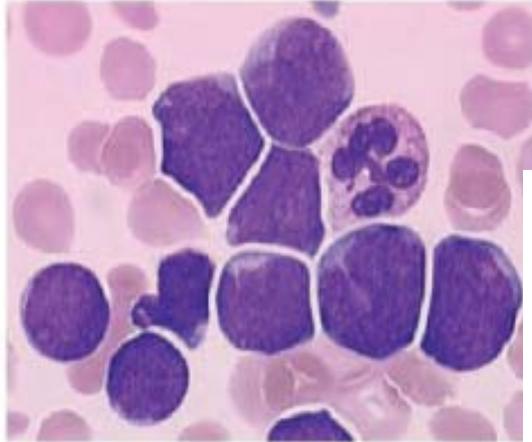


# Preclinical safety evaluation of self-inactivating lentiviral vectors for SCID-X1 gene therapy

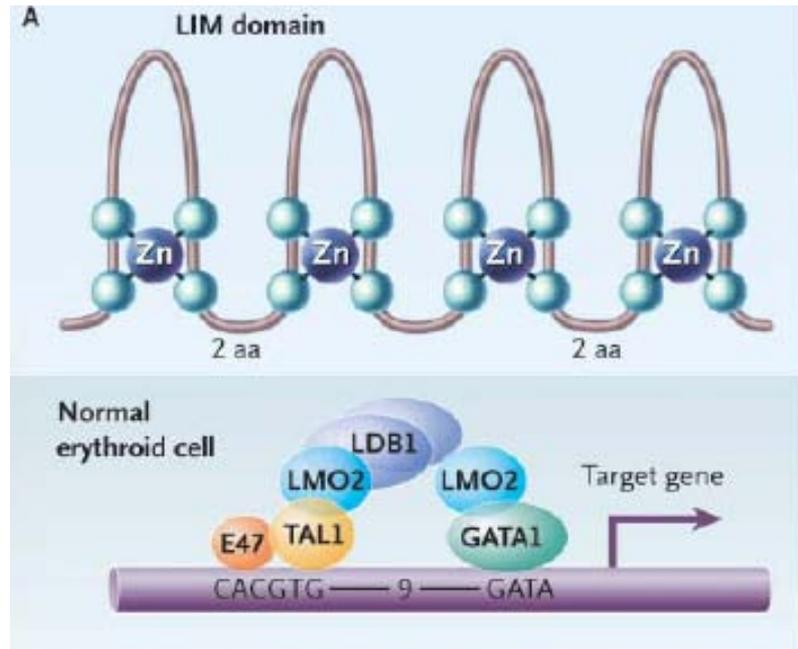
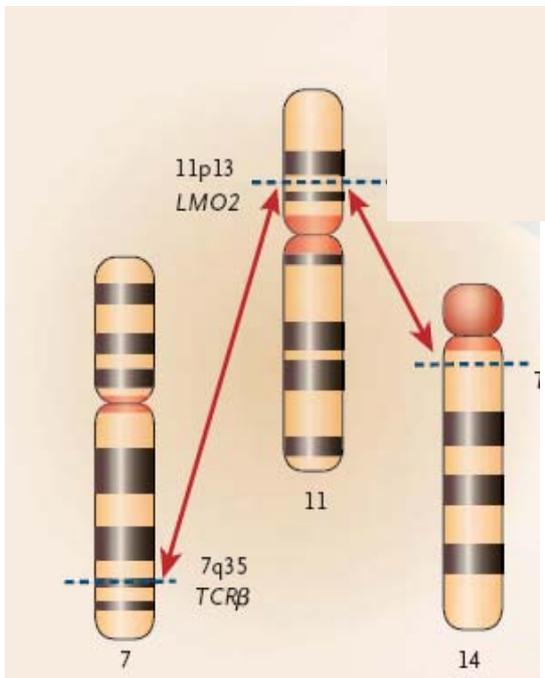
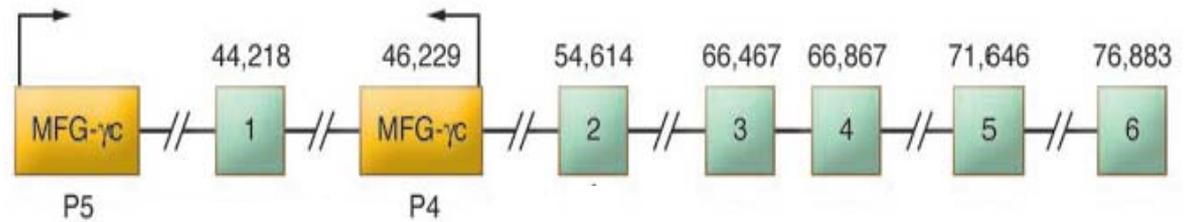
**Brian Sorrentino, M.D., Dept. of Hematology**



# Insertional activation of LMO2 in SCID-X1 gene therapy



5/20 gene Rx patients developed T-cell leukemia;  
4 cases with LMO2 activation

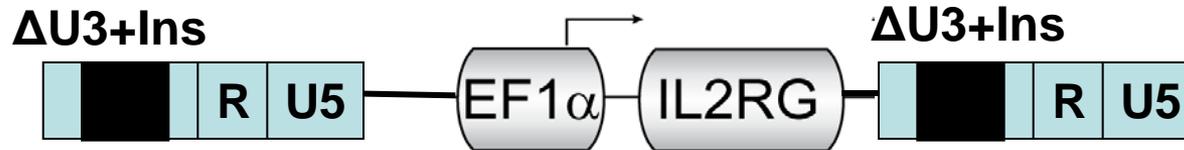


Deichmann A et al, JCI, 2007



# SCID-X1 lentiviral vectors designed for improved safety

## CL20-i4-EF1 $\alpha$ -hy $_c$ -OPT

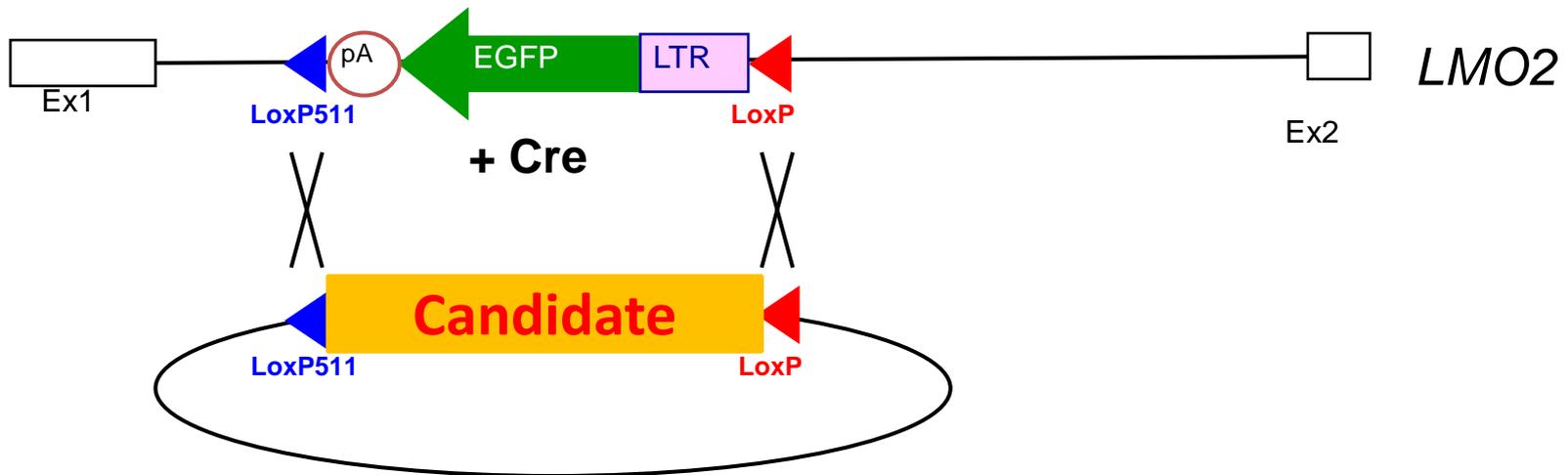
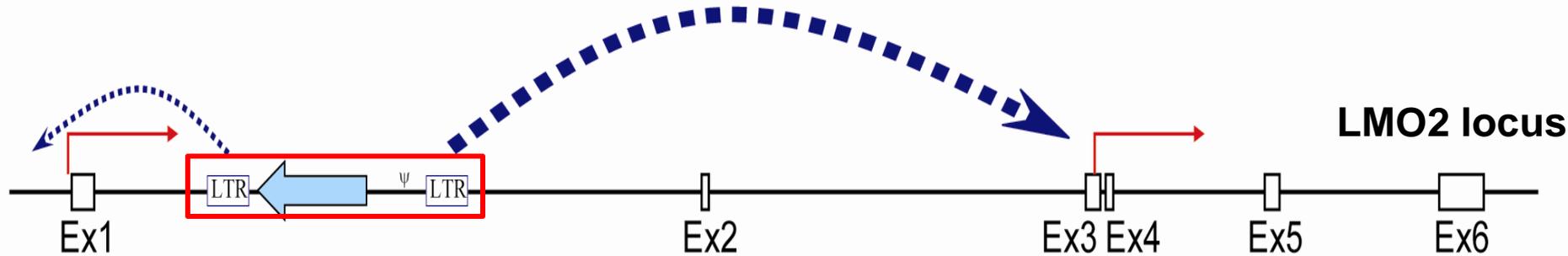


- HIV-1 based lentiviral backbone developed in Nienhuis Lab
- Deleted LTR, self-inactivating, no viral transcription elements
- Incorporated 400 bp cHS4 insulator
- 233 bp EF1 $\alpha$  cellular promoter
- Codon optimized hy $_c$  cDNA
- Titer  $1 \times 10^8$  unconcentrated

# Jurkat T cell LMO2 activation assay

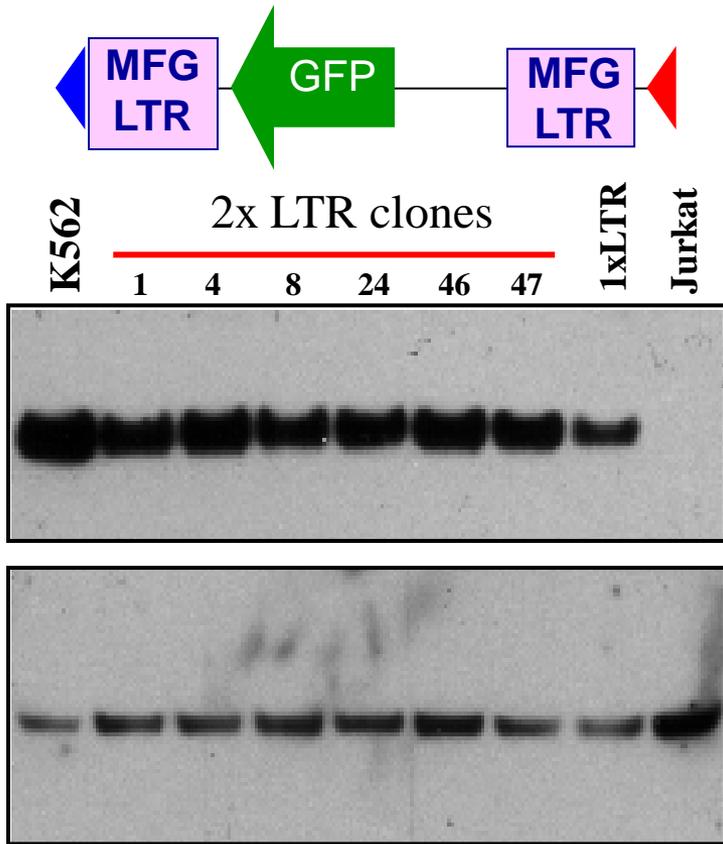
Reproduce insertion in Pt 4 from French trial in the human Jurkat T cell line

## A. Initial Clinical Vector

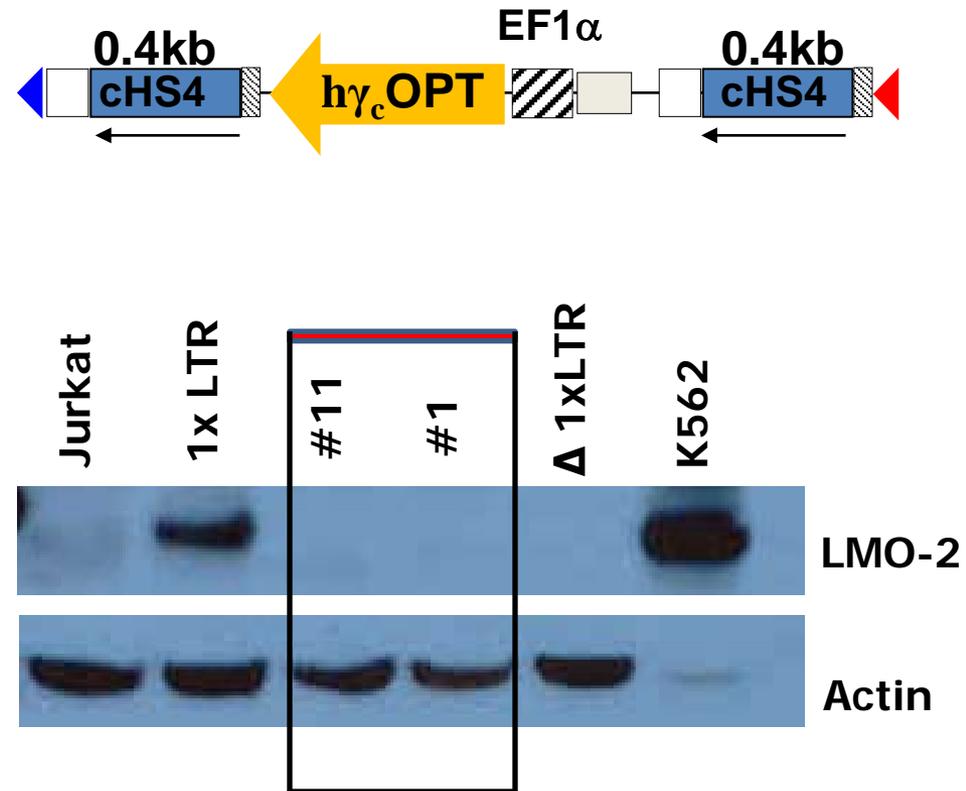


Ryu et al, Blood, 2008

# Lack of LMO2 activation with an insulated EF1 $\alpha$ XSCID vector

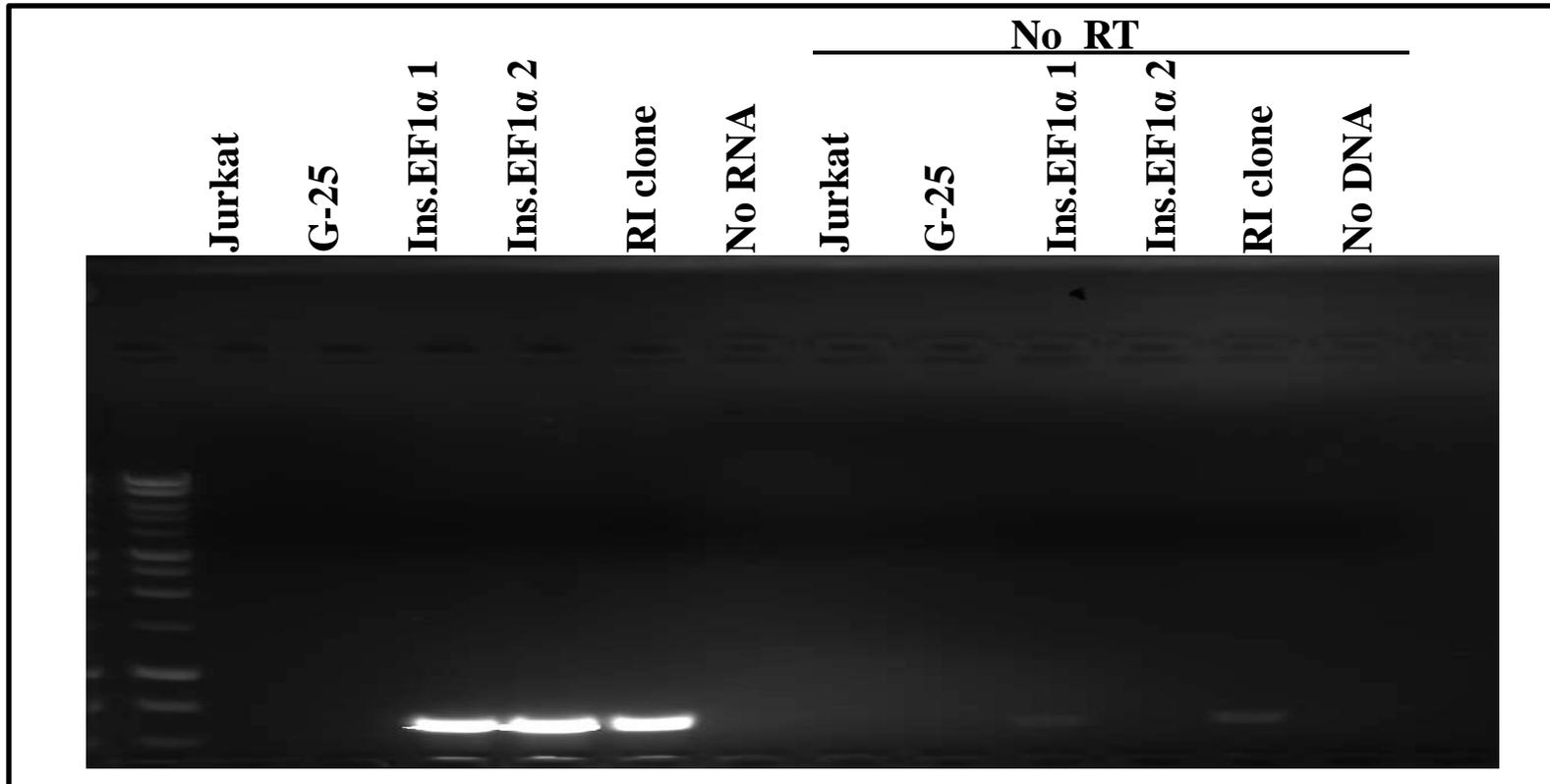


Ryu et al, Blood, 2008

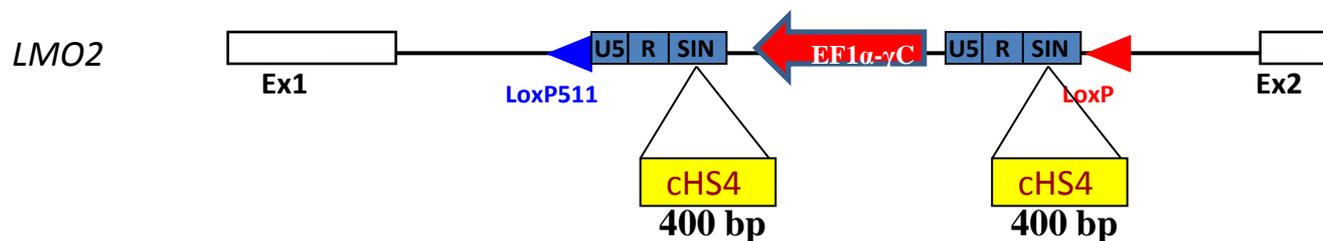


Zhou S et al, Blood, 2010

# Codon-optimized gamma-chain transgene is expressed in insulated EF1a clones

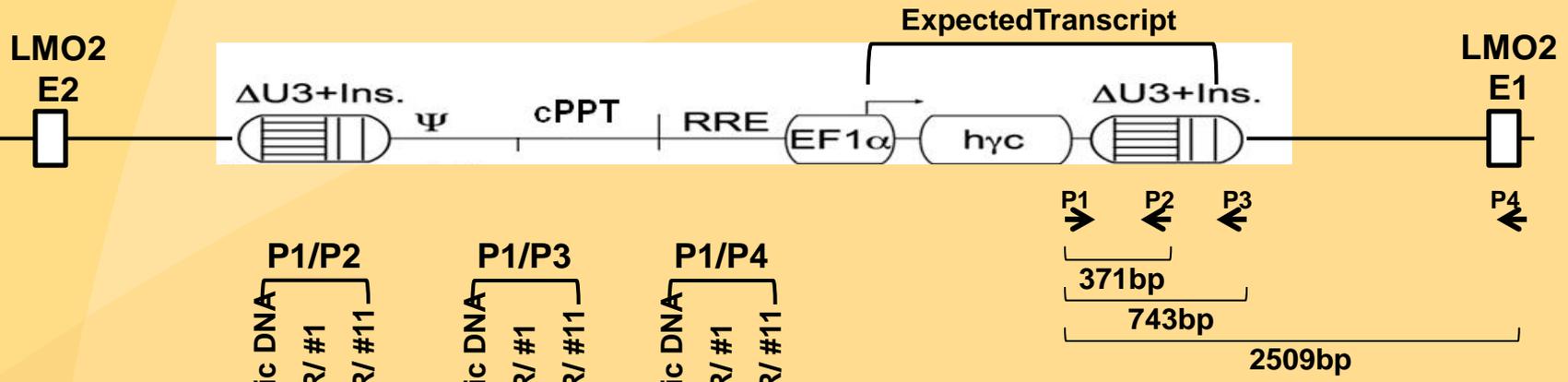


## RT-PCR analysis



# No detection of transcriptional readthrough with the CL20-4i-EF1 $\alpha$ -hyc<sub>OPT</sub> vector inserted into the LMO2 gene

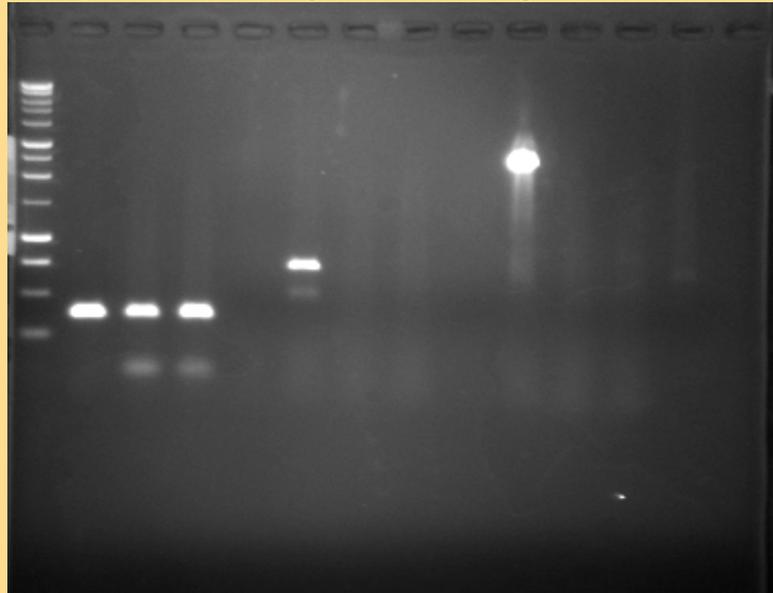
Finding cures. Saving children.



**P1/P2**  
 Genomic DNA  
 RT-PCR/ #1  
 RT-PCR/ #11

**P1/P3**  
 Genomic DNA  
 RT-PCR/ #1  
 RT-PCR/ #11

**P1/P4**  
 Genomic DNA  
 RT-PCR/ #1  
 RT-PCR/ #11



## Limitations of the Jurkat-LMO2 assay

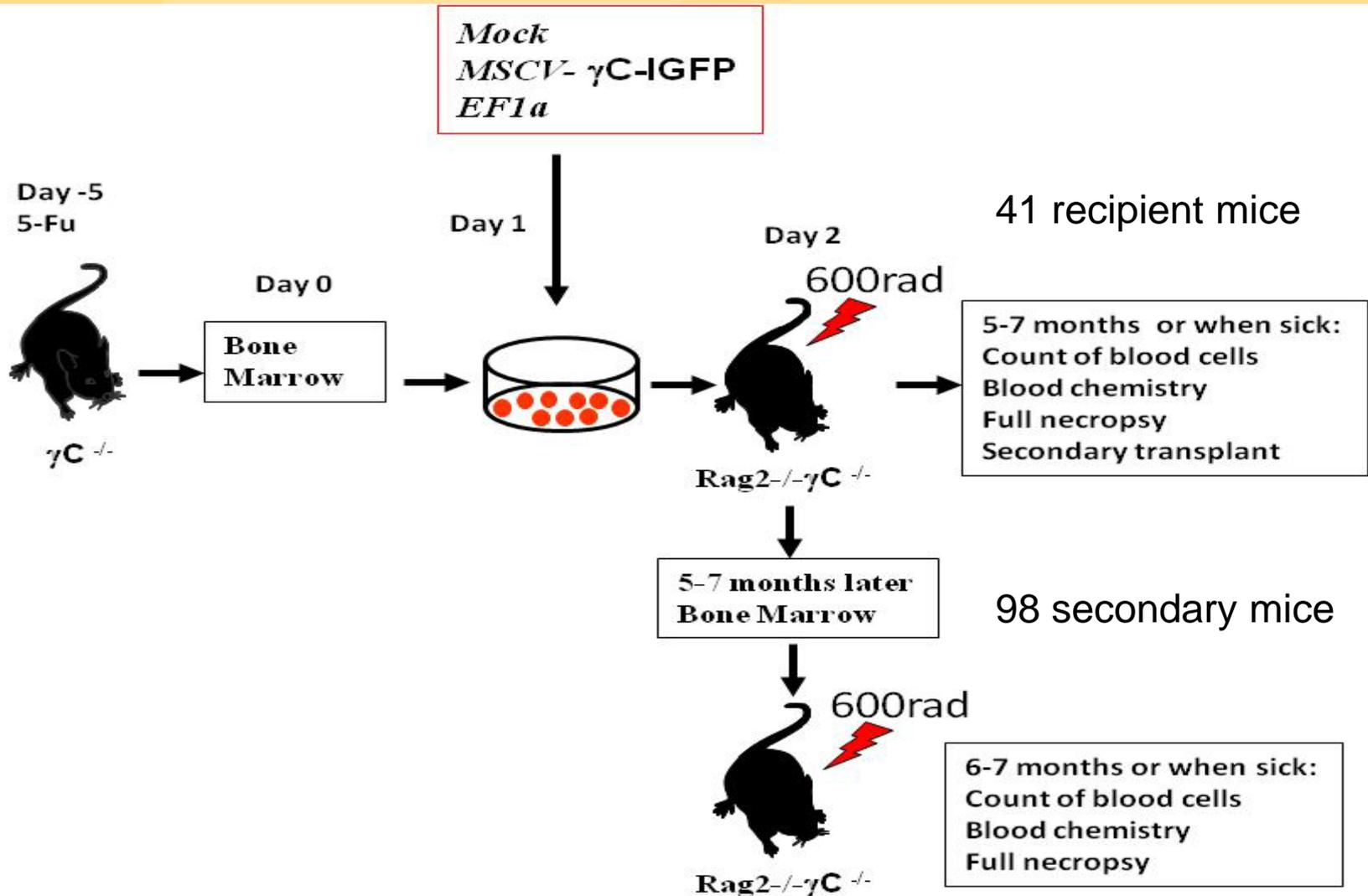
- Only tests for activation at one predefined insertion site, but the overall approach can be used to recreate other insertions.
- Only evaluates activation of LMO2 and not other proto-oncogenes, but this approach can be applied to other “problem” proto-oncogenes (exp: HMGA2).
- Relatively time consuming and cloning efficiencies are variable.

## EF1 $\alpha$ -h $\gamma_c$ -OPT vector lacks myeloid immortalization activity

	<u>Positive wells on 96 well plate</u>		copy number
	100cells/well	500 cells/well	
<b>Experiment 1</b>	n=1	n=1	
Mock	0	0	
EF1 $\alpha$ -h $\gamma_c$ OPT	0	0	1.57
RV-SFFV-GFP	6	16	2.32
MSCV-h $\gamma_c$	0	5	1.46
<b>Experiment 2</b>	n=3	n=3	
Mock	0, 0, 0	0, 0, 0	
EF1 $\alpha$ -h $\gamma_c$ OPT	0, 0, 0	0, 0, 0	1.64
RV-SFeGFP	3, 3, 6	14, 21, 13	1.45

# Serial mouse transplant assay to assess XSCID LV vector safety

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Histology of primary recipients: Mock group (Page 2)			
ID of Primary recipients	ID of Histology		
526	09-5332	Spleen:	Follicular hypoplasia, marked, chronic EMH, moderate, chronic
		Small intestine:	Focal adenoma
528	5336	Lung	Acidophil macrophage pneumonia, multifocal, moderate, chronic *possible pneumocystis Lymphocytic perivascular inflammation, mild
		Liver:	Lymphocytic portal and perivascular inflammation, mild
		Salivary gland:	Degeneration, multifocal, moderate
		Spleen:	Follicular hypoplasia, mild to moderate
		Thymus:	not submitted
		Pancreas:	Exocrine acinar atrophy, severe
		Eyes:	Unilateral lens degeneration with cataracts, iris synechia, and retinal degeneration
		Harderian gland:	Suppurative adenitis, focally extensive, mild (same eye as above)
529	09-5534	Liver:	Extramedullary hematopoiesis (EMH), multifocal, mild, chronic
		Spleen:	Follicular hypoplasia, moderate to severe, chronic EMH, diffuse, severe, chronic
		Lymph nodes:	Hypoplasia, moderate, chronic
		Small intestine:	Adenoma, focal
		Colon:	Pleocellular colitis (macrophages, neutrophils, lymphocytes, plasma cells), diffuse, moderate to marked, subacute
		Skin:	Focal sebaceous hamartoma
530	09-5535	Heart:	Atrial thrombus, moderate, subacute
		Liver:	EMH, multifocal, mild
		Spleen:	Follicular hypoplasia, moderate to severe, chronic EMH, diffuse, severe, chronic
		Lymph nodes:	Hypoplasia, moderate, chronic
		Small intestine:	Focal ulcerated adenocarcinoma
		Harderian gland:	Unilateral degeneration and necrosis (possibly secondary to retro-orbital bleeds)
531	5536	Spleen:	Absence of follicles
		Lymph nodes:	Hypoplasia, marked
		Thymus:	not submitted
		Small intestine:	Adenoma, focal

Necropsy results  
Primary recipis  
Mock group

Histology of secondary recipients at autopsy: CL204i-EF1 $\alpha$ -hyc-OPT group (page2)

Necropsy results  
Secondary recipis  
EF1 $\alpha$  group

3 B-cell lymphomas

ID of secondary recipients	ID of Histology	organs	key findings
882	RS10-830	Lung	Perivascular lymphocytic, histiocytic, and suppurative inflammation, mild
		Liver	Portal and perivascular lymphocytic, histiocytic, and suppurative inflammation, mild
		Lymph nodes:	Suppurative and histiocytic lymphadenitis, multifocal, marked
		Spleen:	Follicular hypoplasia, mild
		Thymus:	not submitted
		Jejunum:	Suppurative and histiocytic enteritis, segmental, mild
		Nasal mucosa:	Submucosal gland suppurative and fibrotic adenitis, focal, moderate
883	RS10-868	Lung:	Acidophil macrophage pneumonia, diffuse, moderate to severe
			Perivascular lymphocytic inflammation, mild
		Liver	Periportal and perivascular lymphocytic inflammation, mild
		Thymus:	not submitted
		Lymph nodes:	not present on slide
884	RS10-900	Lung:	Acidophil macrophage pneumonia, diffuse, mild to moderate
			Perivascular lymphocytic inflammation, mild
			Suppurative portal hepatitis, multifocal, moderate, with portal fibrosis and biliary hyperplasia
		Liver:	hyperplasia
		Kidney:	Perivascular lymphocytic and suppurative inflammation, mild
		Ovary:	Unilateral hemorrhagic follicular cyst

203	AP10-1711	Heart:	Multifocal intravascular lymphoma cells
		Lungs:	Lymphoma cells mildly thicken alveolar septal walls
		Liver:	Perivascular, periportal and sinusoidal lymphoma, moderate
		Spleen:	B-cell lymphoma, Pax5+
204	AP10-2025	Lungs:	Perivascular inflammatory infiltrate (lymphocytes, macrophages, neutrophils, plasma cells), mild
		Liver:	Portal and perivascular inflammatory infiltrate (lymphocytes, macrophages, neutrophils), mild
		Spleen:	B-cell lymphoma, Pax5+
		Stomach:	Squamous hyperkeratosis, mild
205	AP10-1830	Lungs:	Perivascular inflammatory infiltrate (lymphocytes, macrophages, plasma cells), mild
			Acidophil macrophage pneumonia, diffuse, mild
		Liver:	Portal and perivascular inflammatory infiltrate (lymphocytes, macrophages,
		Spleen:	B-cell lymphoma, Pax5+
		Lymph nodes:	not present
		Thymus:	not submitted

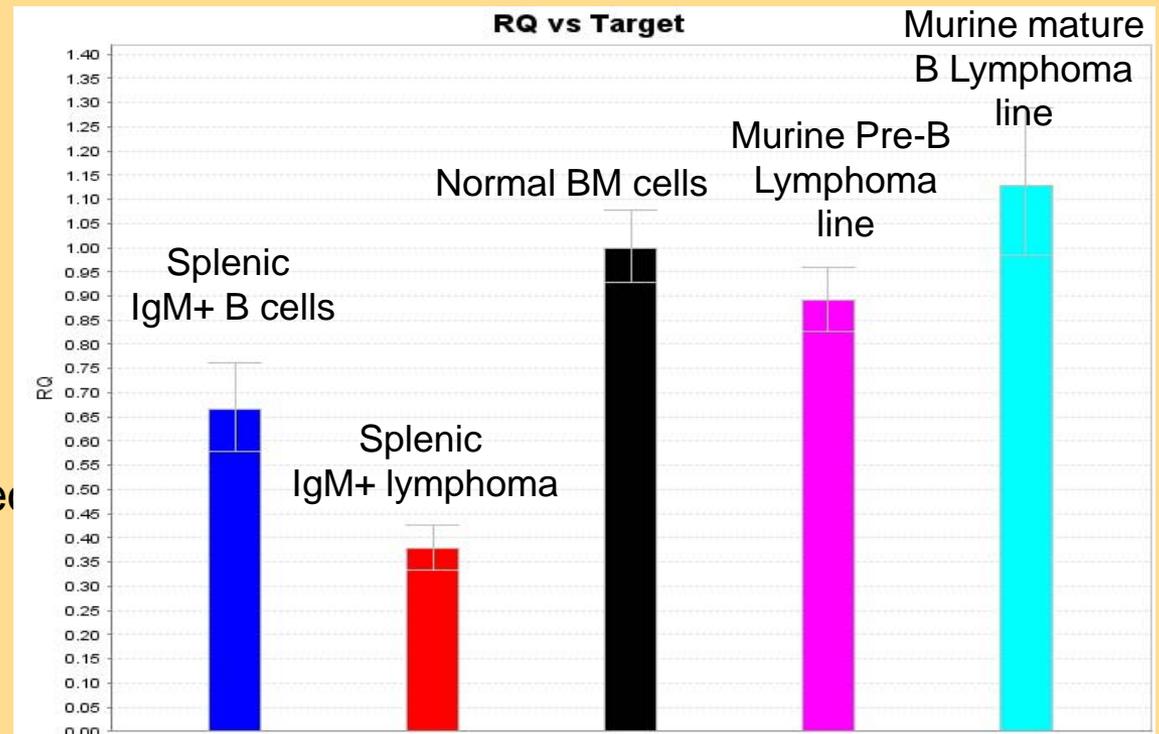
# Insertion of EF1a vector in 1<sup>st</sup> intron of Pkn2 gene

EF1a vector insertion

UCSC Genome Browser v232



- Ser/Thr kinase and Rho/Rac effector protein
- Regulates entry into mitosis in HeLa cells
- Phosphorylates and activates Cdc25G, a phosphatase required for activation of mitotic cyclin/Cdk1 complexes at the G2/M transition



# Publications related to background transformation rate in mouse assay systems

- Haines DC, Toxicol. Pathol., 2001.
  - 100 B6;129 mice followed for 2 years of age to study pathology
  - Lymphoma observed in 42% of males and 67% of females, B cell origin
  - Also, 10-20% developed hepatocellular, lung, thyroid, and ovarian tumors
  - High incidence of non-neoplastic lesions (glomerulonephritis, amyloid, etc).
- Ginn SL et al, Mol. Ther., 2010.
  - SCID-X1 mouse transplant assay, EF-1 $\alpha$ -hyc vector
  - 4/14 cases of T cell lymphoma occurred independent of retroviral vector insertion
  - Increased incidence of lymphoma due to SCID-X1 background on donor cells
- Will E, Mol. Ther., 2007.
  - 50 mice transplanted with MSCV-MGMT transduced, WT BM cells
  - 6 mice developed lymphoma, no vector detected in tumor cells of 5 mice.
  - Background tumor formation rate increased by irradiation of host

# Summary and Conclusions

- LMO2 is the most frequent CIS involved in SCID-X1 gene therapy transformation events.
- The Jurkat-LMO2 activation assay successfully recapitulates the LTR-driven transactivation seen in the clinical trial leukemia cases.
- The CL20-i4-EF1 $\alpha$ -h $\gamma_c$ -OPT vector does not transactivate LMO2 in the Jurkat assay and leads to no detectable transcriptional readthrough in these clones.
- Clinical trial is proposed based on these results and will be necessary to determine if other mechanisms of transformation can occur and, if so, at what frequency.

## Conclusions (2)

- The only tumor seen in our transplant experiment occurred in 3 secondary mice derived from a single primary transplanted animal. The vector insertion into the Pkn2 gene most likely represents a background transformation event.
- Serial mouse transplantation assays using SCID-X1 mice are time consuming, relatively expensive, limited by high background rates of tumor formation, and lack sensitivity using the MSCV gamma-RV positive control. Mouse transplant assays also lack of the ability to reproduce Lmo2 activation events.
- Copy number measurements done at a clonal level using human CFU-C derived from NOG mice shows a Gaussian distribution and raises the question about how to report “safe copy numbers”.

# Acknowledgments

*Finding cures. Saving children.*

- Sheng Zhou
- Disha Mody
- Mike Greene
- Harry Malech
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