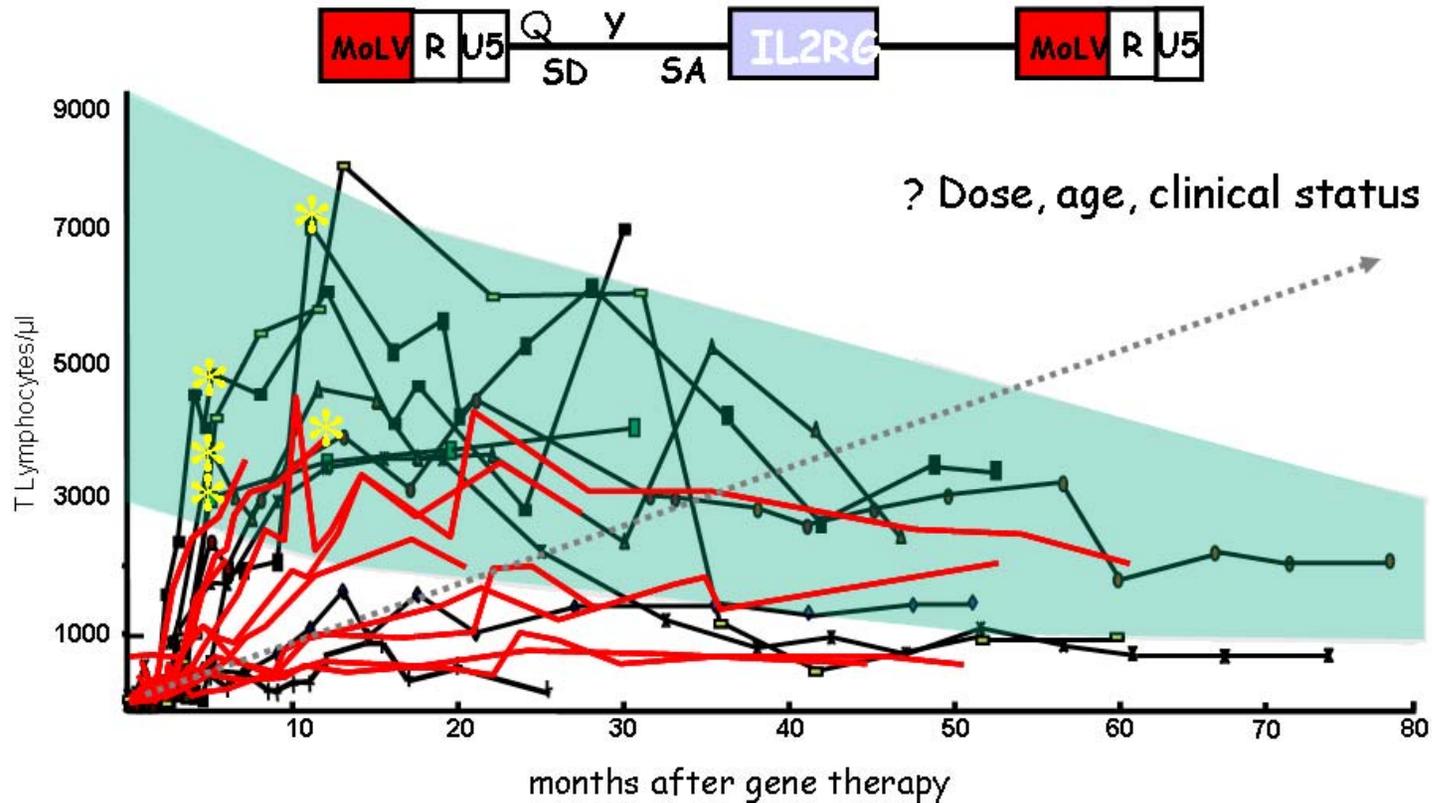
Nexell gas permeable flexible containers

Retronectin coating

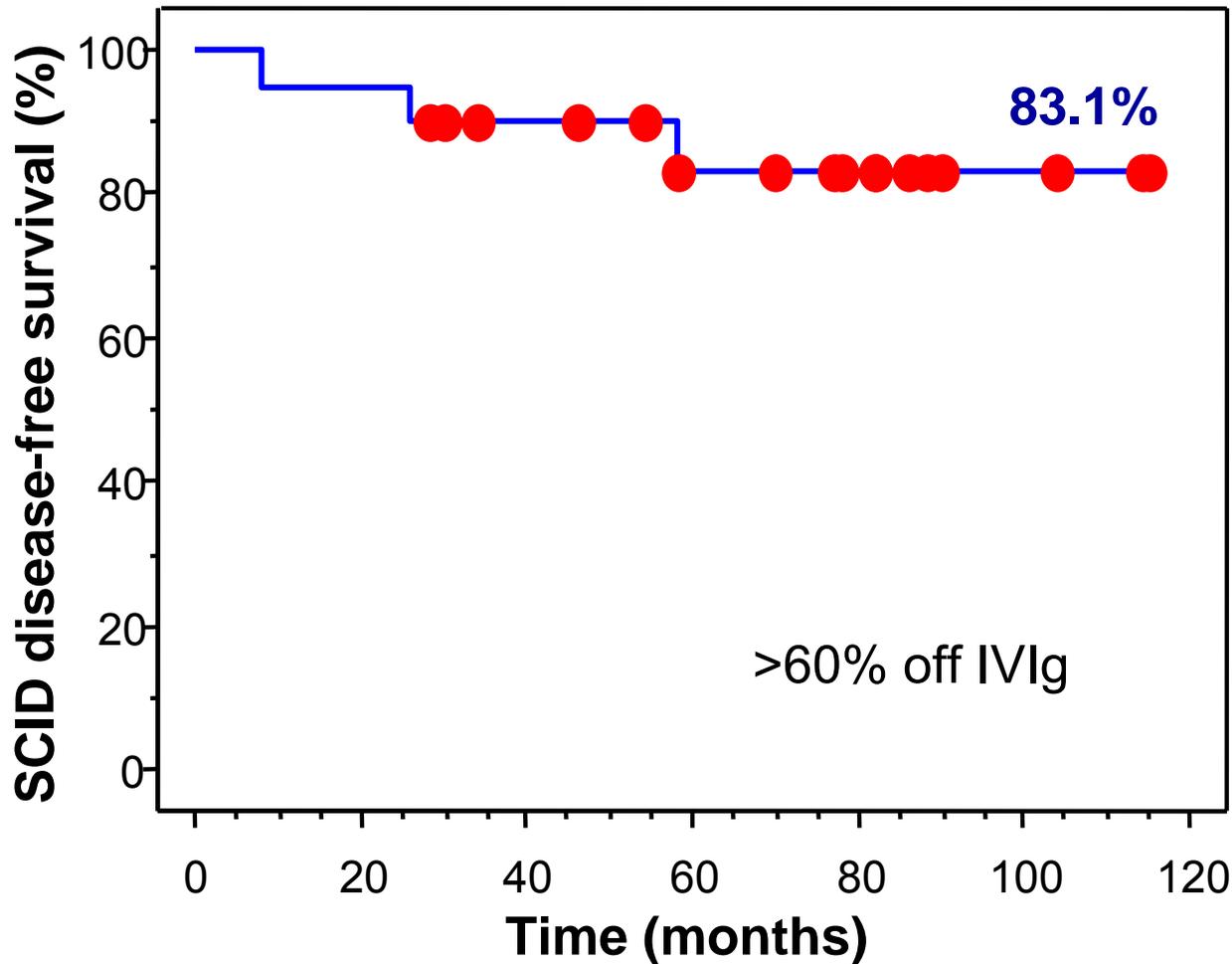
Virus pre-loading

Infusion

It should be noted that RAC guidance calls for the use of the term “gene transfer” rather than “gene therapy.” Is the clinical evidence of therapeutic effect specifically related to SCID sufficiently robust to merit use of the term “therapy” in this consent document ?



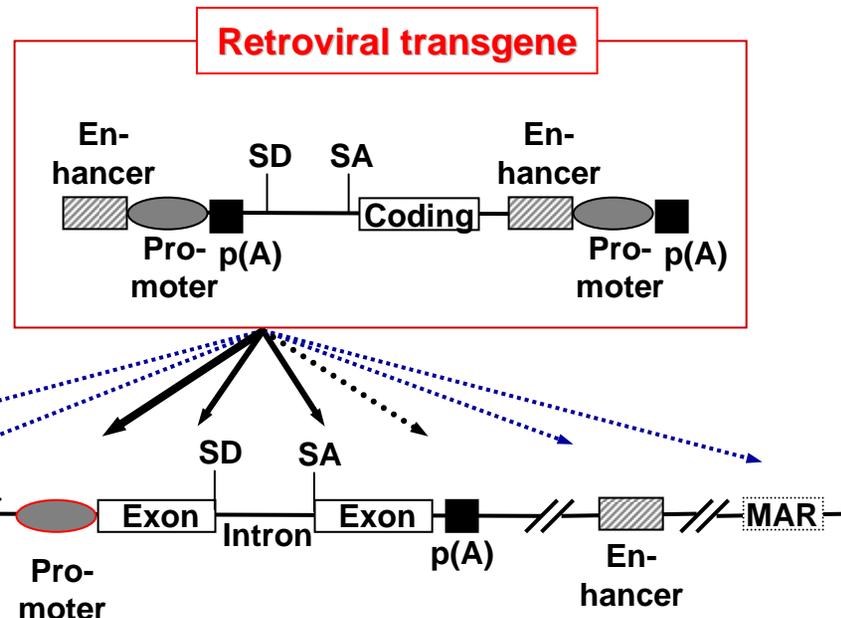
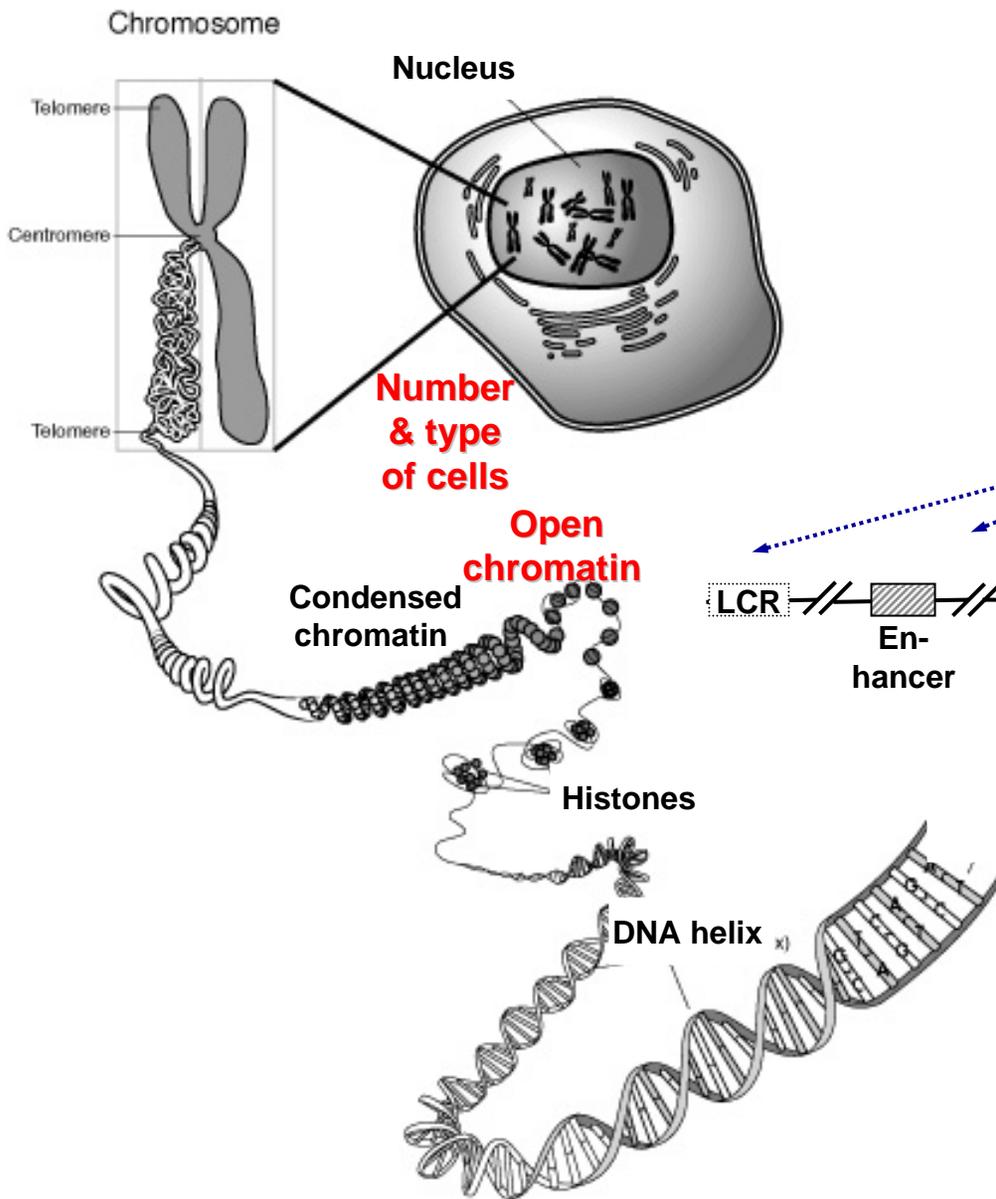
Outcome of gene therapy trials in 20 patients with SCIDX1 (Paris-London data combined)





SIN vector development and validation

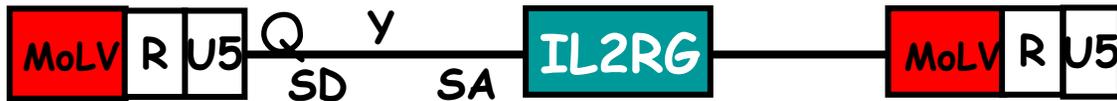
Risk factors of insertional mutagenesis



Integration pattern

	MLV	HIV
Within RefSeq	34.2%	57.8%
+/- 5kb of TSS	20.2%	10.8%
+/- 1kb of DNaseI HSS	10x	1x

Transduction protocol....



Harvest

CliniMacs CD34+ bone marrow

Pre-activation (40hours)

X-Vivo10 (serum free)

SCF 300ng/ml, FL 300ng/ml, TPO

100ng/ml, IL-3 20ng/ml

Transduction (3 cycles over 72 hours)

Nexell gas permeable flexible containers

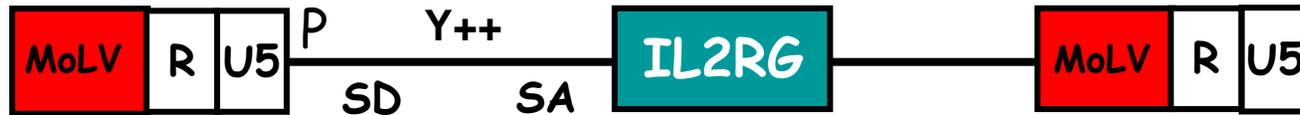
Retronectin coating

Virus pre-loading

Infusion

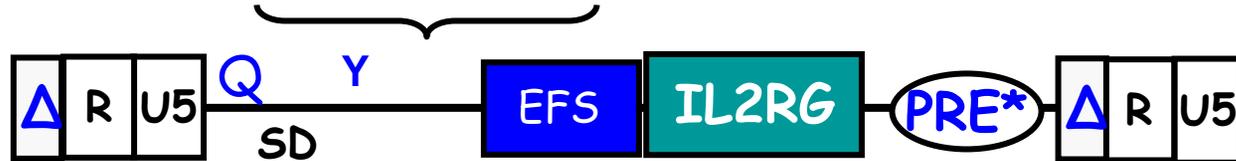


LTR-driven gammaretroviral vector: MFG gC

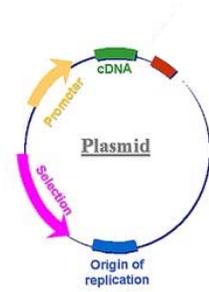
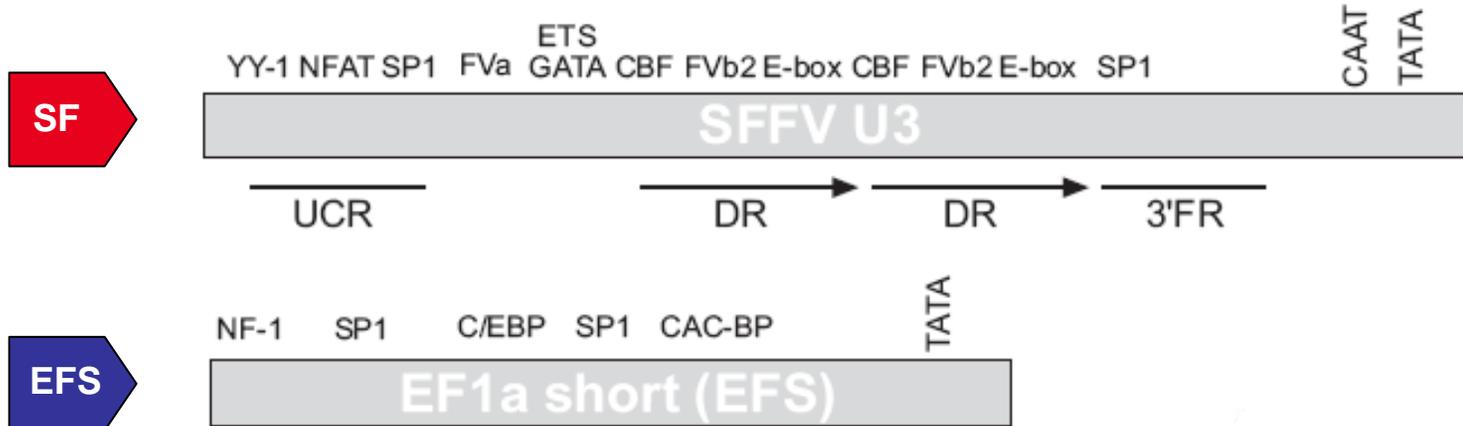


New gammaretroviral SIN vectors: SRS11

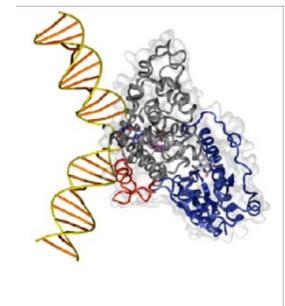
No gag, pol or env residues



Assays to analyze the transactivating potential of different vector configurations and internal promoters



1. Plasmid-based reporter assay
2. Stable, retrovirally transduced reporter assay
3. Clonal dominance assay



Safer vector design for gene therapy

No gag, pol or env residues

Sin.**SF**

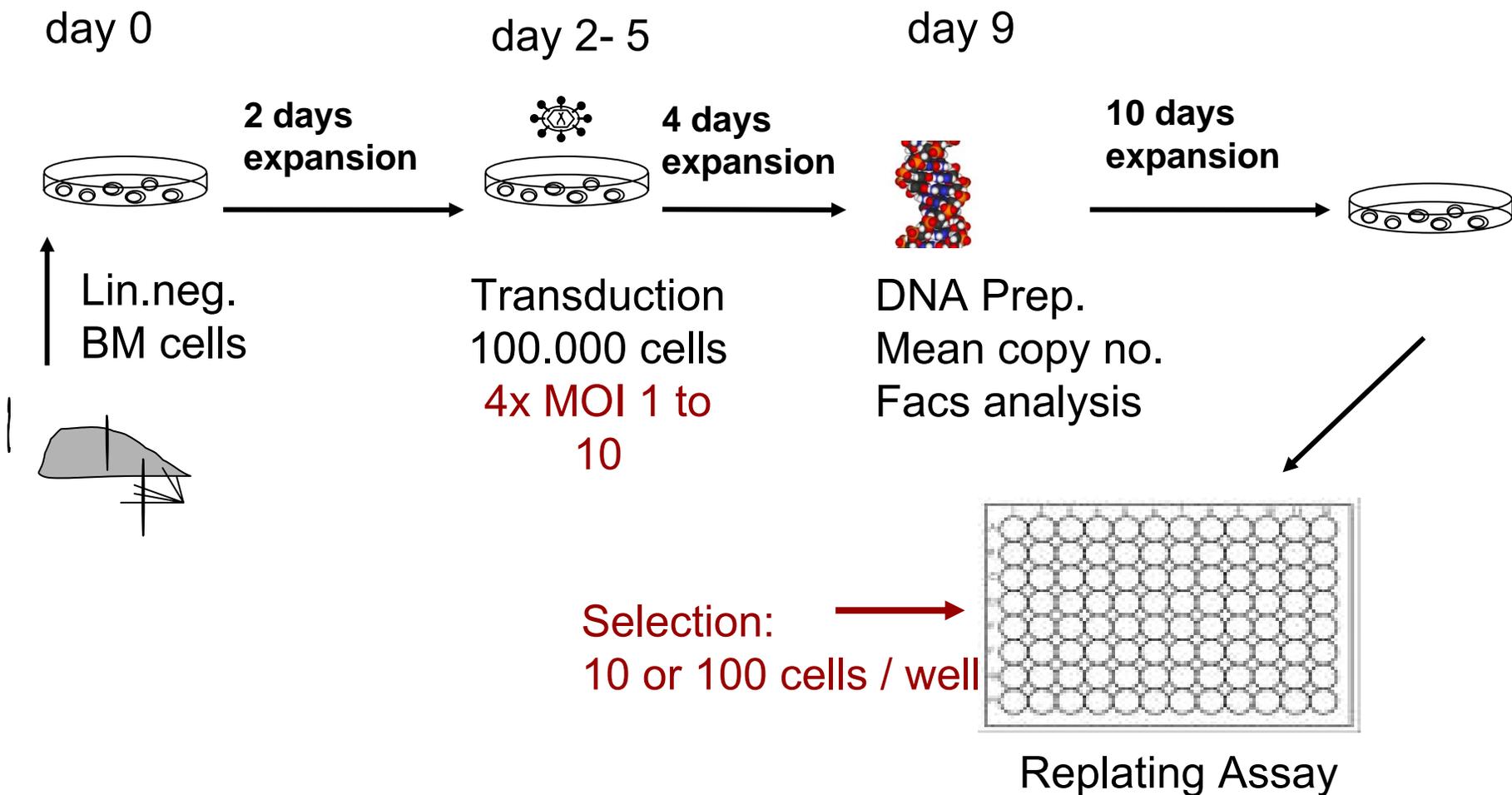


Cellular promoter

Sin11.**EFS**.P



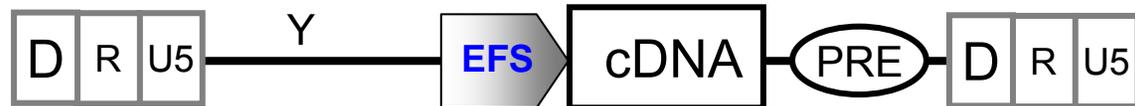
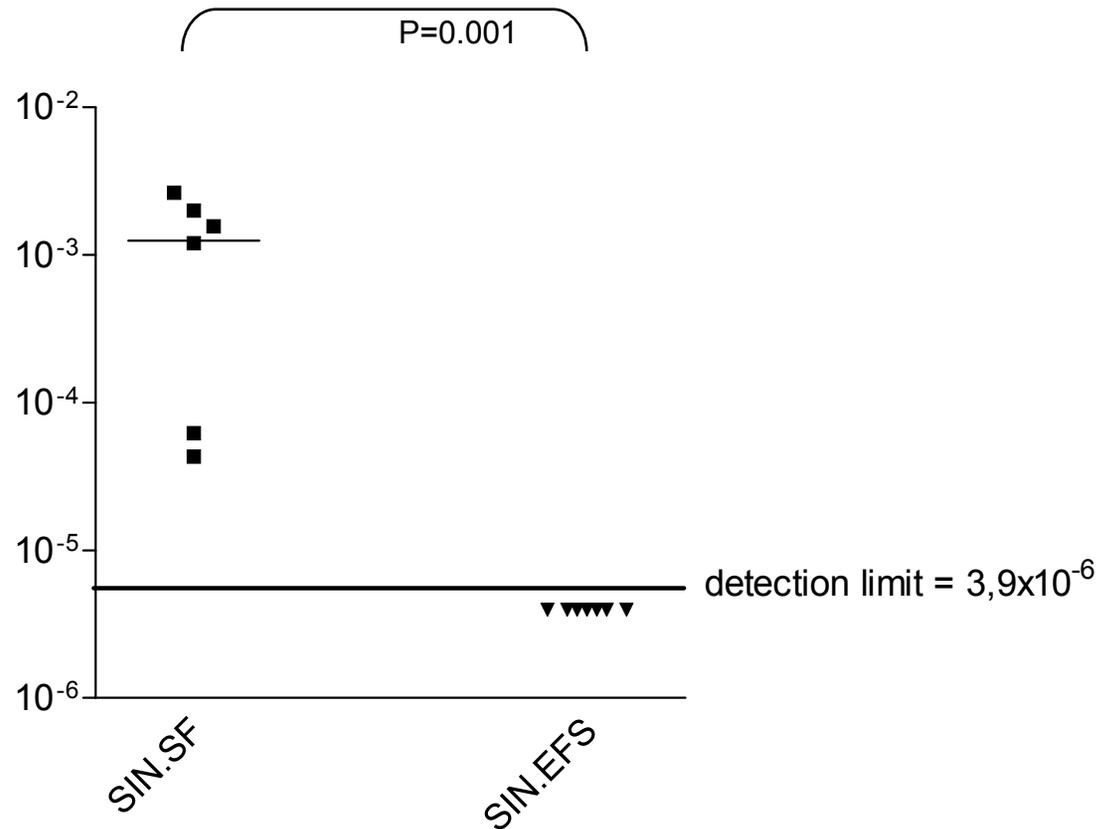
Clonal Dominance Assay



**Medium conditions:
IMDM + 10%FCS + SCF, Flt3L, IL11,**

Cellular promoter (EFS) is far less mutagenic than retroviral promoter

Ratio
Replating frequency
per copy number
on day 4

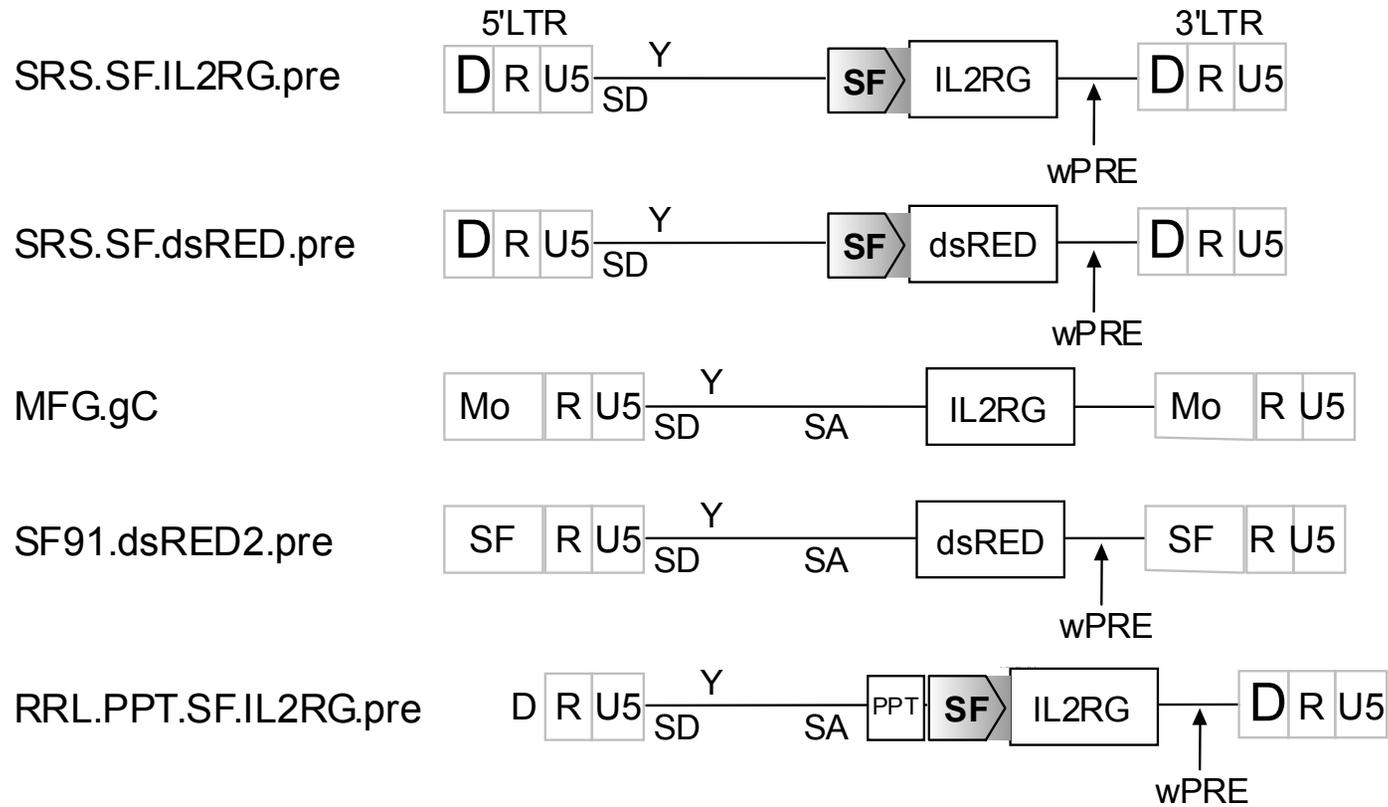


Summary

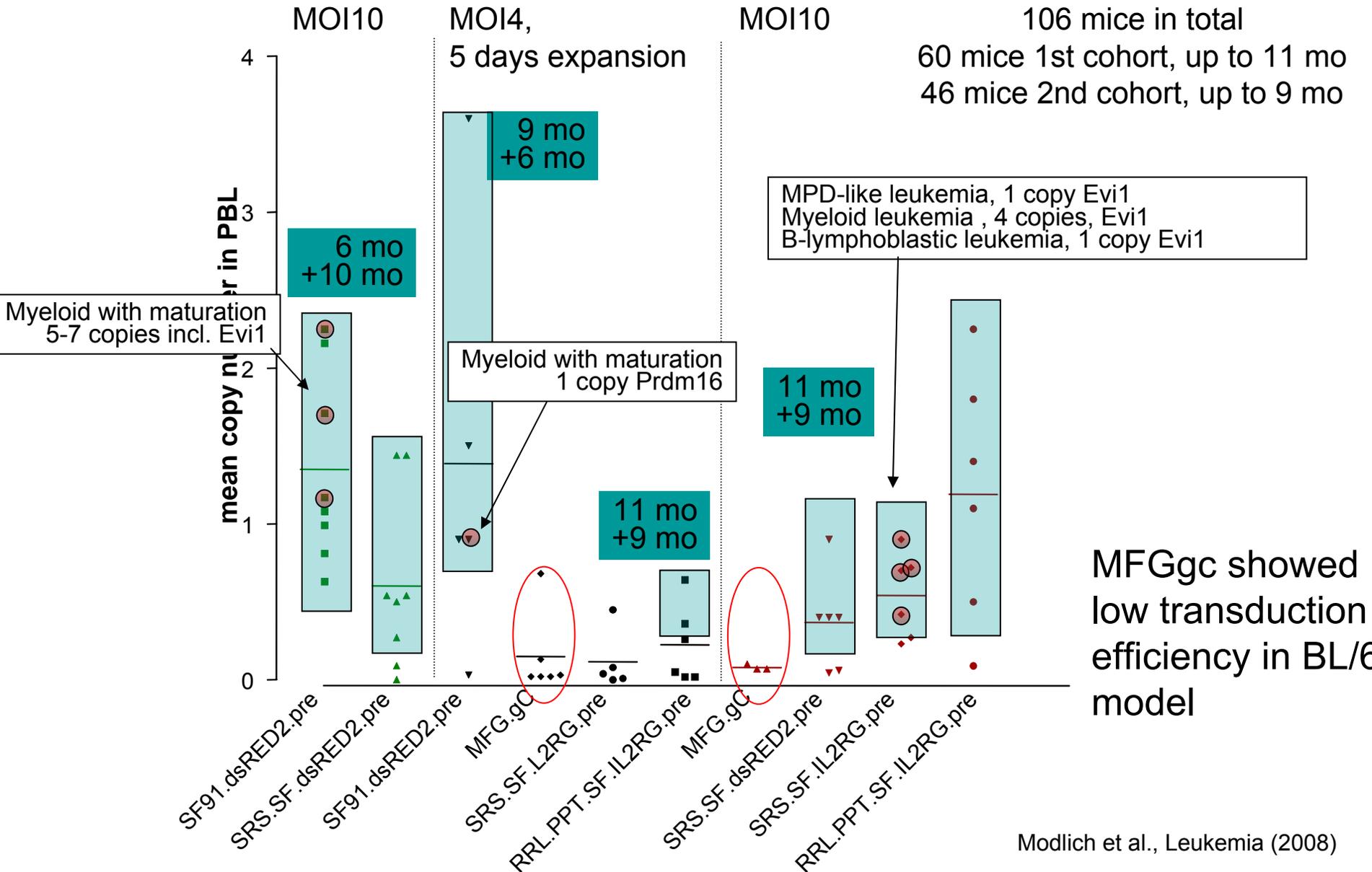
- The short EF1a promoter (EFS) shows low enhancer activity in reporter assays based on transient transfection or stable integration
- SIN vectors containing EFS are unable to transform cells in the clonal dominance assay and do not activate the Evi1 allele in mass cultures
- More than 30 copies of the Sin.EFS vector are less mutagenic than 2 copies of the Sin.SF vector (= 1 copy of an LTR.SF vector)
- Median frequency of replating cells/copy number is > 100x reduced:
Sin.SF = 4×10^{-3} ; SinEFS = $< 4 \times 10^{-6}$ $P < 0.001$; n=6-7

C57BL/6 serial BMT model:

Gammaretroviral and lentiviral vectors expressing IL2RG

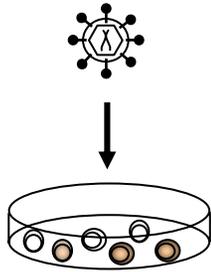


A single insertion in Evi1 or Prdm16 suffices to induce leukemia

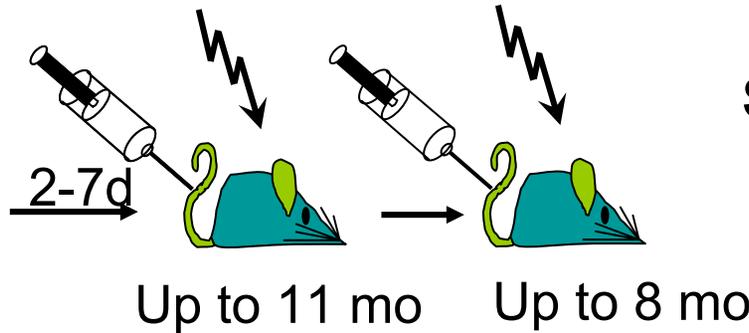


Ongoing: BL/6 mouse serial BMT model

Vectors encoding cell surface markers, fluorescent proteins or therapeutic genes



Low dose versus High dose

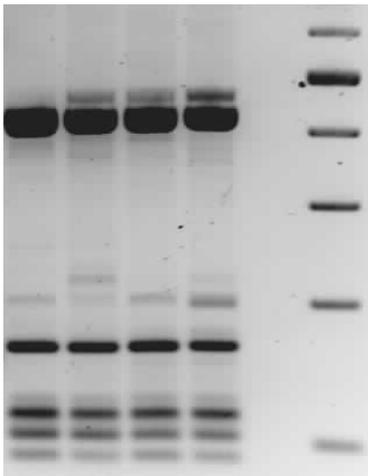


Vector insertions mark genes that enhance stem cell fitness

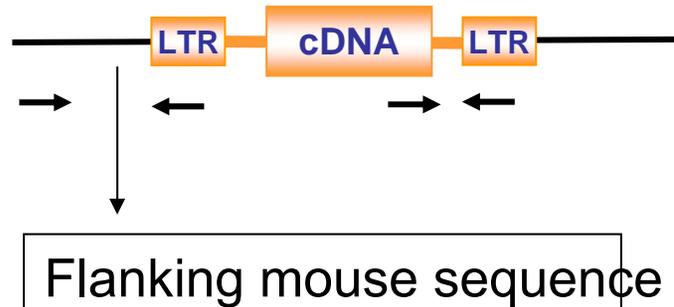
Kustikova et al.,
Science 2005

Skewed integrome as molecular marker

Kustikova et al.,
Blood 2007



Southern blot
LM-PCR



Number of mice under evaluation

SRS11 **SF** P = 10
SRS11.**EFS**.P = 22

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The objectives of this proposal are to initiate a trial of somatic gene therapy for patients with SCID-X1 in whom HLA-matched family donors are unavailable or in whom underlying clinical problems would exclude chemotherapy conditioning.

Protocol change from previous = SIN configuration

UK GTAC approved

Study endpoints

Primary endpoints

1. Immunological reconstitution defined as absolute number of CD3+ cells $>300/\text{mL}$ and PHA response stimulation index >50 at 6 months post-infusion
2. Incidence of life-threatening adverse reactions related to the gene therapy procedure

Secondary endpoints

1. Molecular characterization of gene transfer
2. Normalization of nutritional status, growth, and development

Inclusion criteria

Up to 20 patients will be recruited on the basis of:

1. Diagnosis of SCID-X1 based on immunophenotype (<200 CD3⁺ autologous T cells/mL) and confirmed by DNA sequencing

AND at least one of the following

- 2a. No readily available (within 6 weeks, with ability to transplant within 3 months) HLA identical (A, B, C, DR, DQ) related or unrelated donor
- 2b. Patients with active, therapy-resistant infection or other medical conditions that significantly increase the risk of allogeneic transplant

CONTINUED:

2b. Patients with active, therapy-resistant infection or other medical conditions that significantly increase the risk of allogeneic transplant

- interstitial pneumonia due to adenovirus or parainfluenzae virus 3
- protracted diarrhea requiring total parenteral nutrition
- disseminated BCG infection
- virus-induced lymphoproliferative disease
- any active opportunistic infection (eg, due to *Pneumocystis jiroveci*, cytomegalovirus, cryptosporidium) that does not improve on medical management
- active and progressive pulmonary disease requiring mechanic ventilation.

Exclusion criteria

1. No available molecular diagnosis confirming SCID-X1
2. Major (life-threatening) congenital anomaly...

including, but not limited to: unrepaired cyanotic heart disease, hypoplastic lungs, anencephaly or other major CNS malformations, or other severe non-repairable malformations of the GI or GU tracts that significantly impair organ function.

3. Other conditions which in the opinion of the P.I. or co-investigators, contra-indicate infusion of transduced cells or indicate patient's inability to follow the protocol

Assessment of safety and efficacy (1)

Immunological reconstitution

1. Lymphocyte subsets immunophenotyping
2. Lymphocyte proliferation assays
3. Representation of TCR families (Vb phenotyping and CDR3 Vb spectratyping)
4. Restoration of immunoglobulin levels and antibody response to vaccinations and natural infections

Successful reconstitution:

peripheral blood CD3+ cell count >300/mL

AND

PHA stimulation index >50

at 6 months after infusion of gene-modified cells

Assessment of safety and efficacy (2)

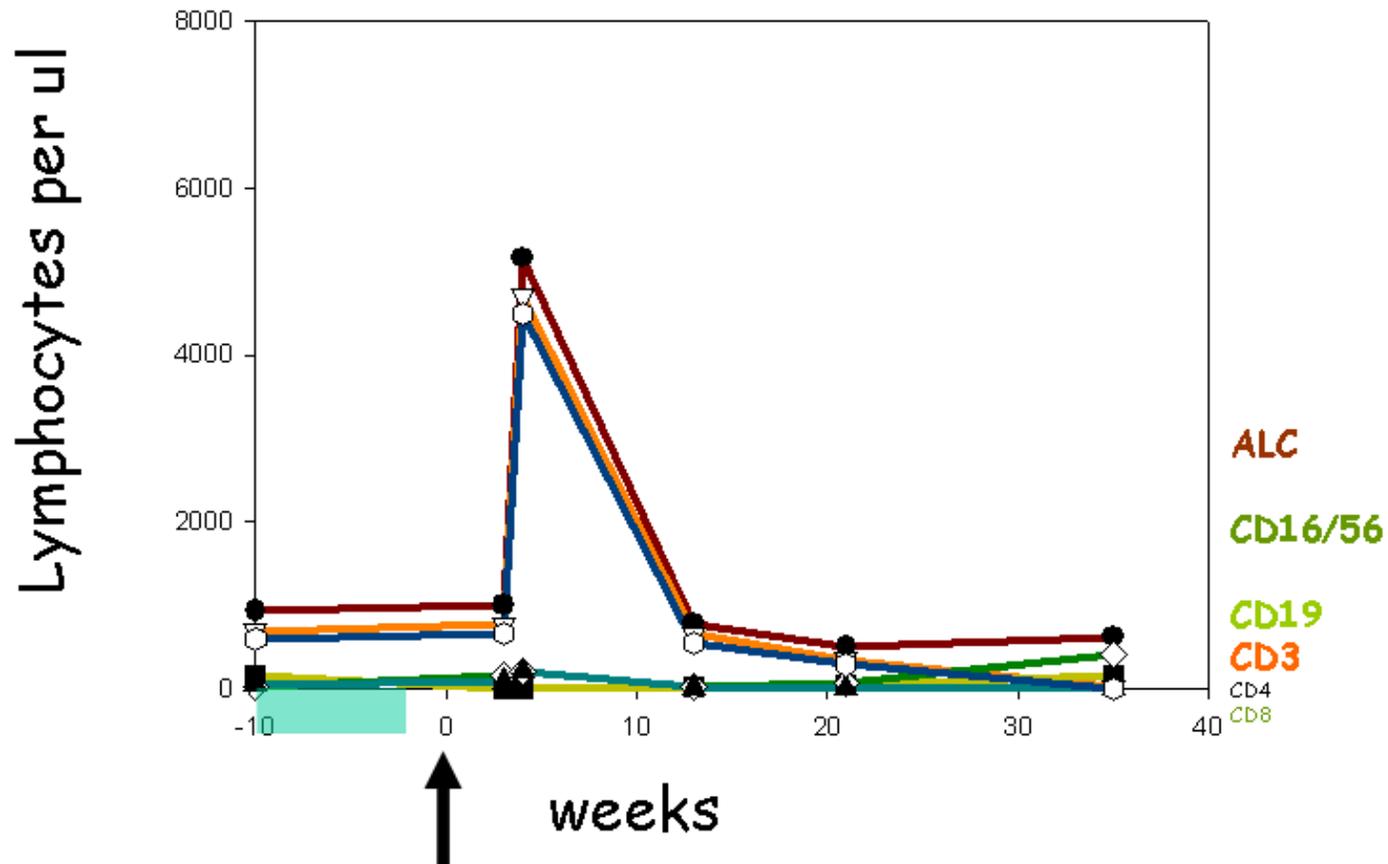
Molecular characterization of gene transfer

1. Quantification of transgene copy number (on sorted populations, by real-time PCR)
2. Peripheral blood clonal analysis (by LAM-PCR)

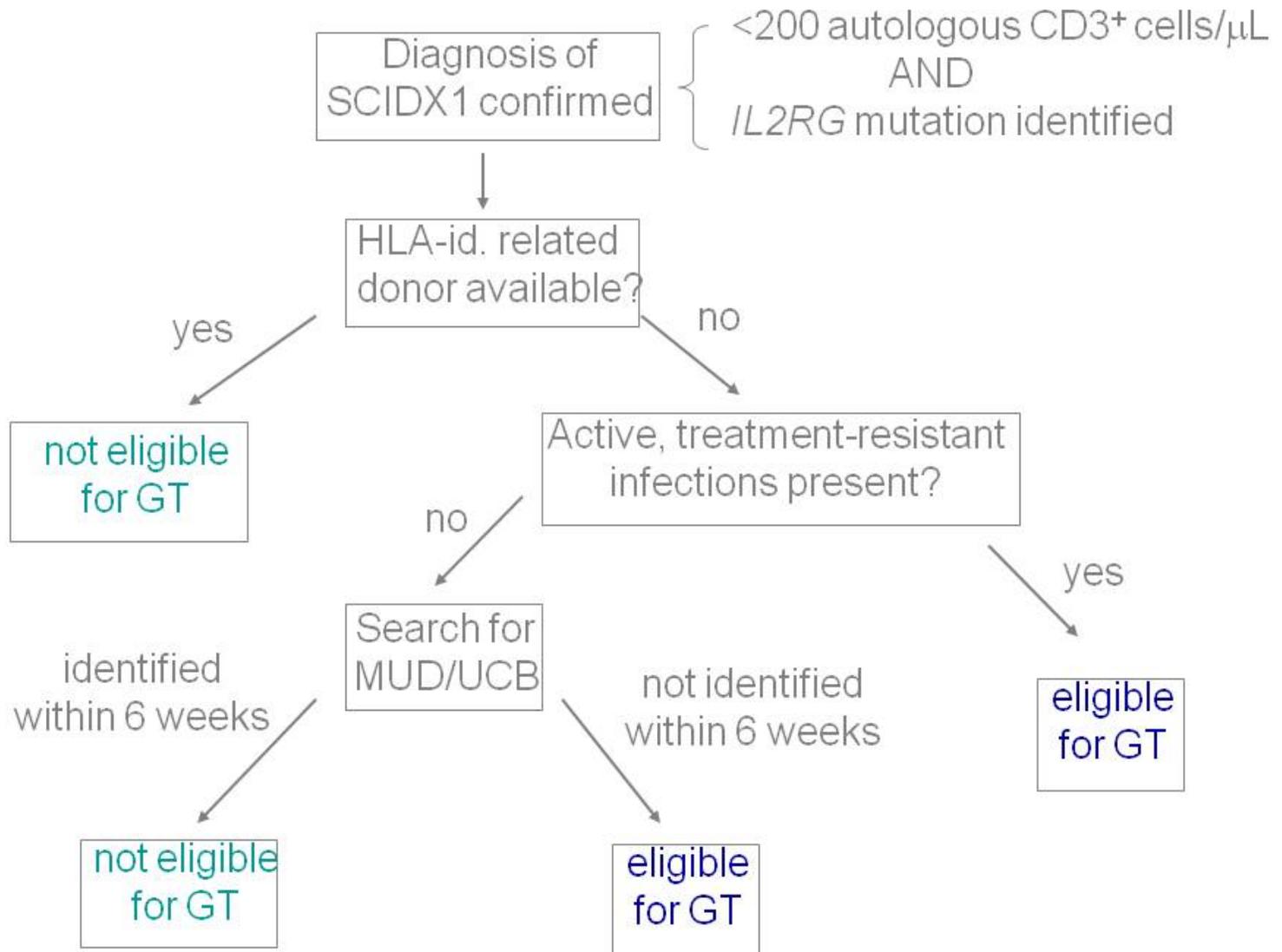
If clonal analysis reveals that **>20% of gene-modified cells are persistently derived from a single clone (and >1% of WBC are gene-marked)**:

- insertion site sequence of the clone and location of site in relation to known loci
- immunophenotypic analysis
- cytogenetic analysis
- bone marrow analysis
- clinical evaluation to rule out malignancy

How was the >20% (of the gene population derived from a single clone) figure selected for initiating additional studies. Would it not be more prudent to select a lower threshold?



Outline of proposed criteria for enrollment



Protocol summary...

SCID-X1 gene therapy trial eligibility criteria
Up to 20 patients will be recruited and will be selected for inclusion on the basis of ALL of the following defined criteria:

Inclusion criteria

1.Diagnosis of SCID-X1 based on immunophenotype (<200 CD3+ autologous T cells,) and confirmed by DNA sequencing.

AND at least one of the following:

2a. No readily available (defined as: within 6 weeks, with ability to transplant within 3 months) HLA identical

(A,B,C,DR,DQ) related or unrelated donor.

2b. Patients with an active, therapy-resistant infection or other medical conditions that significantly increase the risk of allogeneic transplant.

Exclusion criteria

•No available molecular diagnosis confirming SCID-X1.

•Previous gene therapy

•Major (life-threatening) congenital anomaly

•Other conditions which in the opinion of the P.I. or co-investigators, contra-indicate infusion of transduced cells or indicate patient's inability to follow the protocol

Yes

**Consent
Gene therapy Protocol**

Pre-harvest in-patient
stabilization

**D-5 Bone marrow harvest
(general anesthesia)**

**D-5 to D0
Cell processing and
CD34+ cell transduction
(see appendix for details)**

D0: Infusion of transduced cells

D120: Repeat procedure if no T cell recovery and no alternative matched donor source.

D180: Haploidentical or unrelated donor transplantation if no T cell recovery

Patient Monitoring Protocol

	Time points										
	Pre-Gen Therapy	1 Month	3 Month	6 Month	9 Month	12 Month	18 Month	2 Year	3 Year	4 Year	5→15 Year
CBC	X	X	X	X	X	X	X	X	X	X	X
Lymphocyte subsets (LSS) (CD3 #) PHA* Antigen (PPD, candida) * CD3 stimulation*	X	X	X	X X X	X	X	X X X X	X	X	X	X
GAM (IgG IgA IgM)**	X				X	X	X	X	X	X	X
TCR spectratype * TREC * TCR Vbeta panel *	X			X X		X X X	X	X X X	X X X	X X X	X X X
Copy number analysis - transgene expression on sorted lineages	X			X		X	X	X	X	X	X
Transgene site specific integration analysis and frequency per cell (CD3, CD19, CD16/56, CD15 sorted populations)				X		X	X	X	X	X	X
RCR Blood	X		X	X		X		X	X	X	X
Immunology save serum & SCLN (stored cells)	X			X		X	X	X	X	X	X

Data Safety Monitoring Board (DSMB)

Will be appointed by NIH.

Should protocol not obtain NIH funding, a DSMB will be appointed by the Study Sponsor, and will include experts from outside Children's Hospital Boston, one lay person and one biostatistician.

Data Safety Monitoring Plan (DSMP)

Data and safety will be reviewed by the DSMB for each patient entered into the protocol on at least a quarterly basis

Stopping rule for lack of efficacy

Failure of immune reconstitution is determined by poor recovery of T lymphocyte numbers (CD3 <200/ μ l) and function by **day 180**.

We propose to stop the study if we observe 4 or more failures to restore immunological competence at the significance level of 0.016. That is, the probability of observing 4 or more failures is ≤ 0.016 or $\leq 1.6\%$ if the true probability of efficacy is ≥ 0.95 . Therefore if we observe 4 or more failures, the true probability of efficacy is very likely to be ≤ 0.95 (the observed probability of efficacy in the previous SCID-X1 trial) and we will stop the study.

Stopping rule for toxicity

The stopping rule for lack of efficacy under 9.5.1 seems appropriate, but it would be helpful to specify an analogous stopping rule for adverse events (i.e., leukemia). While the typical lag time may make this a moot point, the study is expected to last for a long time period so this may still be prudent. Please comment.

We agree with the Reviewer that a stopping rule should be specified for serious adverse events that include the following: any leukemia potentially related to provirus insertion or treatment-related death. We propose that the protocol should be immediately stopped and reviewed by the DSMB, IRB and FDA if one of these serious adverse events is observed.

Vector Production

The Vector was produced and will be supplied by the vector production facility of Cincinnati Children's Research Foundation, Division of Experimental Hematology, Cincinnati Children's Hospital Medical Center, Cincinnati OH.

Transduction of autologous CD34+ cells with the retroviral vector pSRS11.EFS.IL2RG.pre* will be carried out in the GMP cell manipulation facilities.

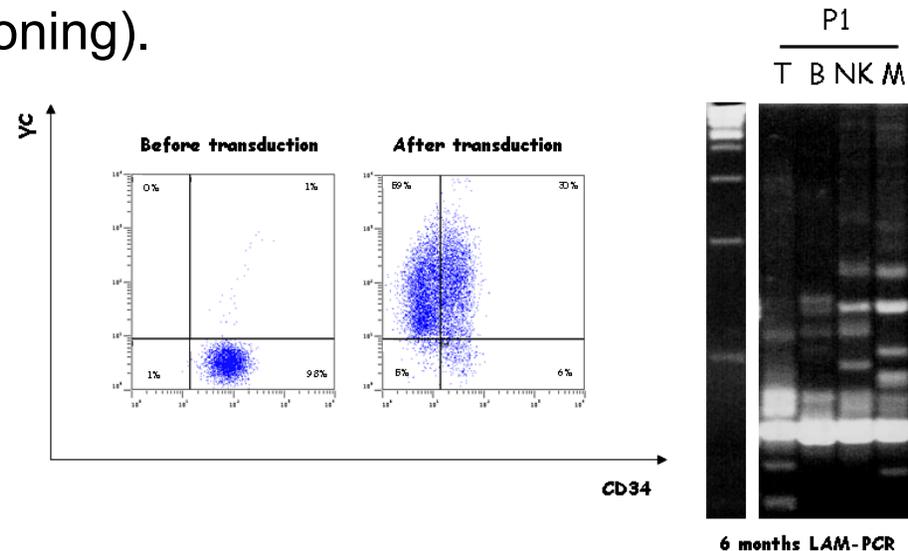
Certification of clinical grade vector supernatant with a titer of $>1 \times 10^6$ transducing units/ml is currently underway (anticipated completion of certification ~3/09).

AGE AT TREATMENT...

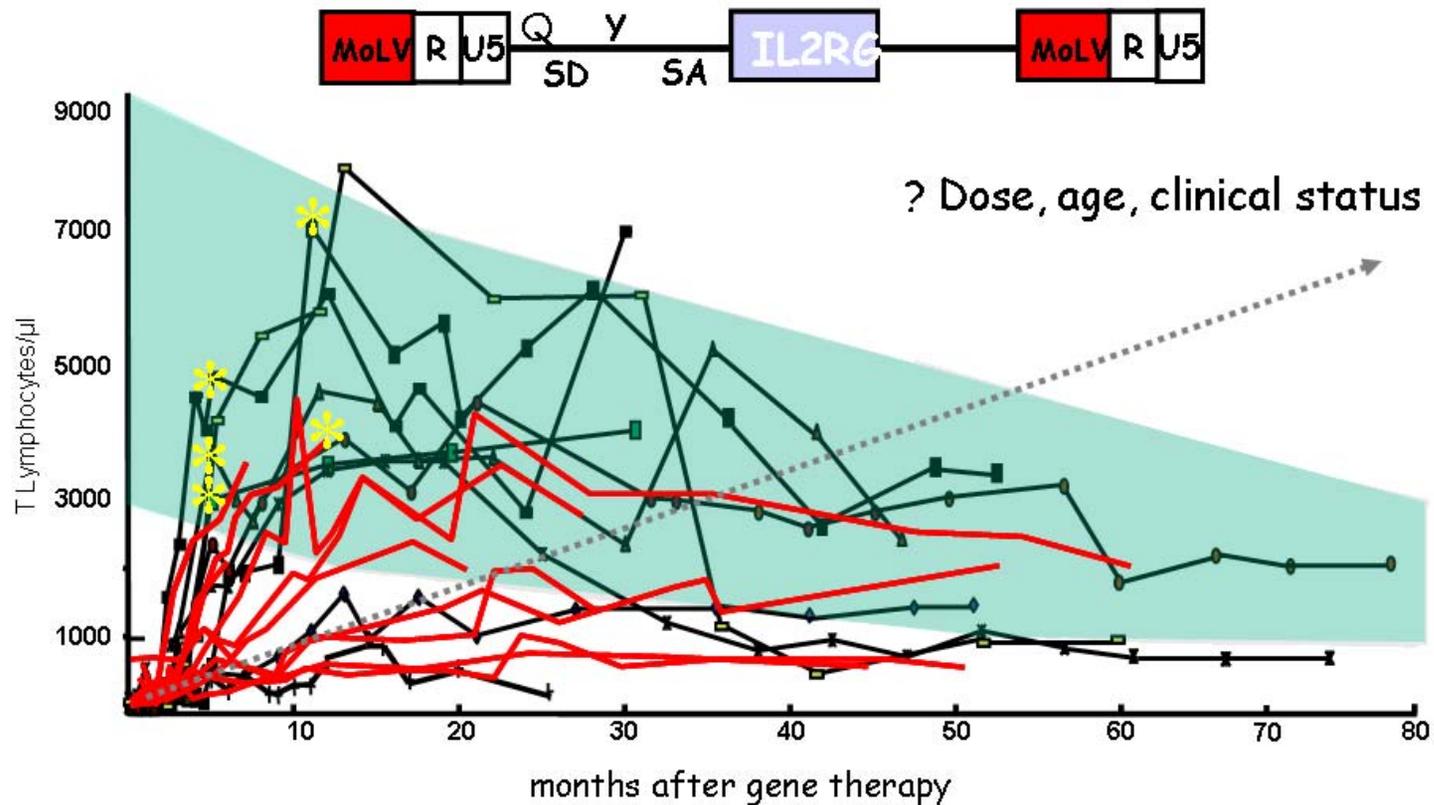
Does the observation that two older patients in prior SCID-X1 trials failed to have immunological reconstitution alter the inclusion criteria with respect to age?

We appreciate this concern. We are aware (and in fact have mentioned in our proposed protocol) that two older patients (aged 15 and 20 years) with SCIDX1 treated in London by gene therapy have failed to attain immune reconstitution (**Thrasher et al., Blood 2005**).

But, also **Chinen et al, 2007** (3 patients with poor function after T-depleted HSCT and no conditioning).



Age effects ?



Transgene leukaemogenicity ?...

Is there any experiment(s) that can exclude the leukemic potential resulting from the forced expression of the IL2RG gene?

Woods et al, *Nature* 2006:

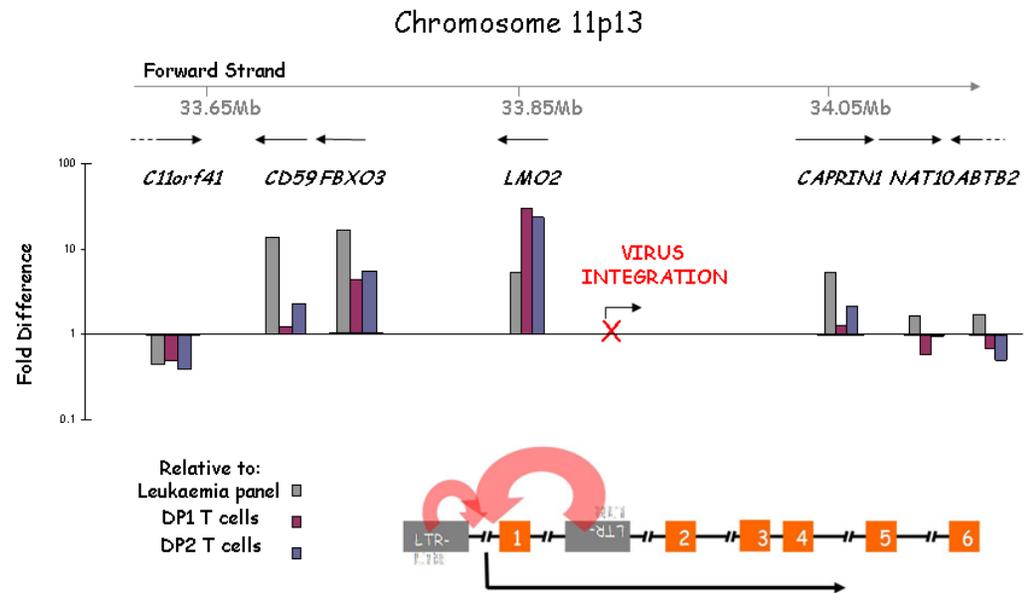
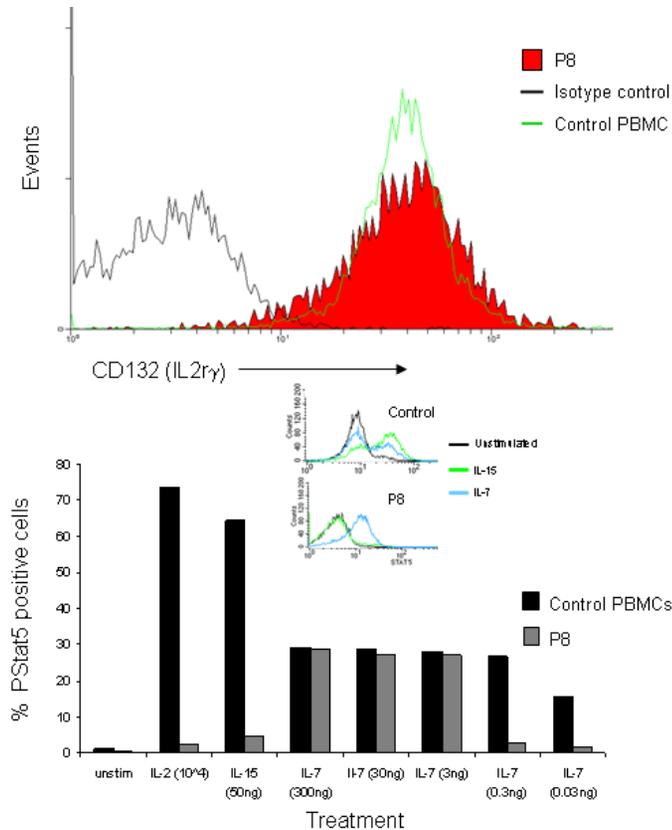
5/15 C57BL6 mice reconstituted with lentiviral vector developed lymphoma

Dave et al, *Science* 2004 (and RTCGD database):
coincident insertions in *Imo-2* and *il2rg* in 2 tumours

BUT no over- expression of gc and in 5' region of Med12 gene

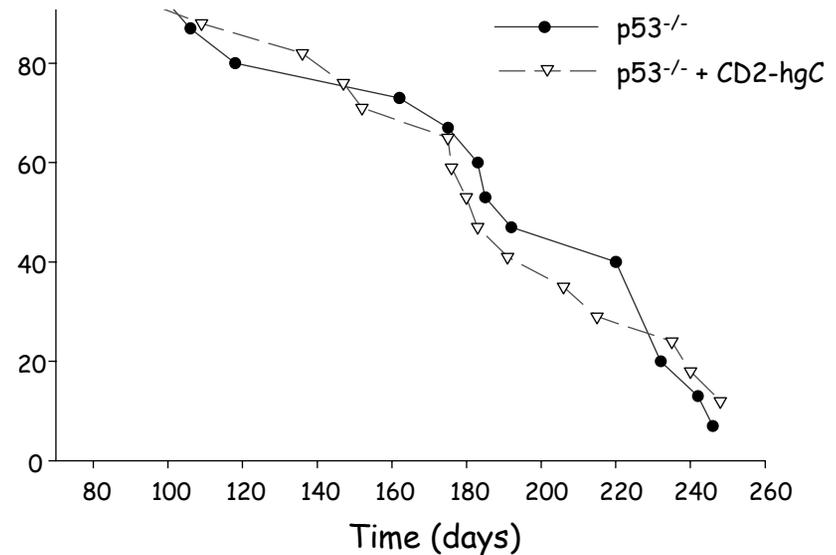
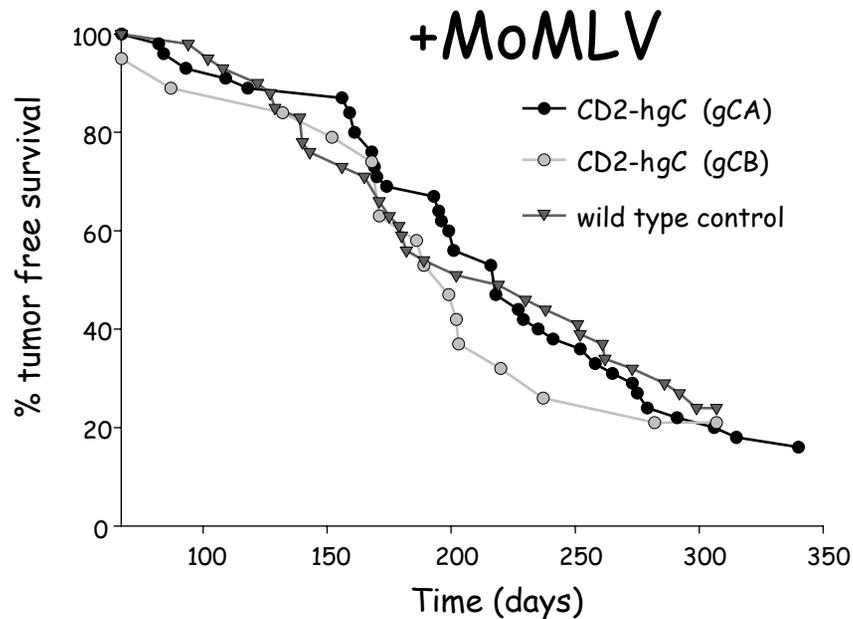
Transgene leukaemogenicity ?...

Is there any experiment(s) that can exclude the leukemic potential resulting from the forced expression of the IL2RG gene?



Is there any experiment(s) that can exclude the leukemic potential resulting from the forced expression of the IL2RG gene?

In 2 transgenic CD2-hIL2RG lines 66 mice (and 48 controls) tumour free for >18 mo, and...



NO EVIDENCE FOR INTRINSIC ONCOGENICITY....

Does the nature of the mutation in IL2RG matter in patient selection?

Patients are selected on basis of genotype and immunophenotype....

Patient details...

Clinical data	Age at therapy (months)	Maternal graft	Mutation	Gamma chain expression	Total cells infused (x10e6)
P1	10	++	R289X	++	180
P2	10	++	S238N	-	180
P3	4	-	Y125C	+/-	78
P4	3y	-	R289X	++	115
P5	10	-	R222C	++	200
P6	10	-	PolyA	-	200
P7	6	-	M1i	-	84
P8	13	-	C182Y	+	207
P9	7	-	S108P	+	160
P10	12	-	del	-	60

Have secondary transplant studies such as those performed with the SF-based vector been performed using the EFS-based vector? If not are such studies planned?

In studies not included in the original RAC submission (but included in pre-IND FDA filing), under similar conditions with long-term observation and serial transplantation, we have not seen any tumor formation associated with vector integration when using the proposed clinical vector.....

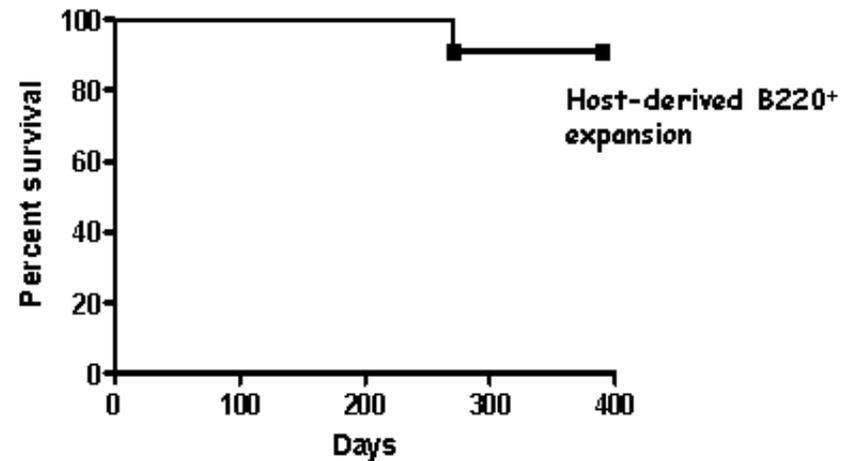
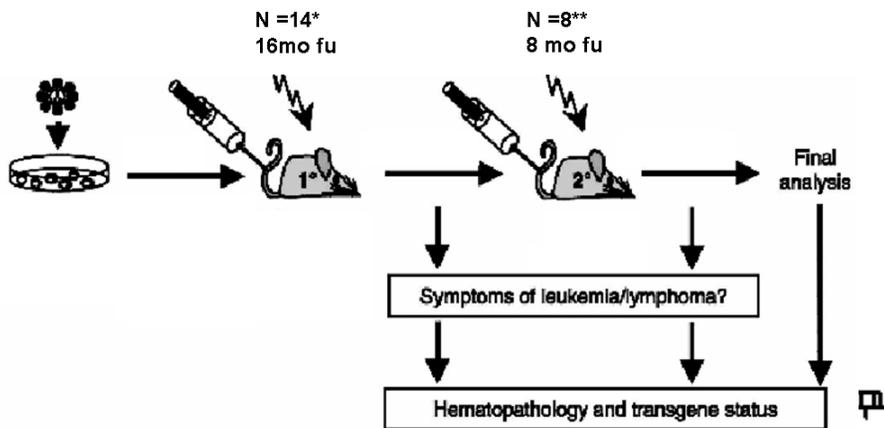
SRS11.EFS.IL2RG.pre*....

C57/Bl6 Ly5.1/Ly5.2 (same as NTP study)

14 animals engrafted long term (marking PB/spleen 5-60%)

4 highest marked (PB/spleen 30-60%) from primary cohort engrafted into **8** secondary recipients.

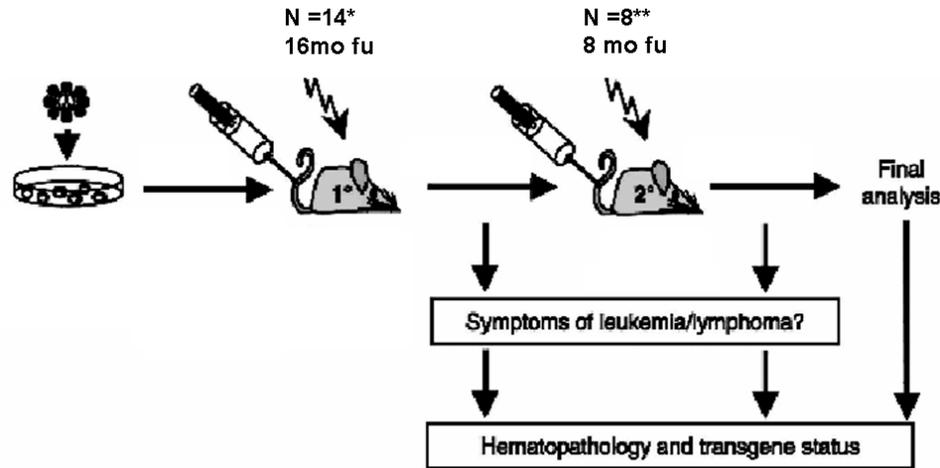
Marking at kill in secondary recipients 4-10%



*1 host-derived B220+ expansion +9mo, 1 non-haematopoietic liver tumour +16mo

** 1 host B220+ expansion (1 non-evaluable), 1 host thymoma

SRS11.EFS.IL2RG.pre* safety summary....



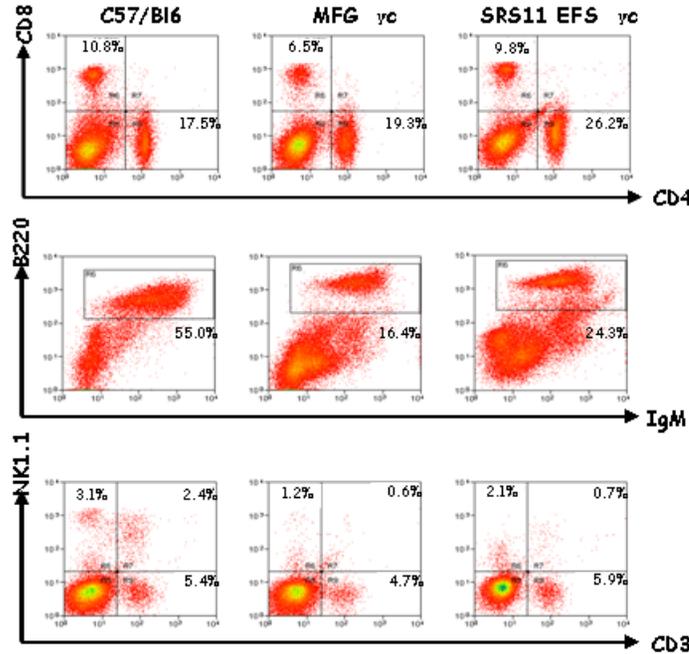
32/33 control C57/Bl6 mice that had received SRS11 EFS γ c-transduced cells tumour free after 10 mo. One host derived tumour noted within this period.

5/8 secondary transplants tumour free after 8 mo

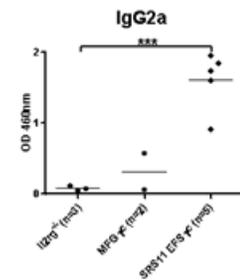
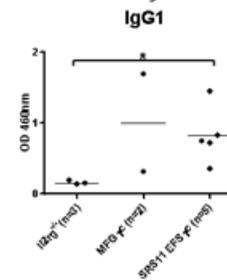
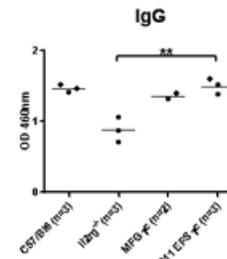
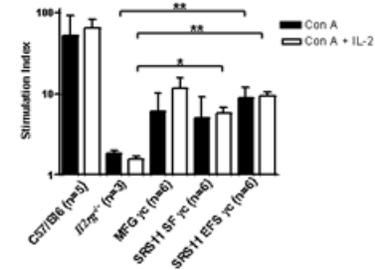
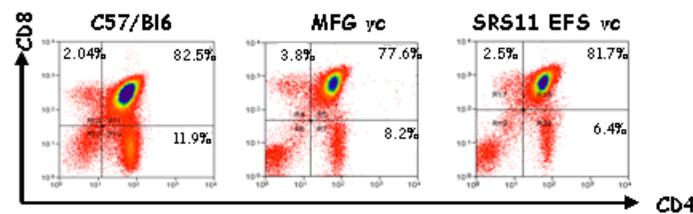
NO DONOR-DERIVED TUMOURS

SRS11.EFS.IL2RG.pre* efficacy summary....

SPLEEN



THYMUS



n=6 n=14
cn 0.47 cn 0.27

Copy number targets

Please discuss how the mouse studies in which average transgene copy numbers of 1.1 copies per cell (Protocol, p. 20) relate to the goal of achieving copy numbers of 2-5 in the transduced cells as discussed in the Response to Appendix M, p. 36.

In our previous human SCID studies, we achieve an average copy number of 1 in peripheral T cells with an initial transduction efficiency of 30-80%. As we will change neither envelope protein nor culture conditions in the transduction protocol, vector dosage for the future patients should be similar as in the previous trials. This was an important consideration in favor of using a gammaretroviral SIN vector.

Conclusions

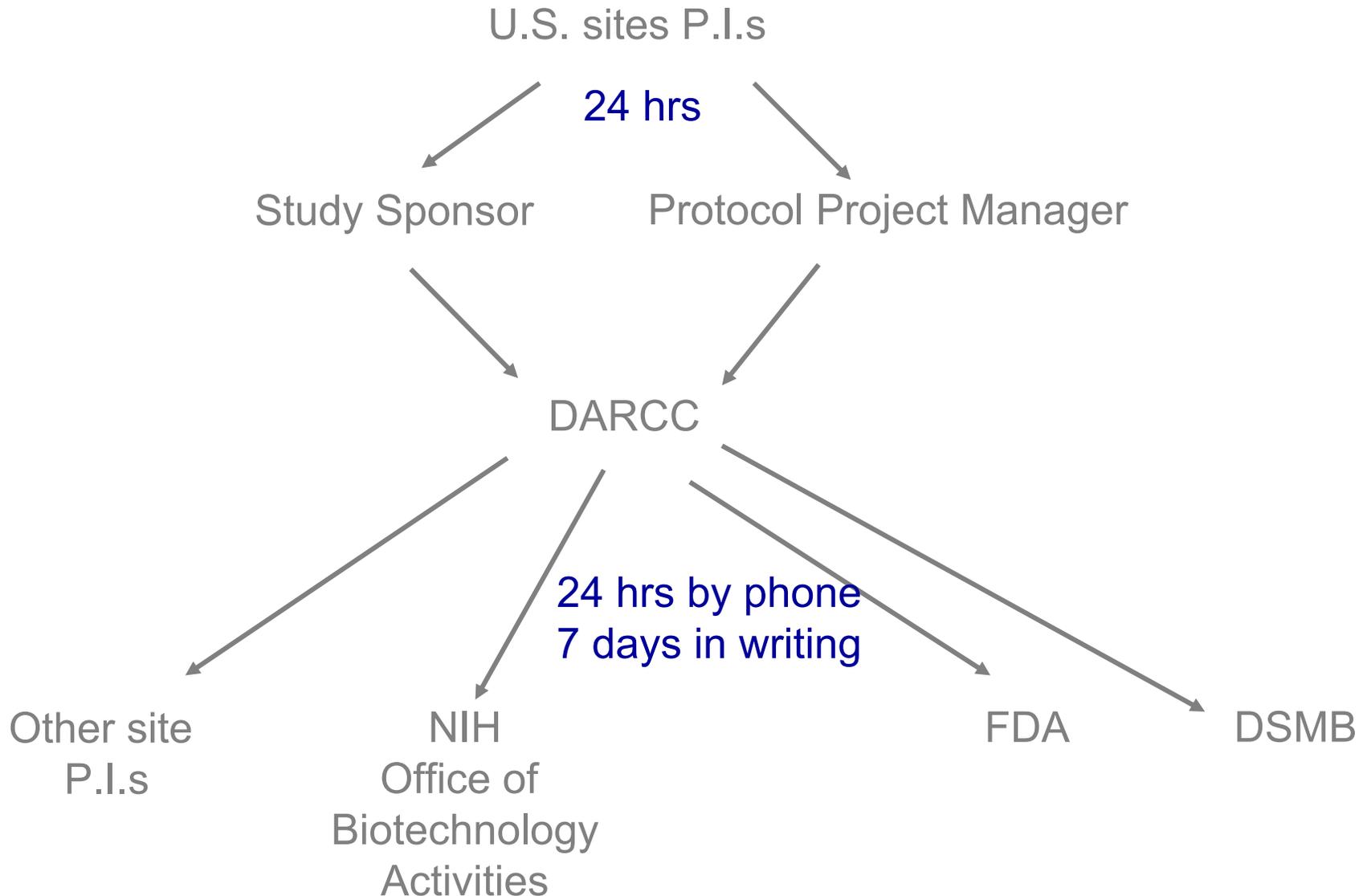
Gene therapy has good therapeutic potential for SCID-X1 and can be implemented rapidly

Toxicity is driven by insertional mutagenesis

New vector is demonstrably less mutagenic in surrogate systems

Change in configuration of vector alone likely to have significant influence on safety profile

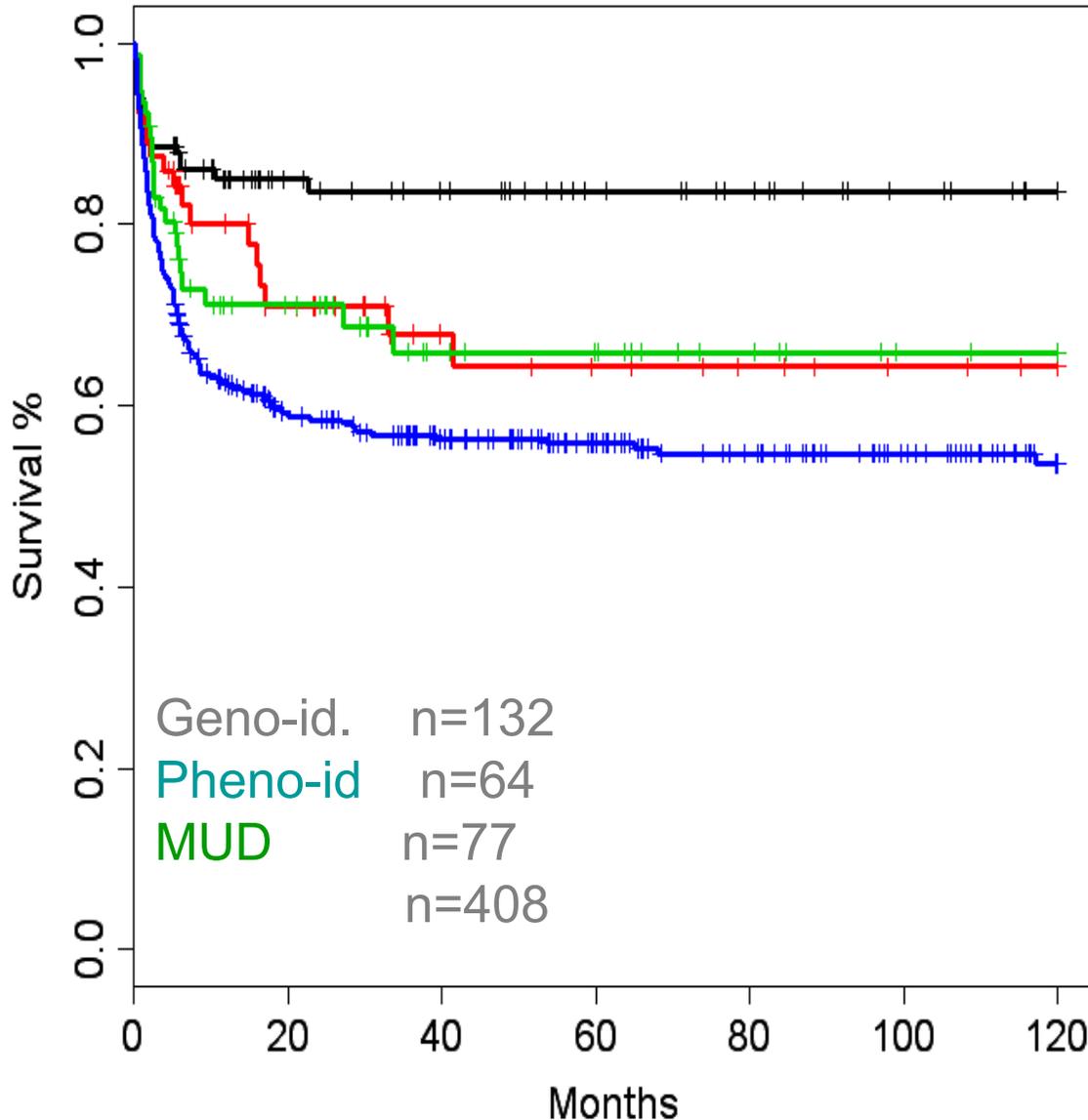
Reporting of Serious Adverse Events



Long term outcome of HCT for SCID-X1 and JAK3 deficiencies (Paris)

1976-2005	n=38	median 13 yrs (2-30)	
		at any time (%)	at last follow up (%)
• free of serious complications			
<i>including HPV disease</i>		16 (42)	22 (58)
<i>excluding HPV disease</i>		23 (60)	28 (73)
• cGVHD		4	2
• colitis/auto immunity		3	2
• severe infections		4	3
• severe HPV disease		9	6
• nutritional support		5	3
• <i>deaths</i>		4	

Probability of survival in SCID patients after HCT according to donor-recipient compatibility



**10 years
Survival rate**

Geno-id : 84%

Pheno-id : 64%

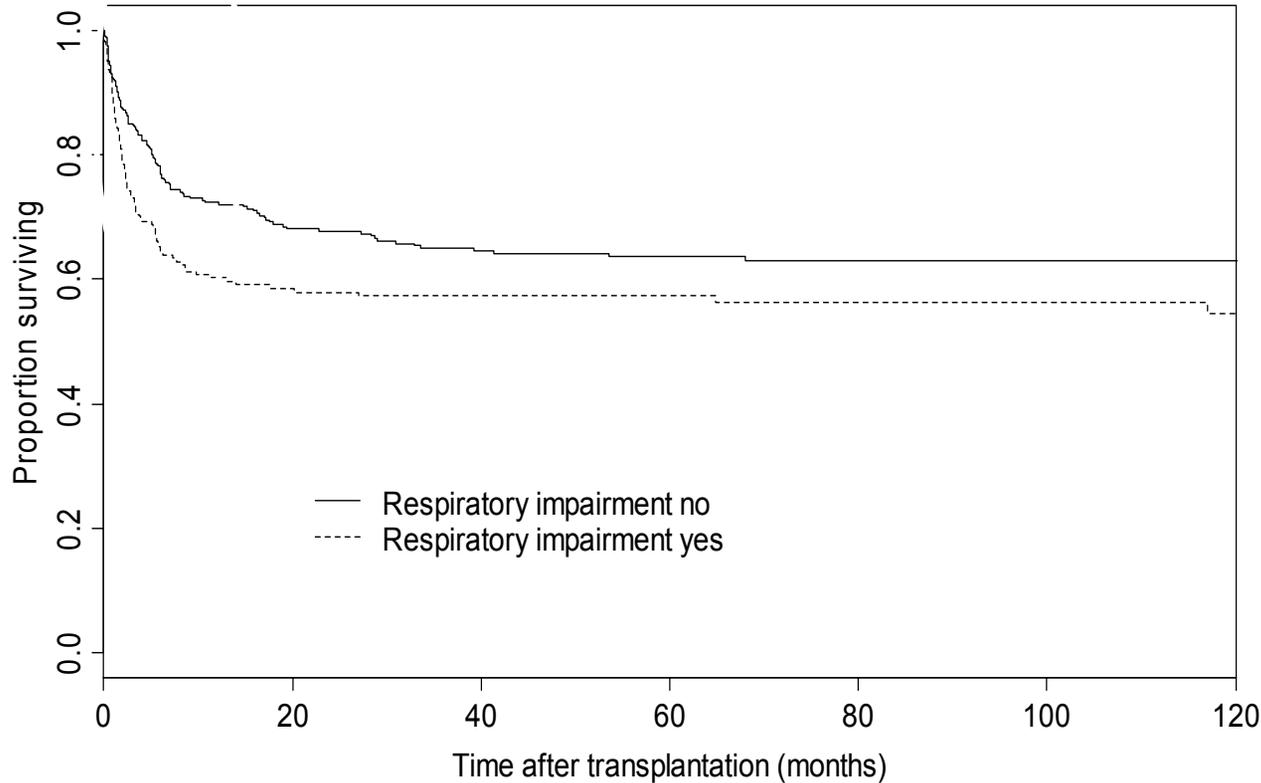
MUD : 66%

$p < 0.0001$

(EBMT Meeting, 2008)

Respiratory infection impacts negatively on survival after HCT for SCID (SCETIDE European Registry)

SCID, all years, Respiratory impairment
 $p=0.006$



**10 years
Survival rate**

Not impaired : 63%

Impaired : 55%

$p=0.006$

Respiratory impairment n=241
No respiratory impairment n=373

(EBMT Meeting, 2008)

Early transplantation results in better outcome

- 35 of 36 (97%) infants transplanted at < 3.5 months survived vs. 67 of 96 (70%) infants transplanted at >3.5 months at Duke

(Buckley, Annu Rev Immunol 2004)

- 20 of 21 (95%) infants transplanted at <28 days survived vs. 71 of 96 (74%) infants transplanted at >28 days at Duke

(Myers et al., Blood 2002)

Safety studies for SRS11.EFS.IL2RG.pre*....

CCHMC IL2RG Animal Data Analysis: 10/1/08					
Recipient	Donor	Vector	Evaluable Mice [^]	Donor Derived Lymphomas	Percent
C57 (CD45.2)	BoyJ (CD45.1)	SRS11.EFS.IL2RG.pre*	19	0	0
C57 (CD45.2)	BoyJ (CD45.1)	SERS11.EFS.GFP.pre*	18	0 ^{^^}	0
C57 (CD45.2)	BoyJ (CD45.1)	Mock	18	0	0
gc ^{-/-} Rag ^{-/-}	gc ^{-/-}	SRS11.EFS.IL2RG.pre*	9	1 (1) ^{^^^}	11.0%
gc ^{-/-} Rag ^{-/-}	BoyJ	SERS11.EFS.GFP.pre*	29	3 (3) ^{^^^}	10.3%
gc ^{-/-} Rag ^{-/-}	BoyJ	Mock/Empty Vector	14	2 (na)	14.3%
gc ^{-/-} Rag ^{-/-}	BoyJ	SERS11.PLESS.EGFPpre*	8	2 (2) ^{^^^}	25.0%
Combined Data from both Models		SRS11.EFS.IL2RG.pre*	28	1	3.6%
		SERS11.EFS.GFP.pre*	48	3	6.3%
		Mock	32	2	6.3%
		SERS11.PLESS.EGFPpre*	8	2	25.0%

[^] Evaluable mice in the C57 recipients were all mice followed for at least 10 months and with more than 30% engraftment at 5 weeks. On average, there was 60-80% engraftment in this group of mice. Evaluable mice in the gc^{-/-} group were mice that survived to 7 months, or those which had tumors prior to 7 months. In the gc^{-/-}Rag^{-/-} transplanted with the IL2RG vector transduced cells, only mice with >2% lymphoid reconstitution were followed.

^{^^} Vector+ tumors. ^{^^}in one mouse tumor (Thymoma), the origin could not be determined; however, the thymoma mass was vector negative. NA=not applicable