St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

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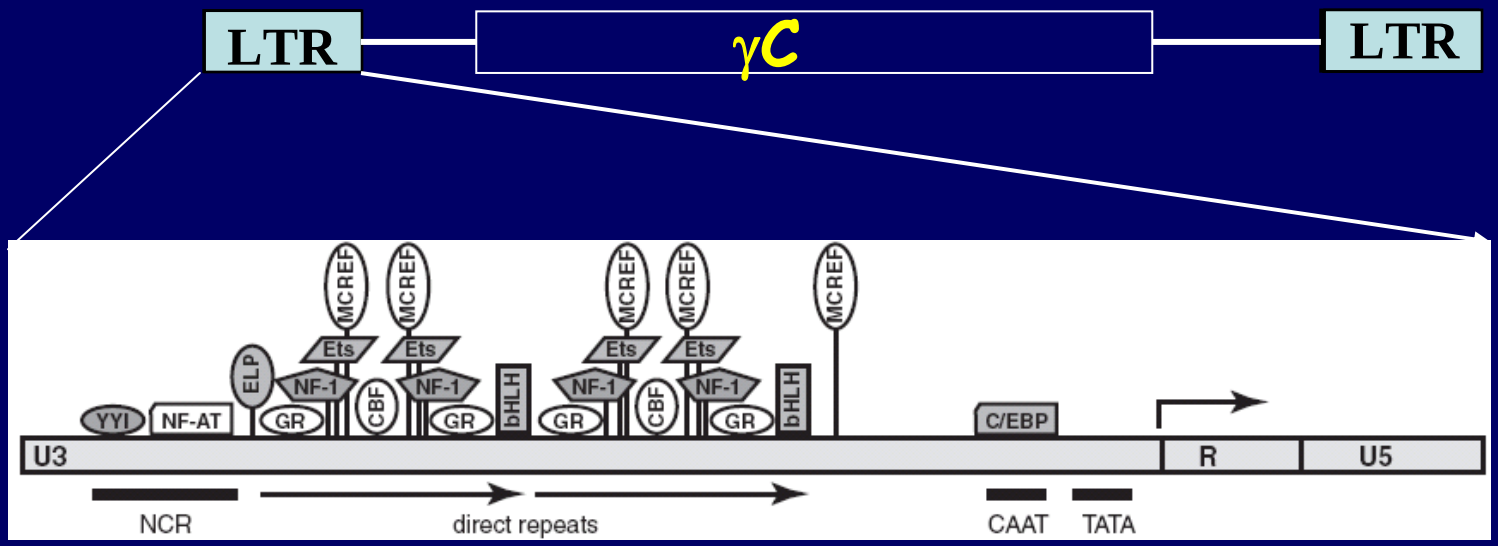
Dept of Hematology
St. Jude Children's Research Hospital

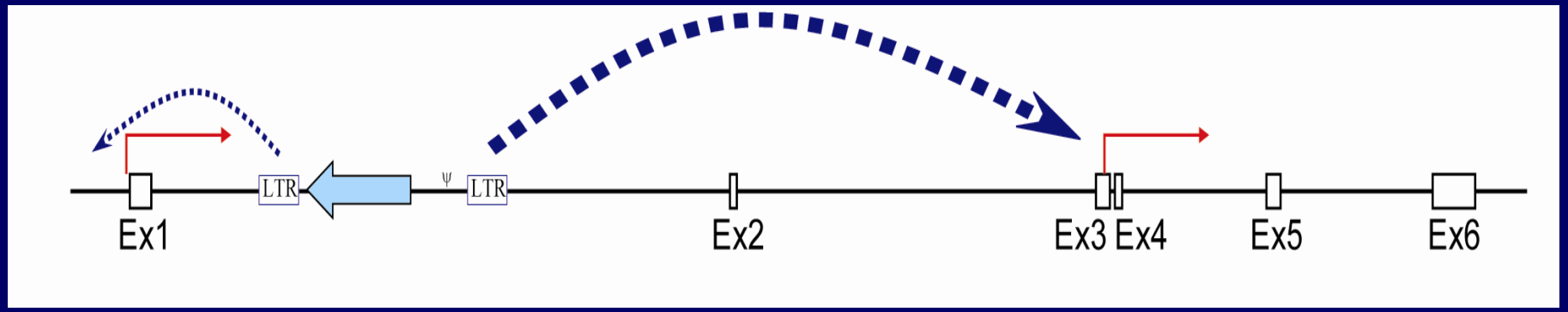
Preclinical Safety and Efficacy Studies

Finding cures. Saving children.

Insertional activation of LMO2 by the LTR enhancer

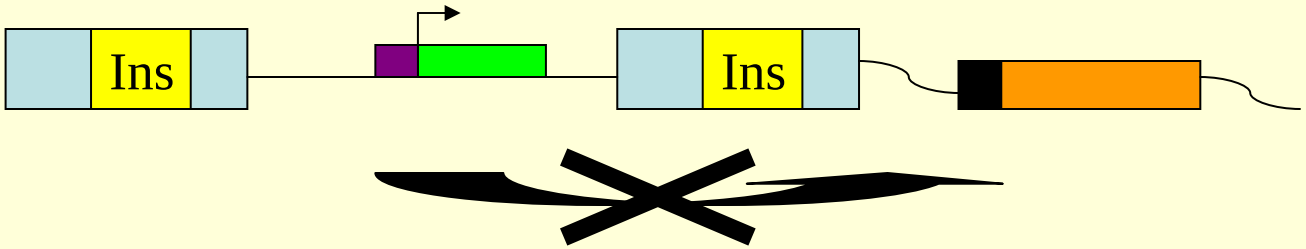


U3 Enhancer



<u>Hypothesis</u>: Self-inactivating HIV vectors that lack enhancer activity and incorporate enhancer blocking elements will increase the safety of XSCID gene therapy

Internal promoter and enhancer blocker



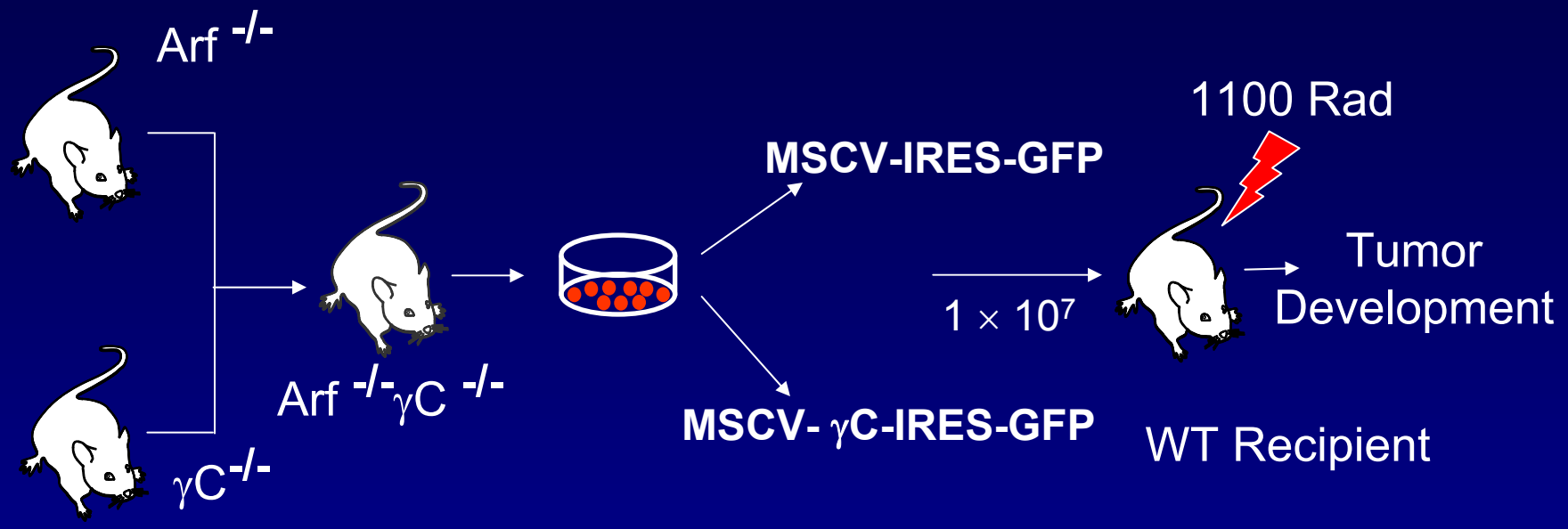
CL20-i4-EF1α-hγ_c-OPT

Potential advantages of lentiviral vectors

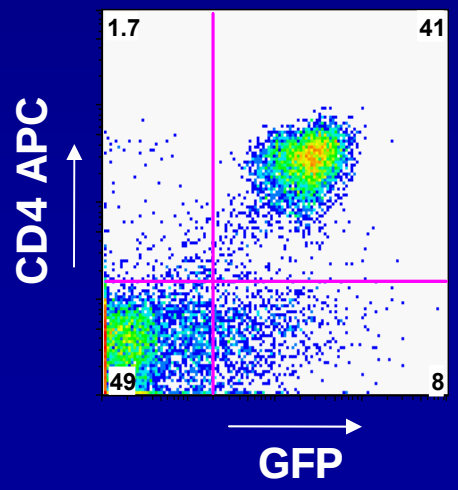
- Unlike MLV, HIV not associated with insertional oncogenesis in the context of wildtype infection.
- Lentiviral vectors have a fundamentally different pattern of genomic integration compared to MLV vectors.
- Lentiviral vectors do not have a predilection for targeting “known problem” cellular oncogenes such as LMO2, PRDM1, EVI1.
- Data from several preclinical models suggest that SIN HIV vectors are less prone to causing activation of cellular proto-oncogenes.
- HIV vectors give superior transduction efficiency in primate hematopoietic stem cells.
- HIV vectors be produced at high titer in SIN configuration, stable producer cell lines now available at St. Jude.

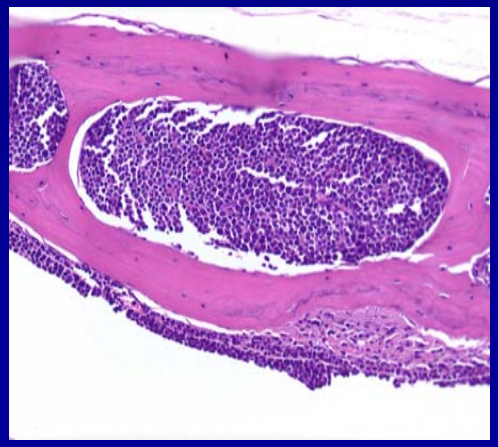
Arf $^{-/-}$ γc$^{-/-}$ mouse model

Shou Y et al, PNAS, 2006

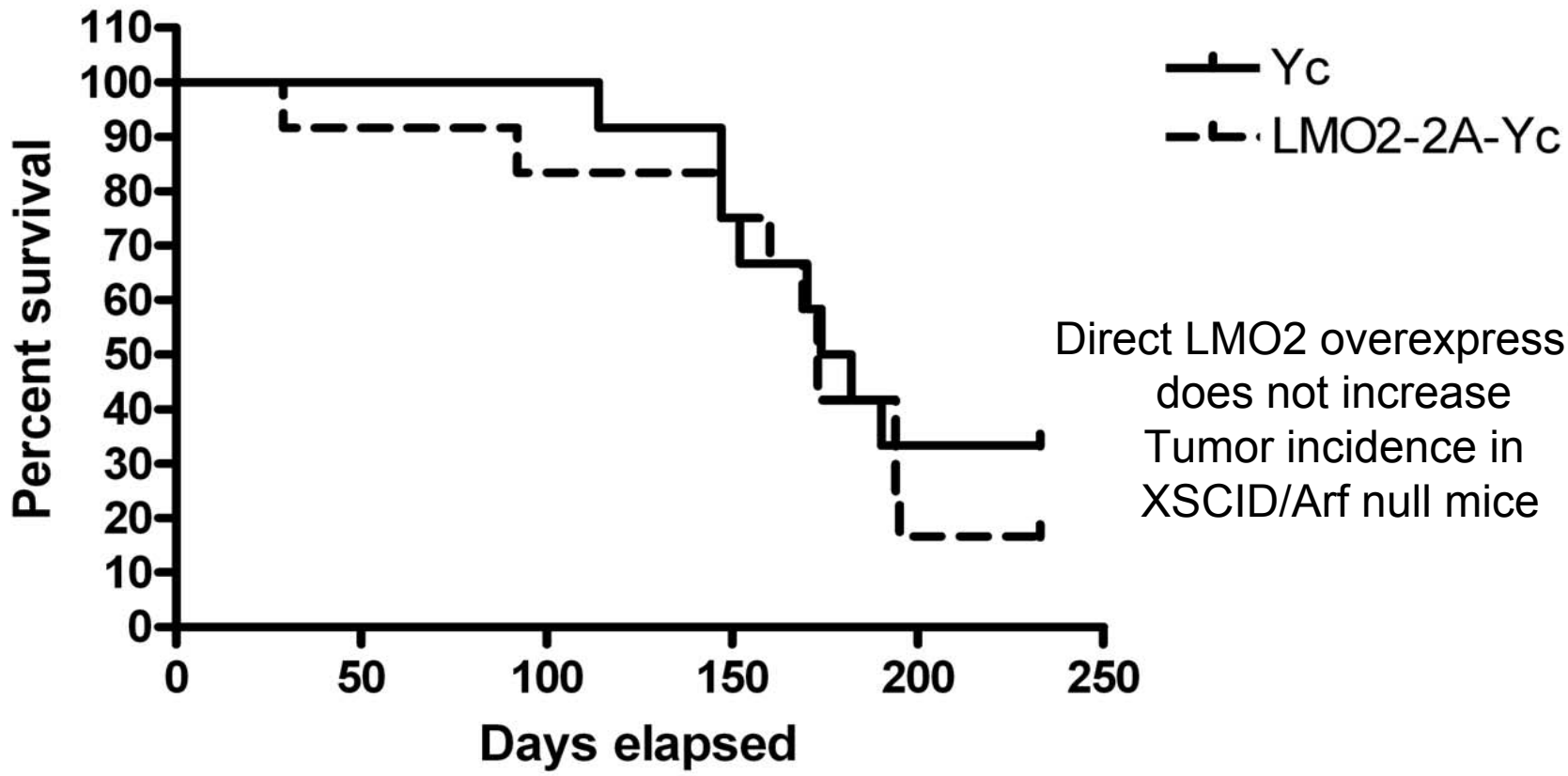


Bone marrow





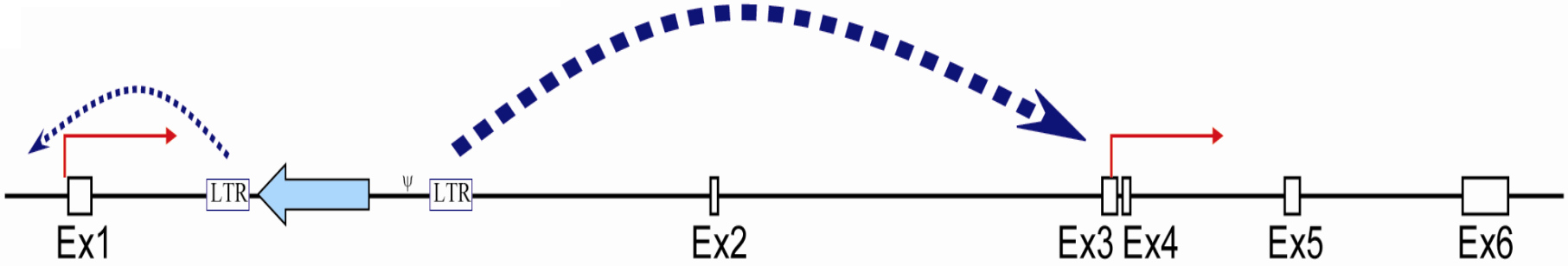
Does the Arf/XSCID model detect activation of relevant proto-oncogenes ?

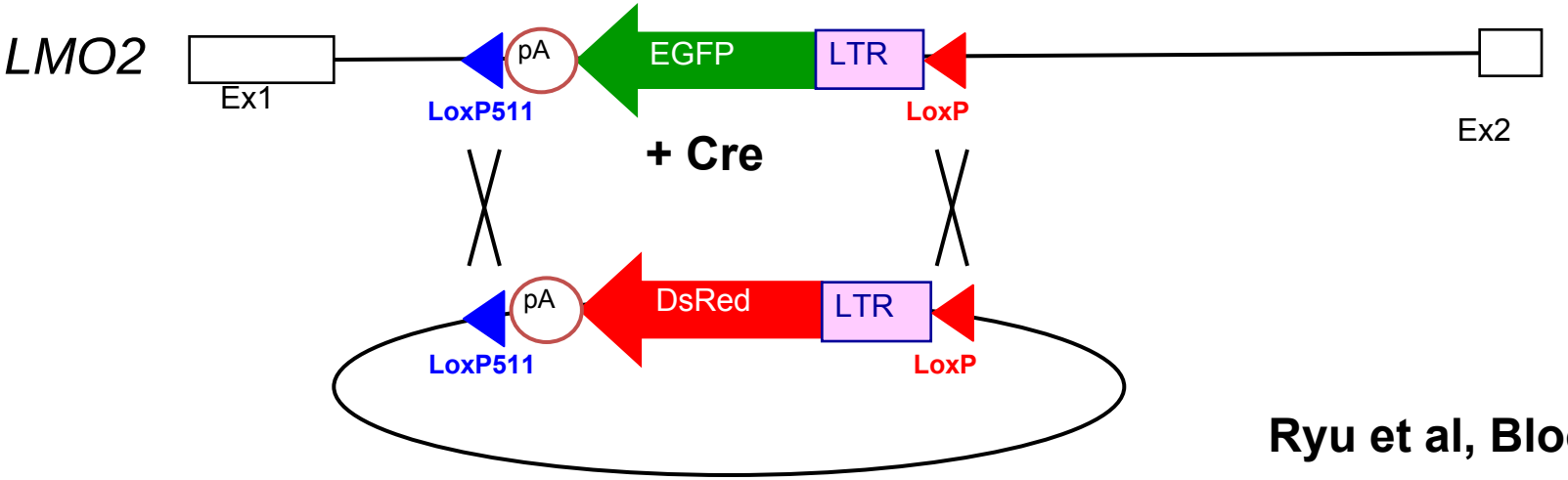


Direct LMO2 overexpression does not increase Tumor incidence in XSCID/Arf null mice

Jurkat/ LMO2 safety assay (Nienhuis lab)

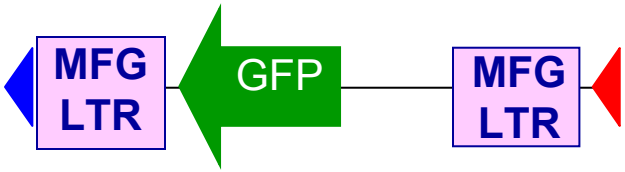
Recreate insertion in Pt4 from French Trial

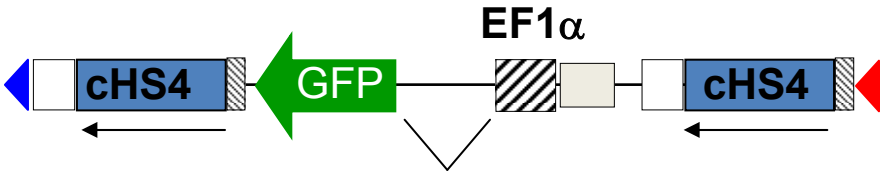


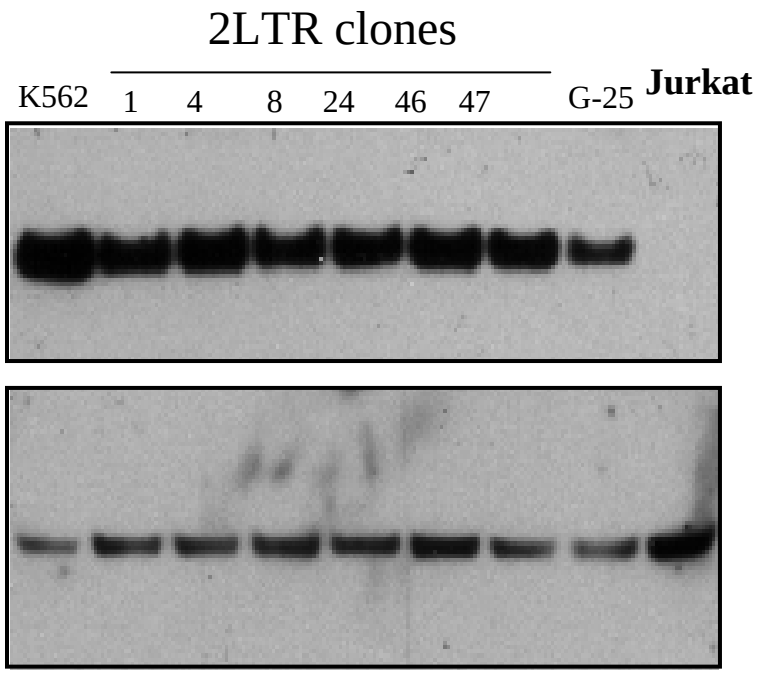


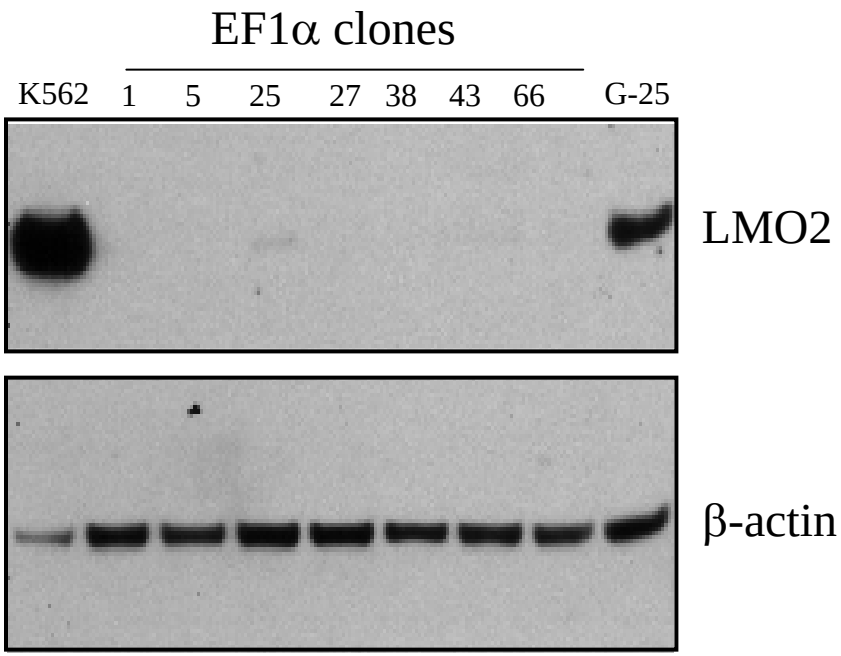
Ryu et al, Blood, 2008

Lack of LMO2 activation with an insulated EF1α HIV vector









Ryu et al, Blood, 2008

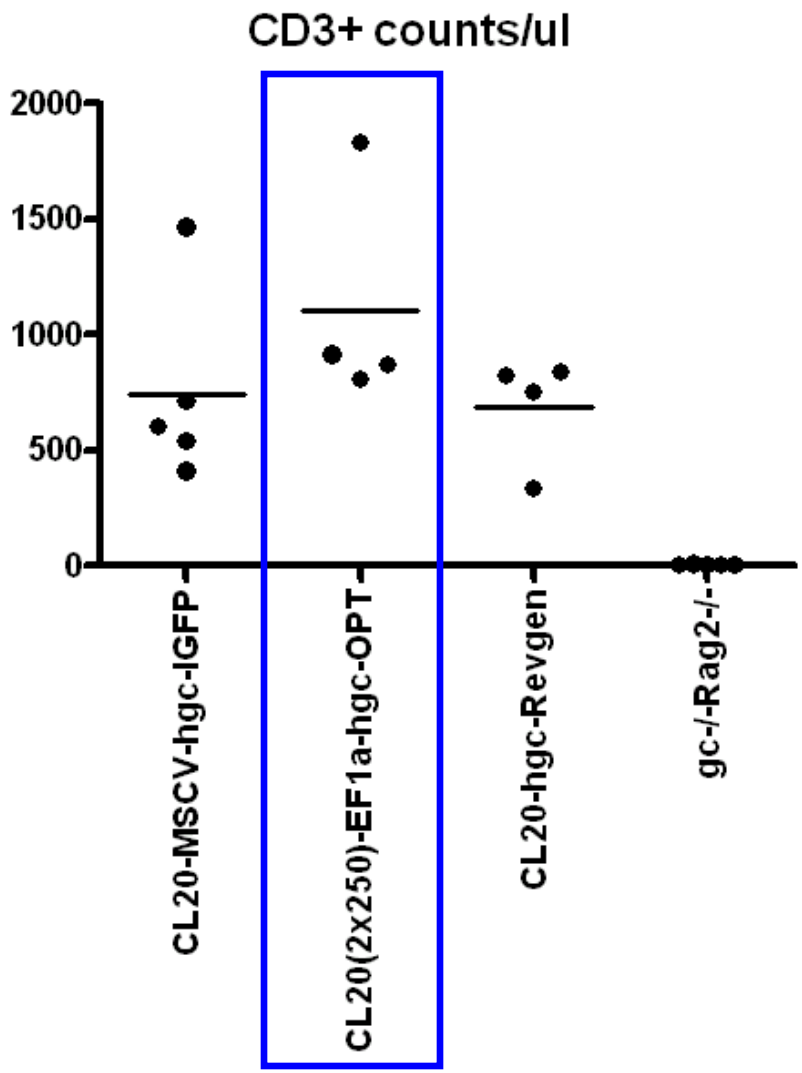
The CL20i4-EF1α-hγ$_c$-OPT vector gives no clones in a myeloid immortalization assay

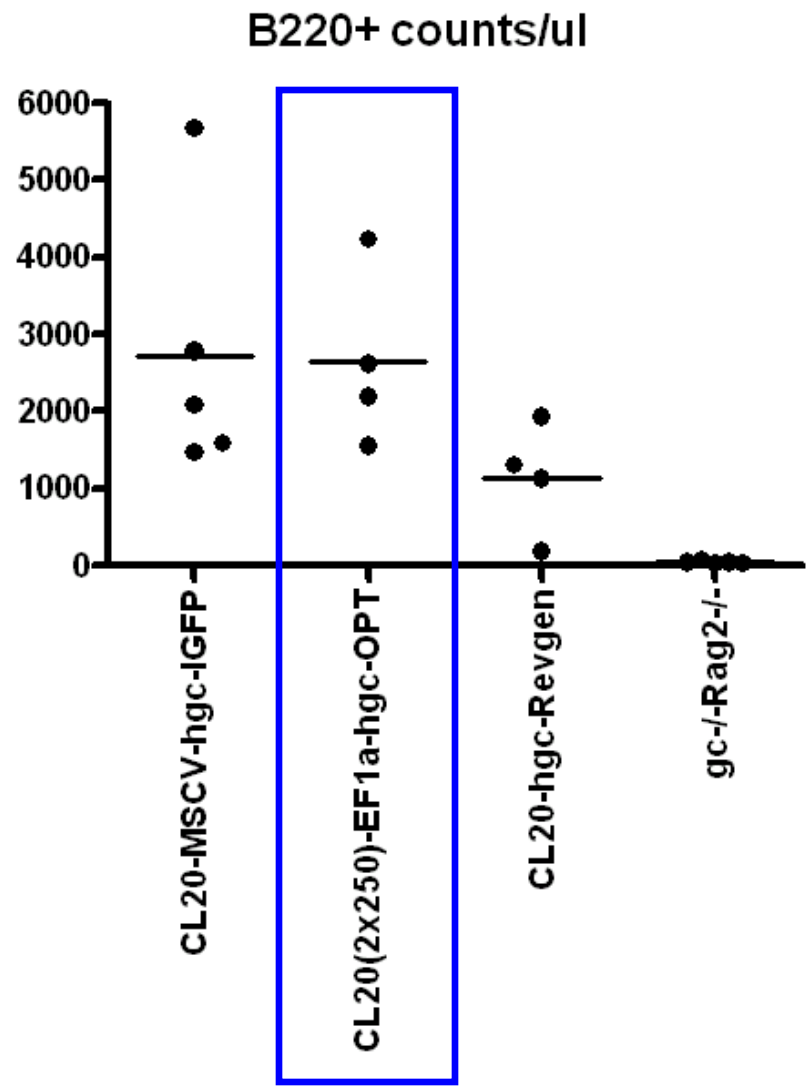
	100cells/well	500 cells/well	Copy Number (Taqman)
1. Mock	0	0	0.04
2. CL20-EF1a-hgc-OPT	0	0	1.4
3. CL20i4r-EF1a-hgcOPT	0	0	1.57
4. CL20i4r-EF1a-hgcOPT(low)	0	0	0.71
5. CL20-revgen3	0	0	0.23
6. CL20i4r-revgen3	0	0	0.31
7. CL20-MSCV-rc-IGFP	0	0	0.88
8. CL20i4r-MSCV-rc-IGFP	0	0	2.23
9. SF91: 1x	0	14	2.08 (Southern)
10. SF91: 2x	6	16	2.32 (Southern)
11. SF91: 3x	0	8	2.32 (Southern)
12. SF91: 4x	8	37	
13. MSCV-rc-IFGP(100ul)	0	5	1.46 (Southern)
14. MSCV-rc-IGFP(10ul)	0	0	

Efficacy data for CL20-i4-EF1α-hγ_c-OPT vector in preclinical models

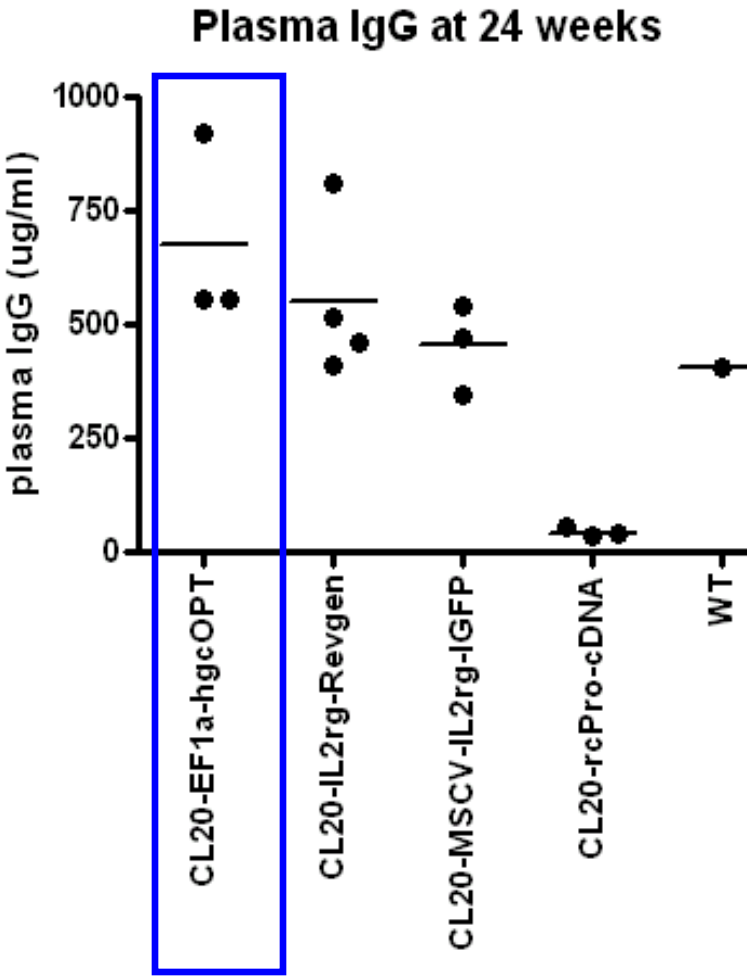
- XSCID mouse models
- XSCID dog model
- Human XSCID CD34+ cells

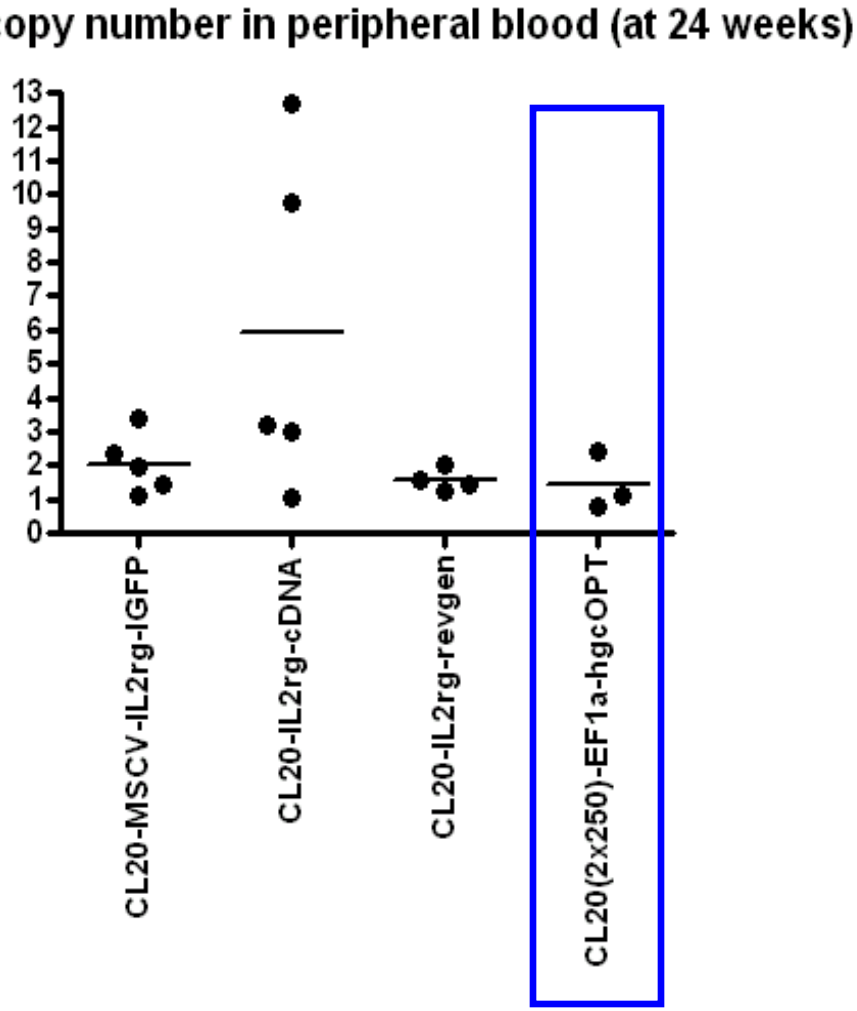
Reconstitution of XSCID Mice with the CL20(2X250)-EF1α-hγ_c-OPT vector 18 weeks after transplant





Reconstitution of XSCID Mice with the CL20(2X250)-EF1α-hγ_c-OPT vector 18 weeks after transplant





Immune Reconstitution of an XSCID Dog Following VSV-G CL20-4i-EF1α-hγc-OPT In Vivo Gene Therapy

Peter Felsburg
Suk See DeRavin

- **Direct IV injection of vector supernatant into newborn puppies**
- **Model established previously using MLV-derived vectors**
- **Selective advantage for transduced cells.**

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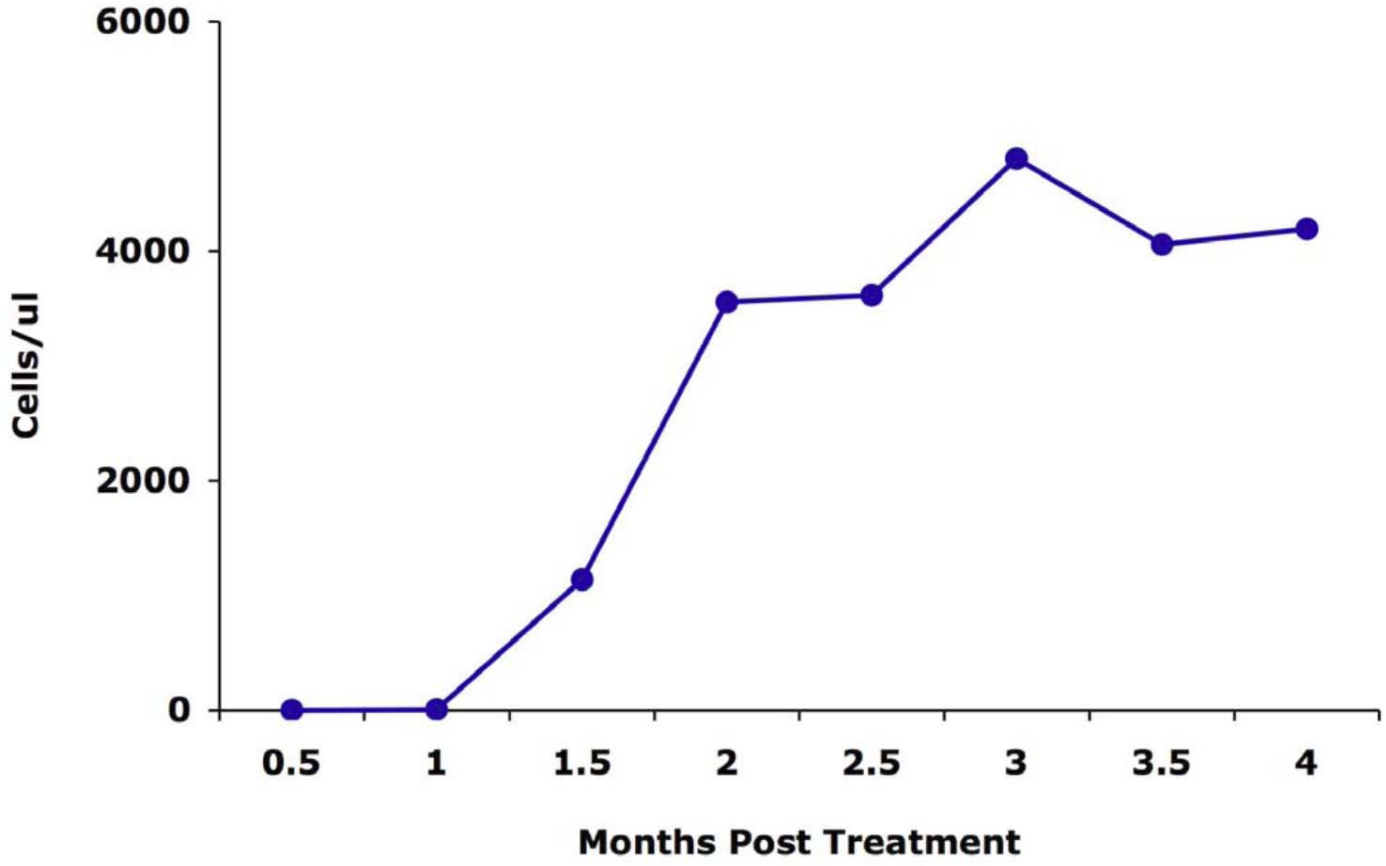
Number of γc^+ T Cells



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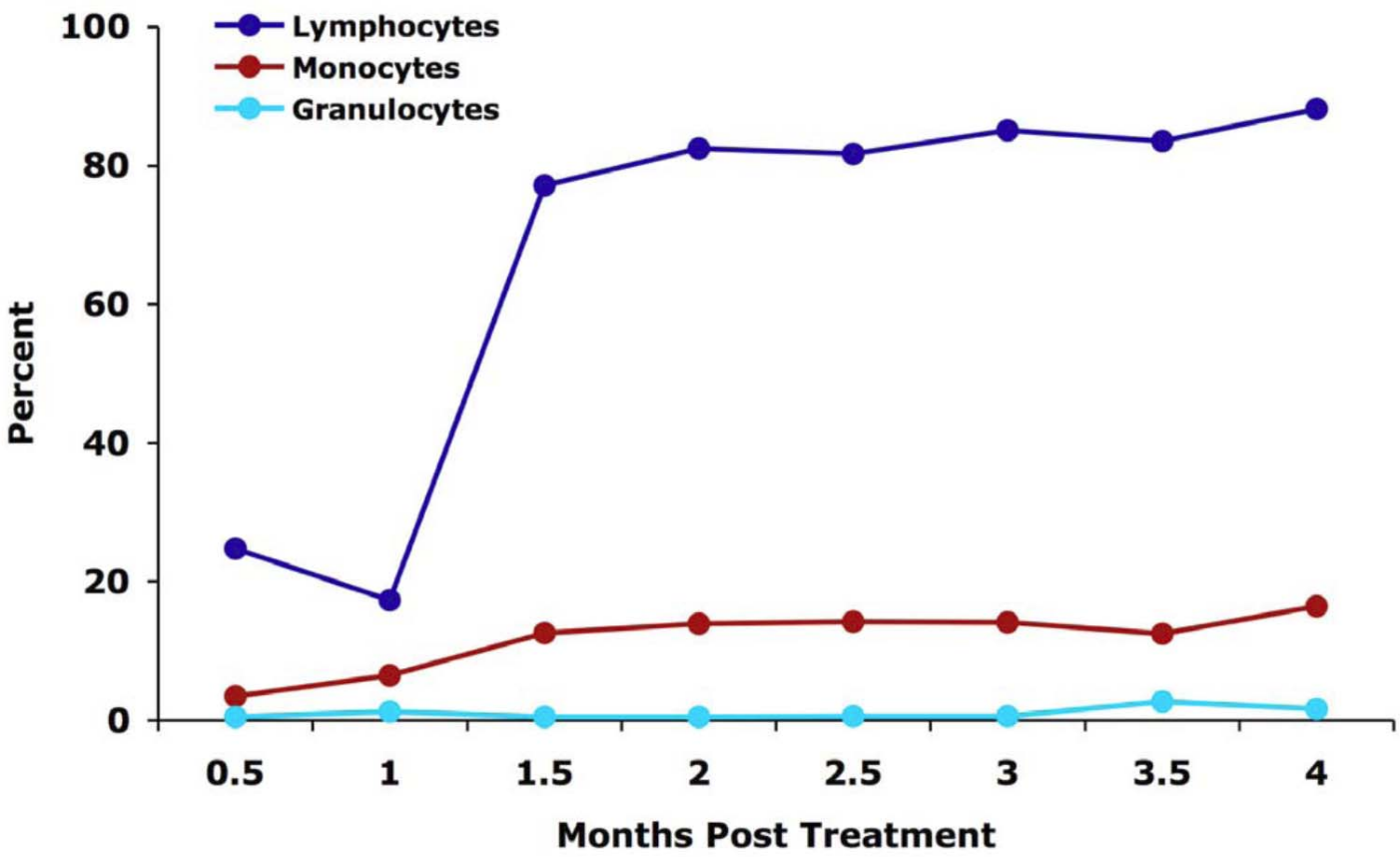
Proportion of γc^+ Cells



Granulocytes = 0.5 to 2.7%.

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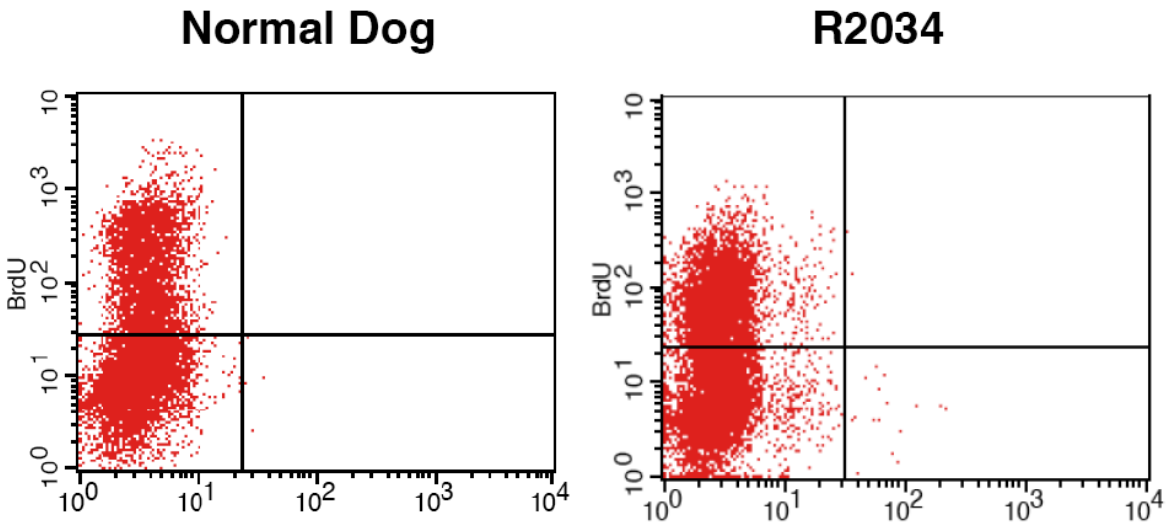
Proliferative Response to PHA
(4 Months Post Treatment)



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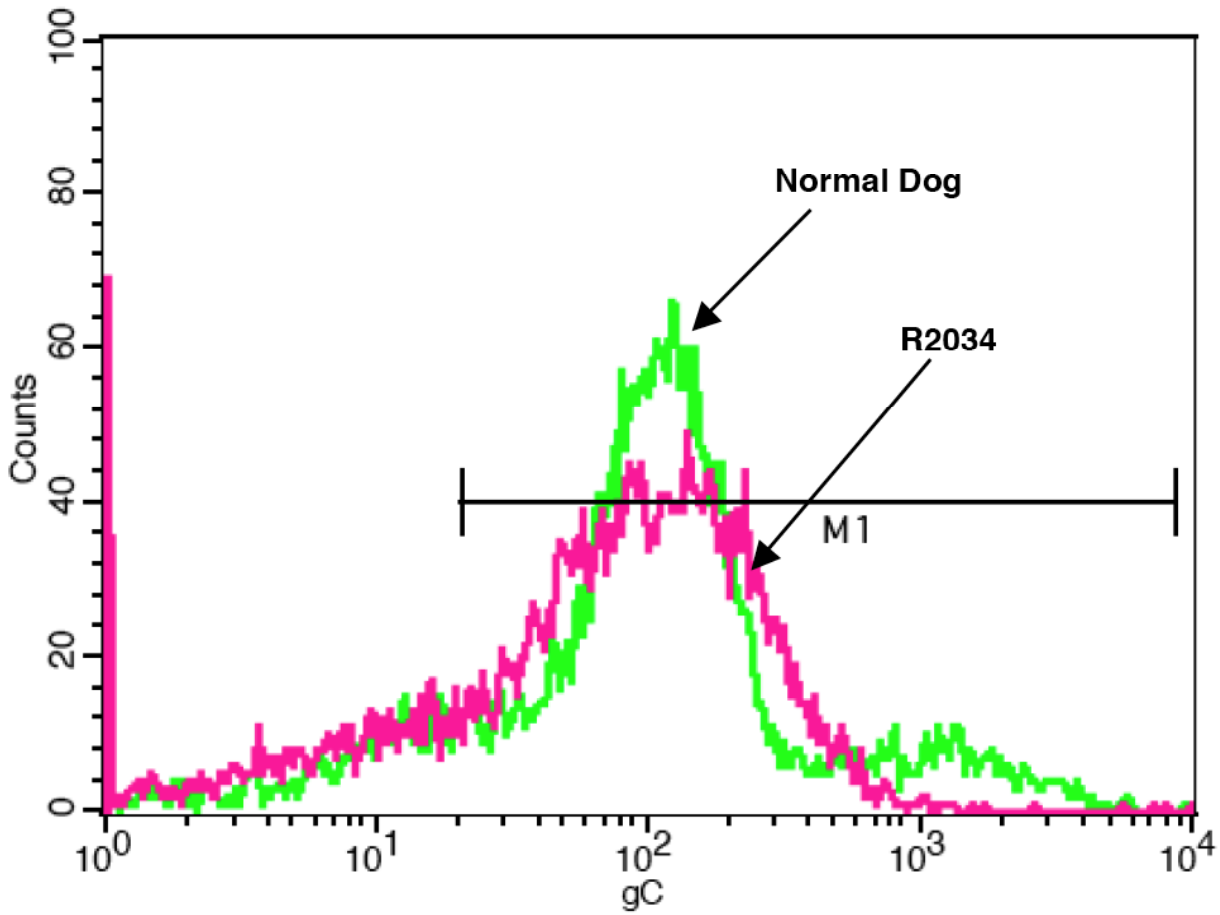
γc Expression
(4 Months Post Treatment)



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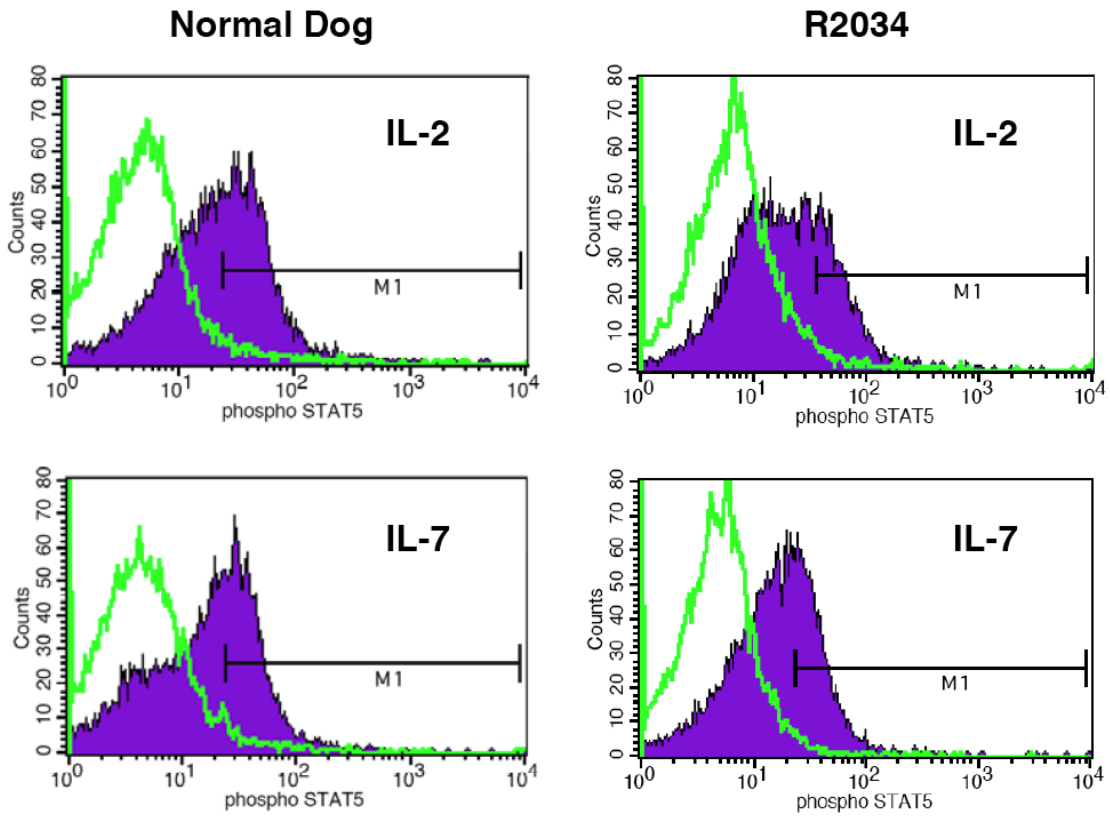
IL-2 and IL-7 Induced STAT5 Phosphorylation
(4 Months Post Treatment)



Green = unstimulated.
Purple = stimulated.

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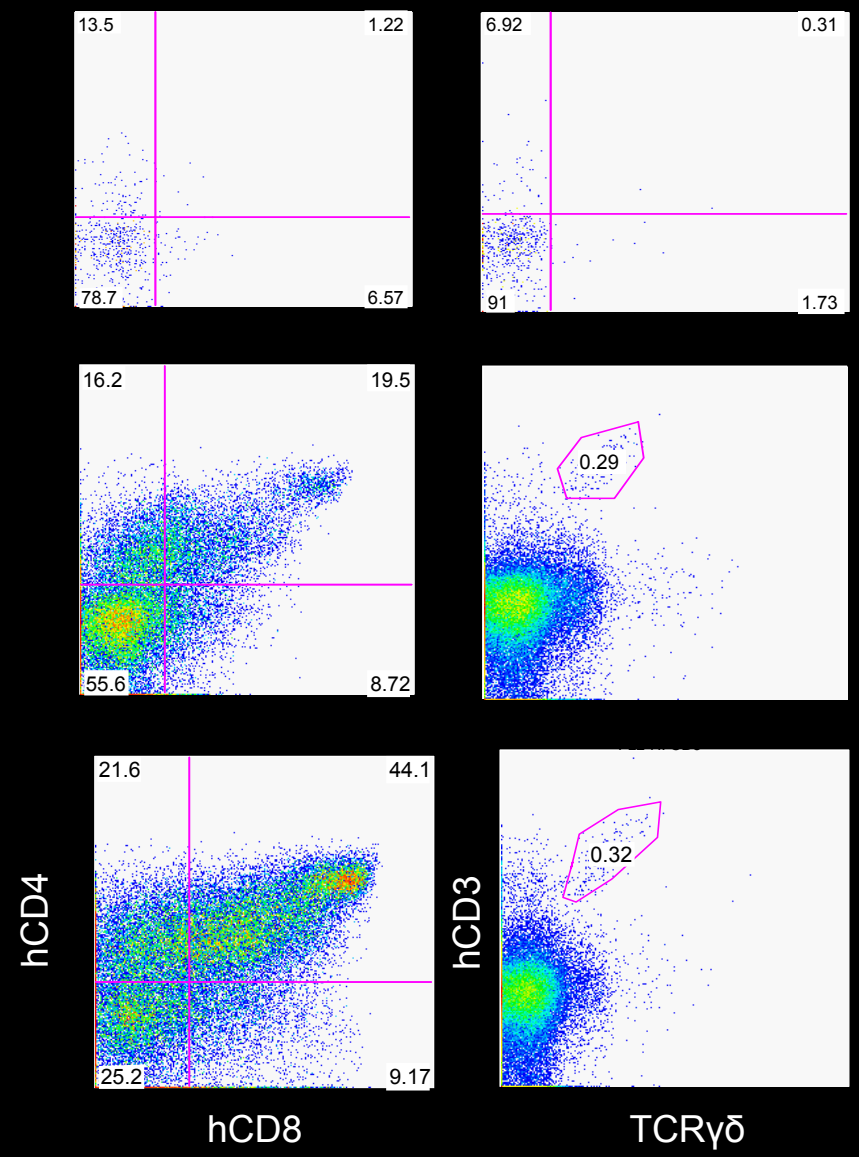
Normal T cell development in vitro using human CD34+ bone marrow cells from a SCID-X1 patient transduced with the CL20-EF1α-hγ$_c$-OPT vector:

OP9-delta1 stroma cell cultures, day 27

Julia Hauer
And Marina
Cavazzano-Calvo



CD34+ XSCID cells

Mock transduced

CD34 +XSCID cells

CL20-EF1α-hγc-OPT

CD34+ normal cells

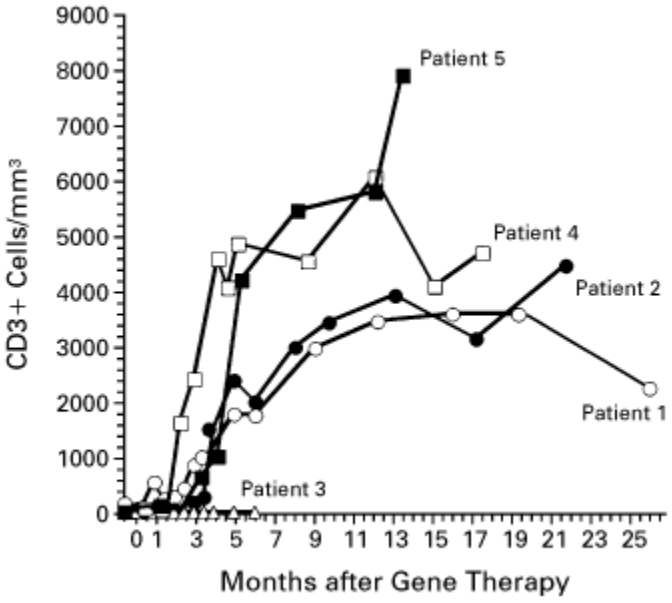
Cord blood

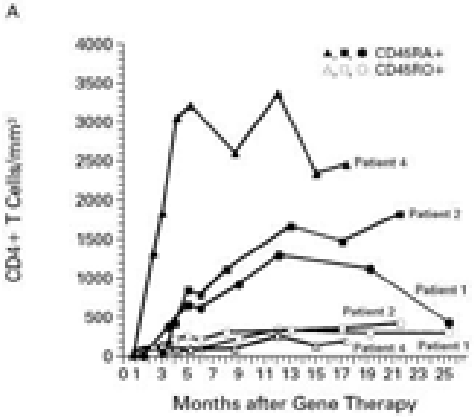
Conclusions

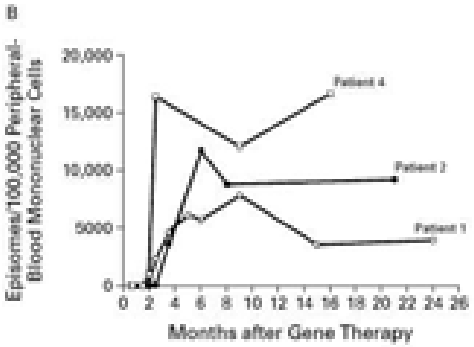
- The XSCID/Arf model shows that the XSCID phenotype and γ_c gene replacement increase the risk of T-cell malignancy, but is too sensitive to detect oncogene activation events due to insertional mutagenesis. These risk factors underscore the need for safer vector systems !

- The CL20-i4-EF1α-hγ_c-OPT vector is less likely to cause oncogene activation compared to LTR MLV vectors based on studies from other labs, based on LMO2 activation studies in Jurkat cells with a similar vector, and based on our own myeloid immortalization data.

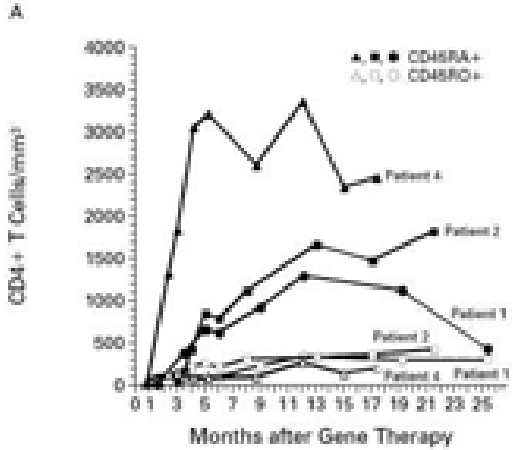
- The CL20-i4-EF1α-hγ_c-OPT vector corrects the phenotype in XSCID mice (2X250), XSCID dogs, and in human XSCID CD34+ cells in vitro.

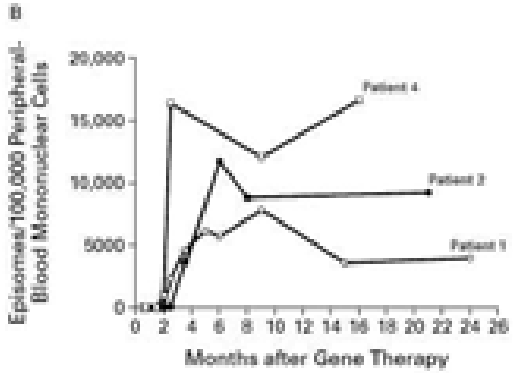

9000
8000
7000
6000
5000
4000
3000
2000
1000
0
CD3+ Cells/mm³
Patient 5
Patient 4
Patient 2
Patient 1
Patient 3
0 1 3 5 7 9 11 13 15 17 19 21 23 25
Months after Gene Therapy


A
CD4+ T Cells/mm³
CD45RA+
CD45RO+
Patient 4
Patient 2
Patient 1
Months after Gene Therapy

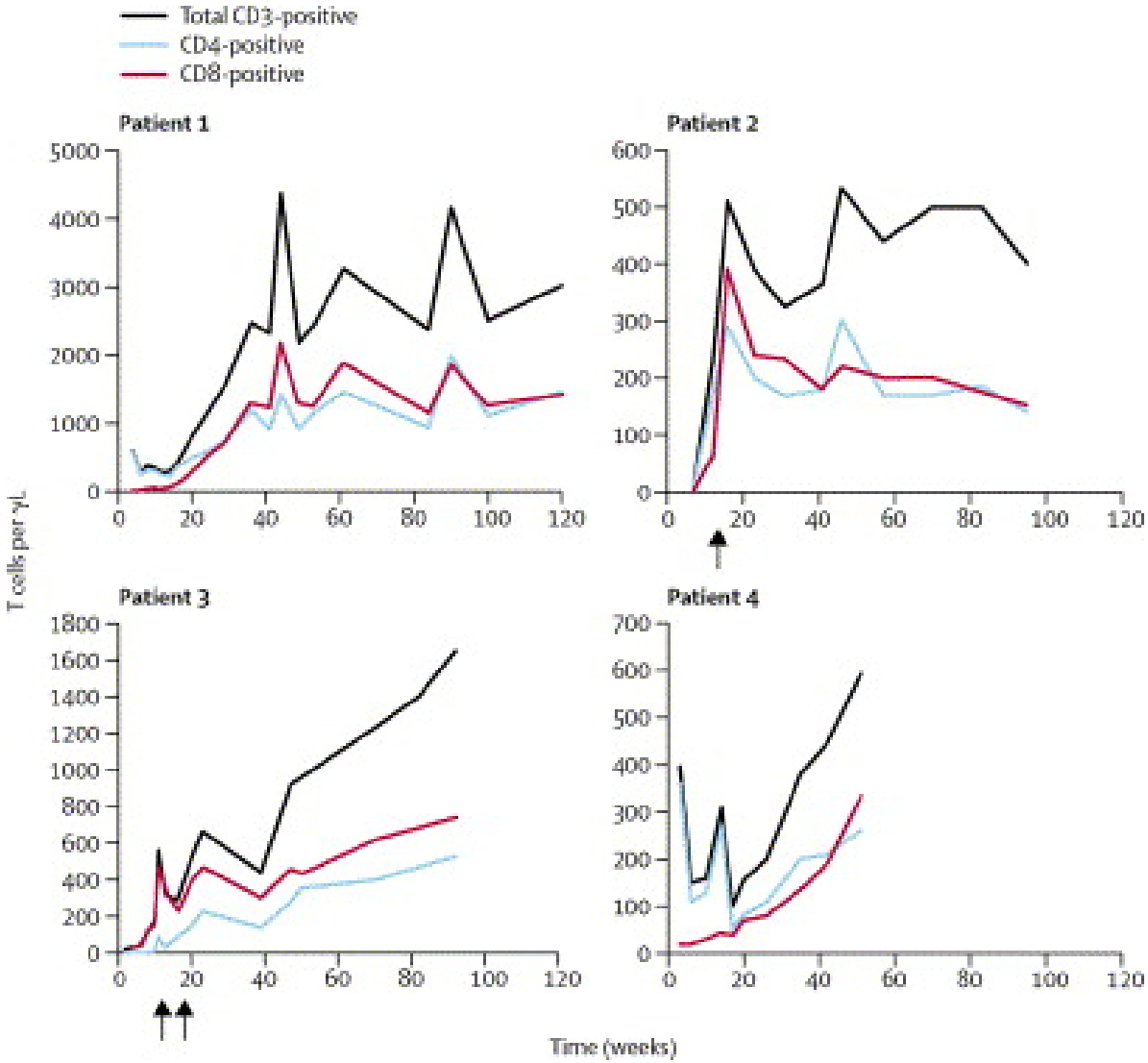

B
Episomes/100,000 Peripheral-Blood Mononuclear Cells
Patient 4
Patient 2
Patient 1
Months after Gene Therapy

C

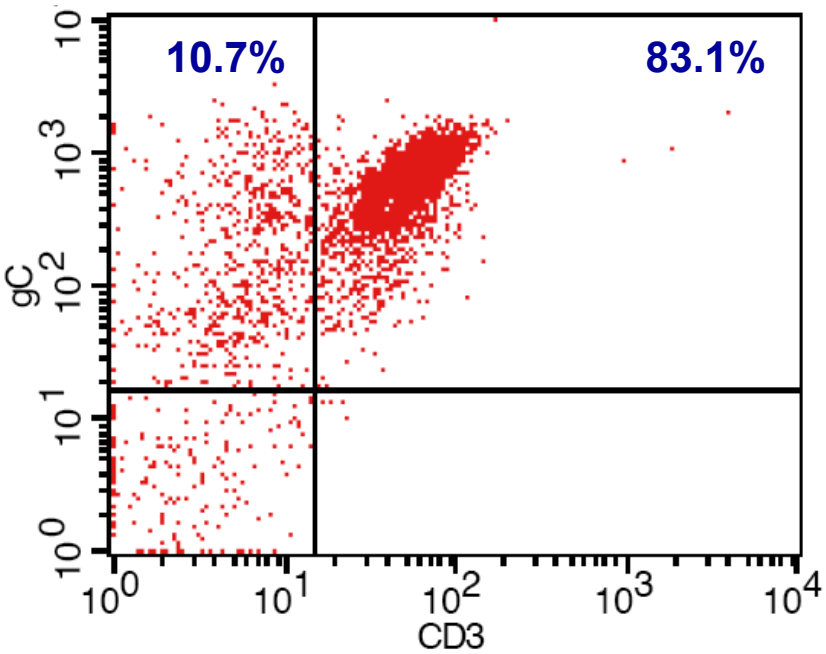

A
CD4+ T Cells/mm³
CD45RA+
CD45RO+
Patient 4
Patient 2
Patient 1
Months after Gene Therapy


B
Episomes/100,000 Peripheral-Blood Mononuclear Cells
Patient 4
Patient 2
Patient 1
Months after Gene Therapy

C


Total CD3-positive
CD4-positive
CD8-positive
Patient 1
Patient 2
Patient 3
Patient 4
T cells per μL
Time (weeks)

Proportion of Gene-Corrected (γc^+) T Cells
(4 Months Post Treatment)



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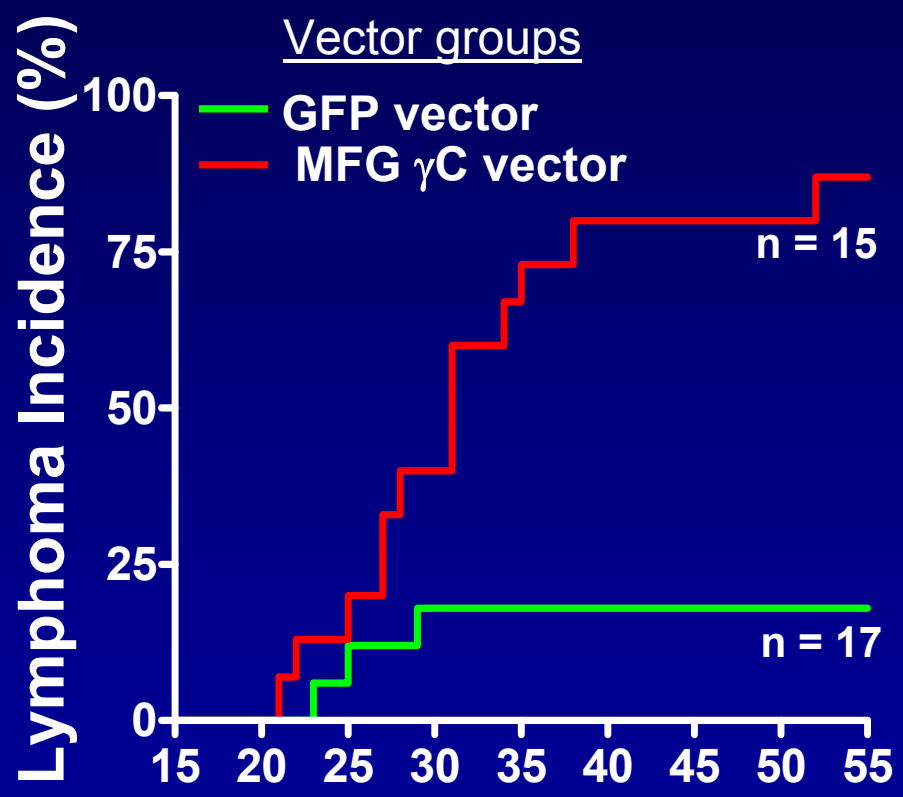
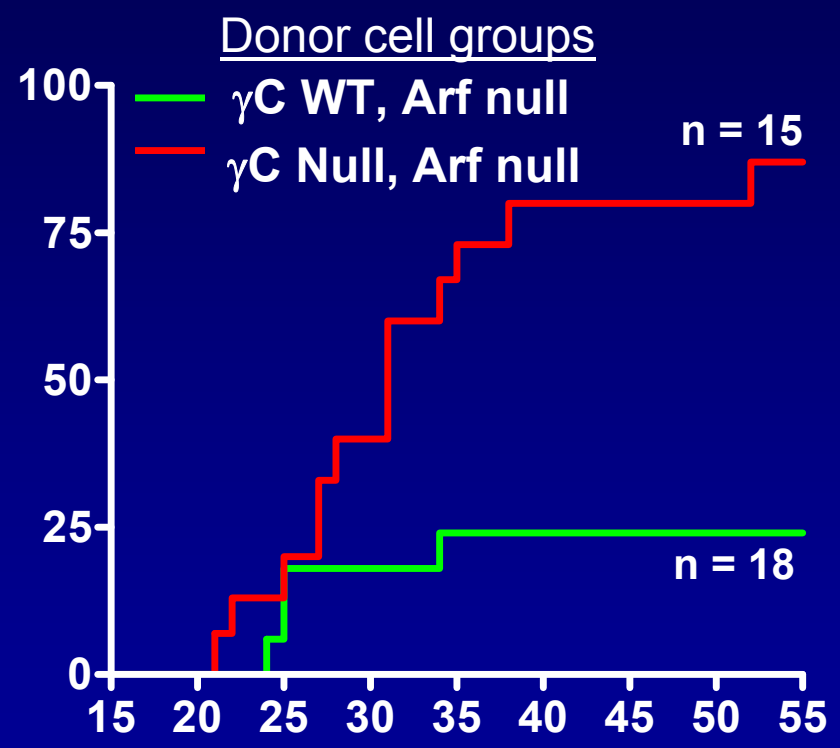
Transduction with a γ_c vector and the XSCID background in donor cells are major risk factors for T cell lymphoma



Both groups with Arf$^{-/-}$, XSCID donor cells



Both groups with MSCV-gamma chain vector