

Insertional mutagenesis induced leukemogenesis in mice and implications for human gene therapy

Neal Copeland

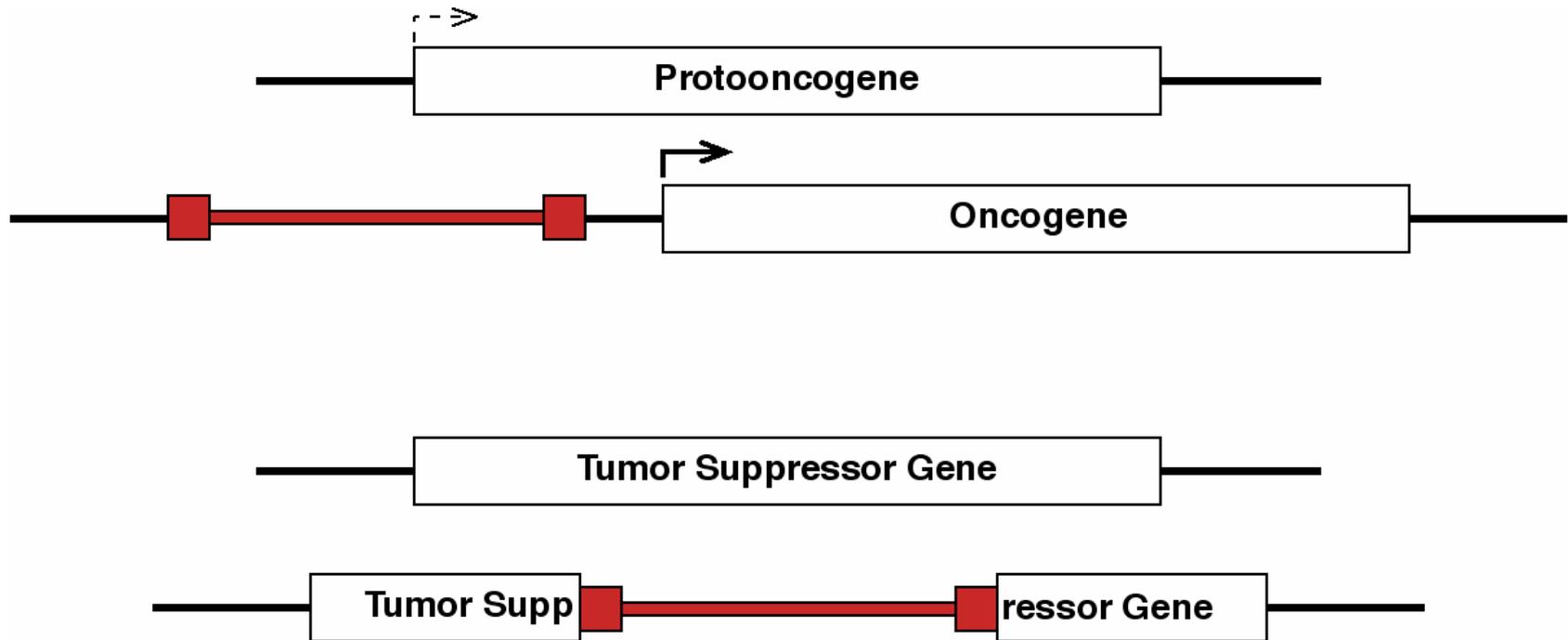
Mouse Cancer Genetics Program

National Cancer Institute

Frederick, MD, USA



Mouse hematopoietic tumors are induced by insertional mutagenesis



RTCGD Internal Database - Model Search

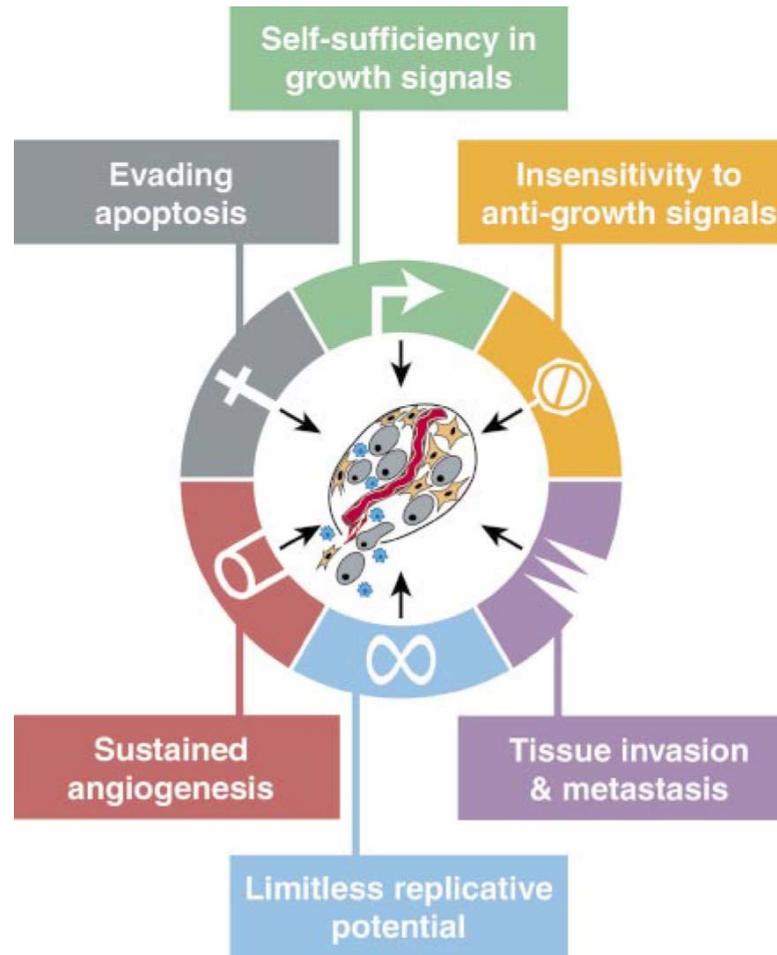
[[Home](#) | [RTCGD2 Search](#) | [Easy Search](#) | [Model Search](#) | [Interaction Search](#) | [Human Cancer Gene](#) | [public RTCGD](#)]

Result of Model Search - Displayed by gene

You Selected TUMOR MODEL: All
You Selected TUMOR TYPE: All
You Selected ONLY CIS: Yes
RESULT number of CIS gene: 482

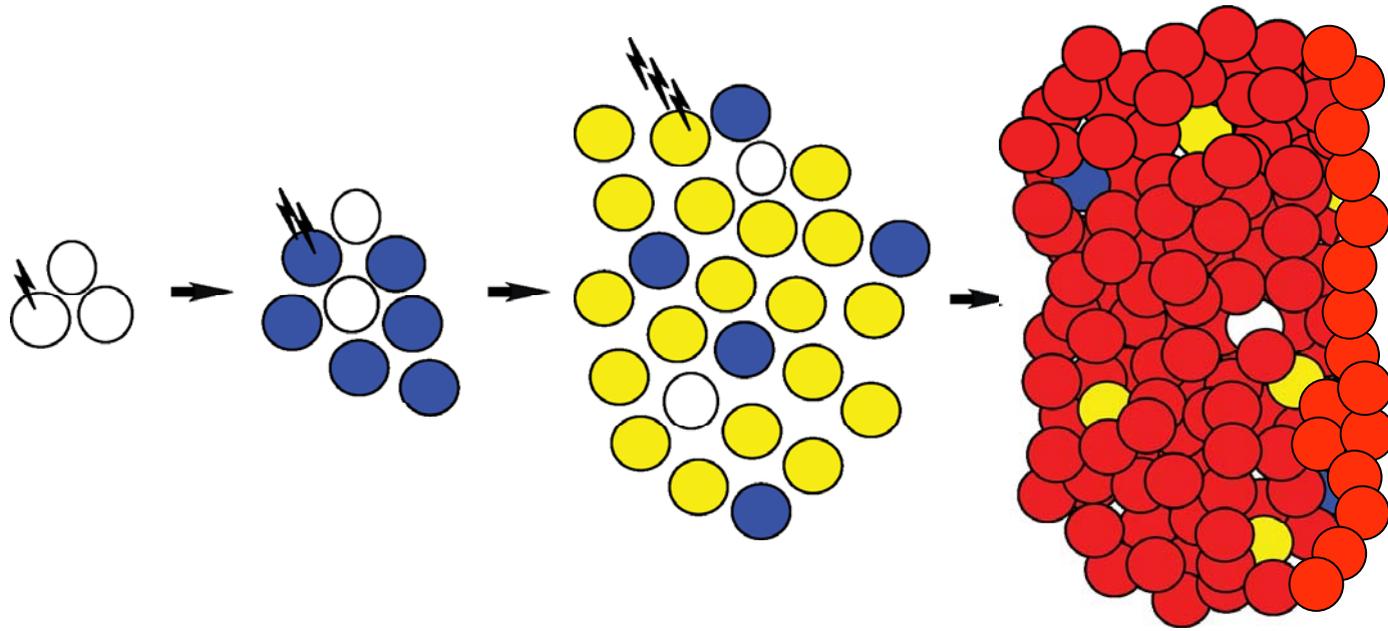
RIS_name	mouse_symbol	product	MGI	mouse_chr	hits
Evi16	Sox4	SRY (sex determining region Y)-box 4	MGI:98366	chr13	107
Gfil	Gfil	growth factor independent 1	MGI:103170	chr5	82
Myc	Myc	myelocytomatosis oncogene	MGI:97250	chr15	77
Myb	Ahi1 Myb	jouberin myeloblastosis proto-oncogene product	MGI:87971 MGI:97249	chr10	51
Lvis1	Hhex	hematopoietically expressed homeobox	MGI:96086	chr19	49
Pim1	Pim1	proviral integration site 1	MGI:97584	chr17	34
Dkmi12	Rras2	related RAS viral (r-ras) oncogene homolog 2	MGI:1914172	chr7	32
Evi18	Rasgrp1	RAS guanyl releasing protein 1	MGI:1314635	chr2	29
Ccnd2	Ccnd2	cyclin D2	MGI:88314	chr6	25
Bmi1	Bmi1	B lymphoma Mo-MLV insertion region 1	MGI:88174	chr2	22
Evi1	Evi1	ecotropic viral integration site 1	MGI:95457	ND	21
Dkmi3	Cebpb Ptpn1	CCAAT/enhancer binding protein beta protein tyrosine phosphatase, non-receptor type	MGI:88373 MGI:97805	chr2	21
Evi7	Hoxa7 Hoxa9	homeobox protein A7 homeobox protein A9	MGI:96179 MGI:96180	chr6	21
Evi54	Notch2	Notch gene homolog 2	MGI:97364	chr3	19

Cancer Results from Multiple Cooperating Mutations



Hanahan and Weinberg
Cell, 2000

Multiple viral mutations



Interaction Search

[[Home](#) | [RTCGD2 Search](#) | [Easy Search](#) | [Model Search](#) | [Interaction Search](#) | [Specificity Search](#) | [Back to public RTCGD](#)]

Interaction Search

If you find a gene of your interest by using Easy Search or Model Search, you can search gene-to-gene interaction by using this tool. This tool displays the genes which reside in the same tumor of your gene. Please type a mouse gene symbol in the text box below and click "Go".

Search Gene Interaction for

Limited to: CISs only

[[Home](#) | [RTCGD2 Search](#) | [Easy Search](#) | [Model Search](#) | [Interaction Search](#) | [Specificity Search](#) | [Back to public RTCGD](#)]

Comments/suggestions? E-mail to: mcgphelp@ncifcrf.gov.

Last modified: Tue Aug 19 2003 12:18:40.

Location: http://genome2.ncifcrf.gov/LabDB/interaction_search.html

Meis1 Cooperating Genes

[[Home](#) | [RTCGD2 Search](#) | [Easy Search](#) | [Model Search](#) | [Interaction Search](#) | [Specificity Search](#) | [Back to public RTCGD](#)]

Interactive Genes For "Meis1" (only common integration sites)

To view NCBI LocusLink, click mouse_symbol.

To view JAX entry, click MGI.

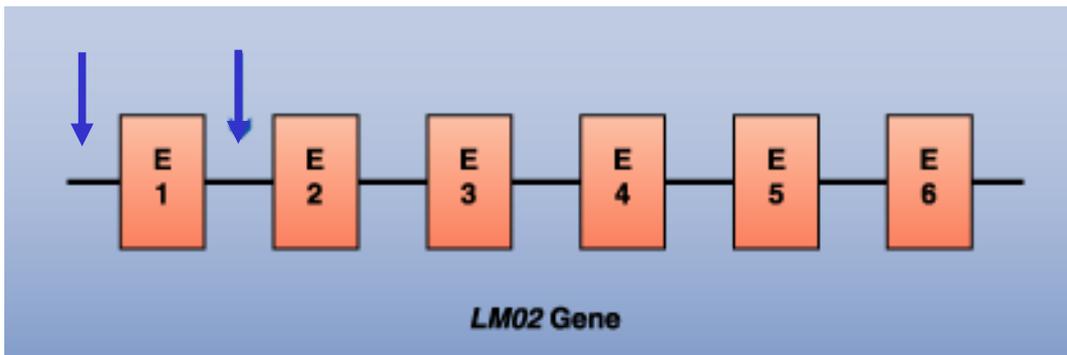
To view tumor list, click Hits.

RIS_name	mouse_symbol	Product	MGI	Mouse_chr	Hits
Evi8	Meis1	myeloid ecotropic viral integration site 1	MGI:104717	chr11	17
Evi7	Hoxa7 Hoxa9	homeobox protein A7 homeobox protein A9	MGI:96179 MGI:96180	chr6	4
Evi16	Sox4	SRY (sex determining region Y)-box 4	MGI:98366	chr13	2
Shc1	Shc1	src homology 2 domain-containing transforming	MGI:98296	chr3	1



Retroviral gene therapy with an *IL2RG*-containing retrovirus cures nine children with severe combined immunodeficiency

Two children develop
T-cell leukemia

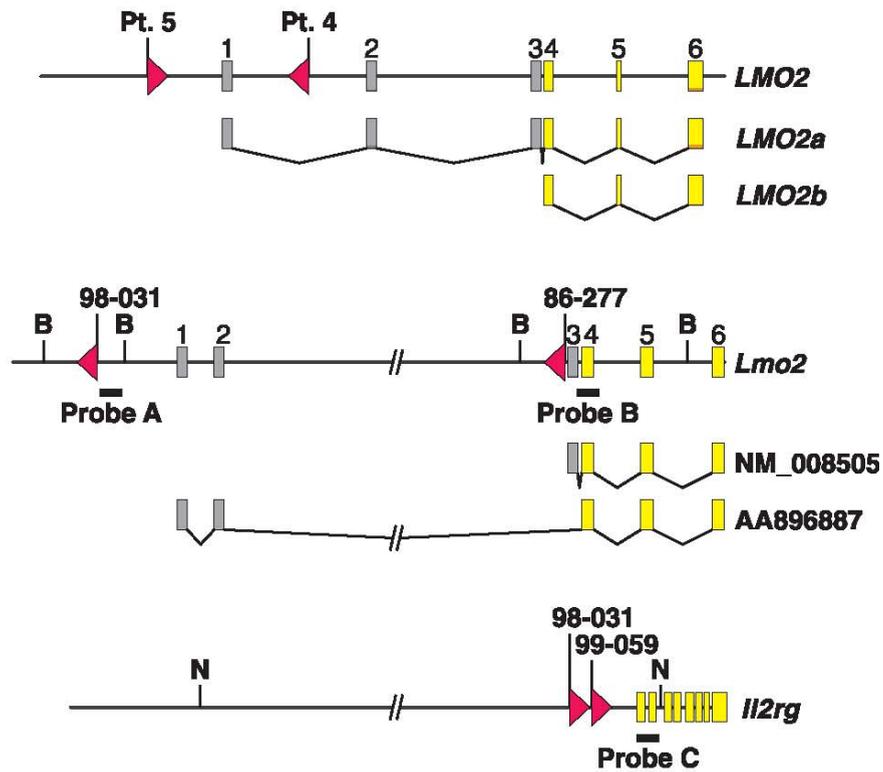


Retroviral vector inserts
itself into or near *LMO2*, a
known human T-cell oncogene

Regulators split on gene therapy as patient shows signs of cancer

Erika Check, Washington

Insights from Mouse Tumors



Dave, Jenkins, Copeland *Science* 303:333, 2004

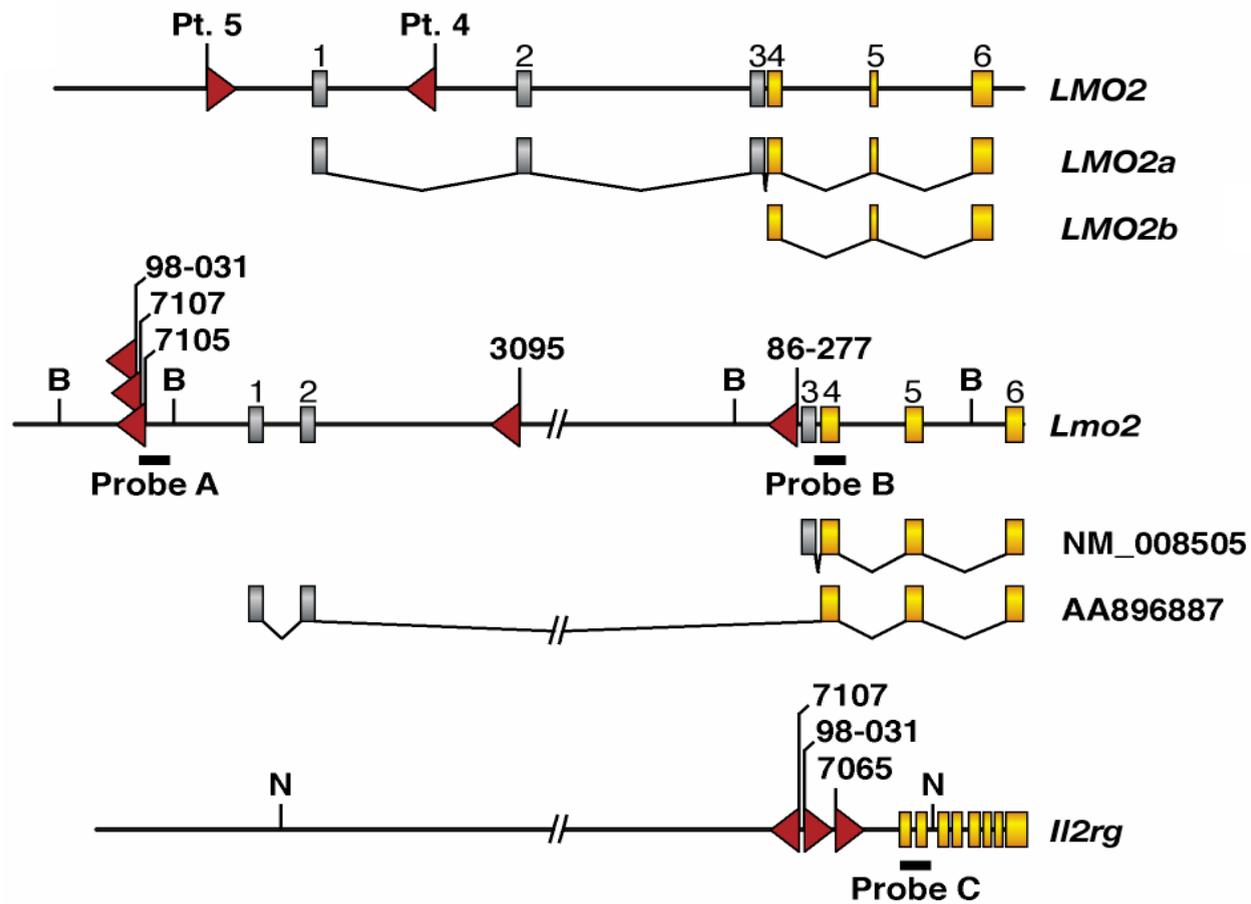
Implications of these results

LMO2 and *IL2RG* cooperate to induce leukemia in SCID patients

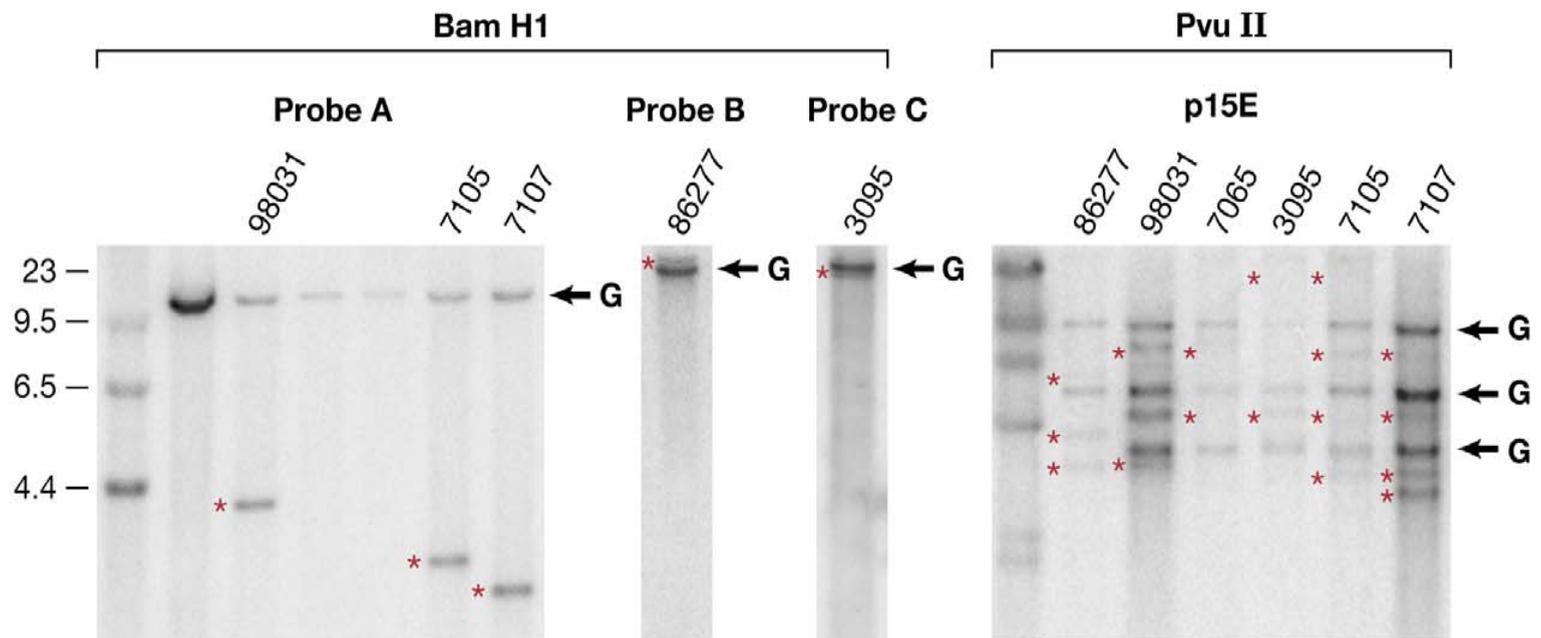
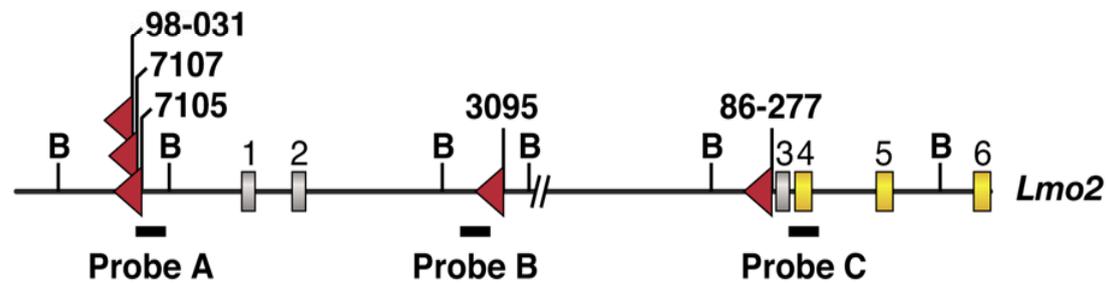
IL2RG provides the first oncogenic hit; *LMO2* the second hit

Bodes well for future gene therapy trials involving other genes as very few genes will likely be oncogenic when expressed in a retrovirus

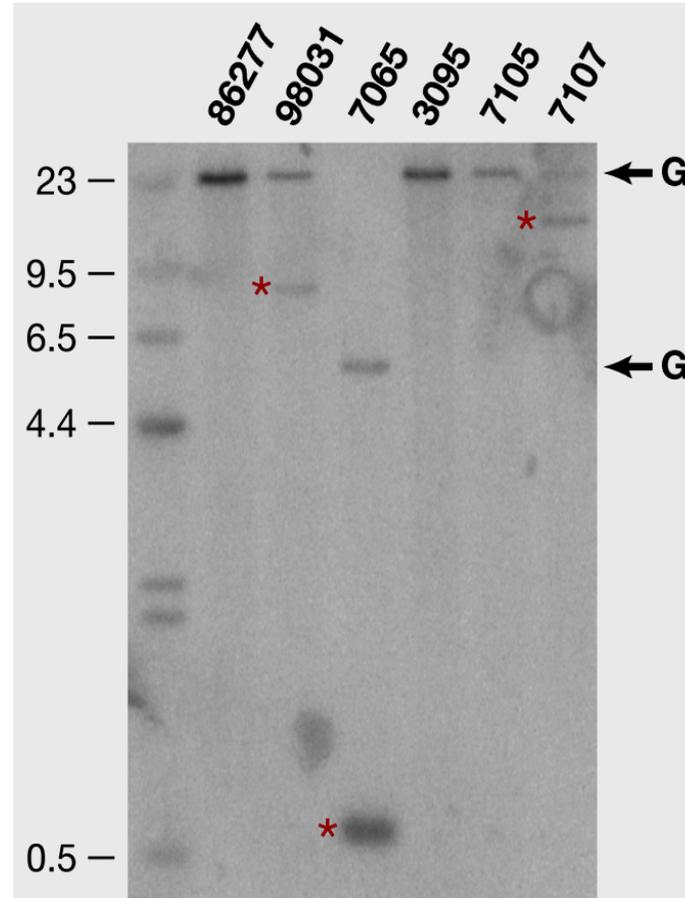
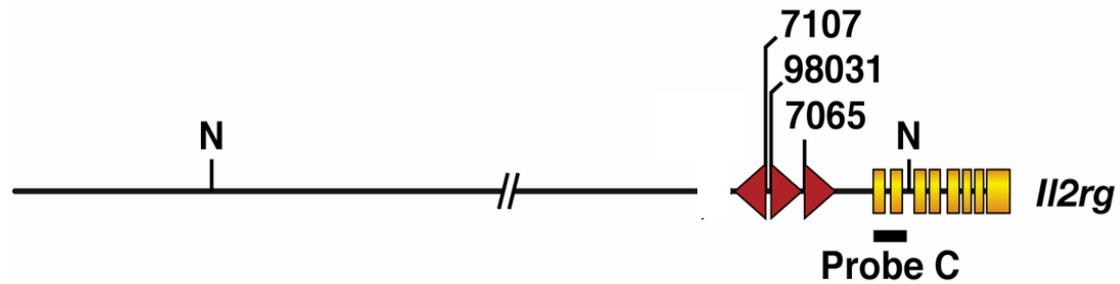
A second tumor with *Lmo2* + *Il2rg* integrations



Lmo2 integrations in tumors are clonal



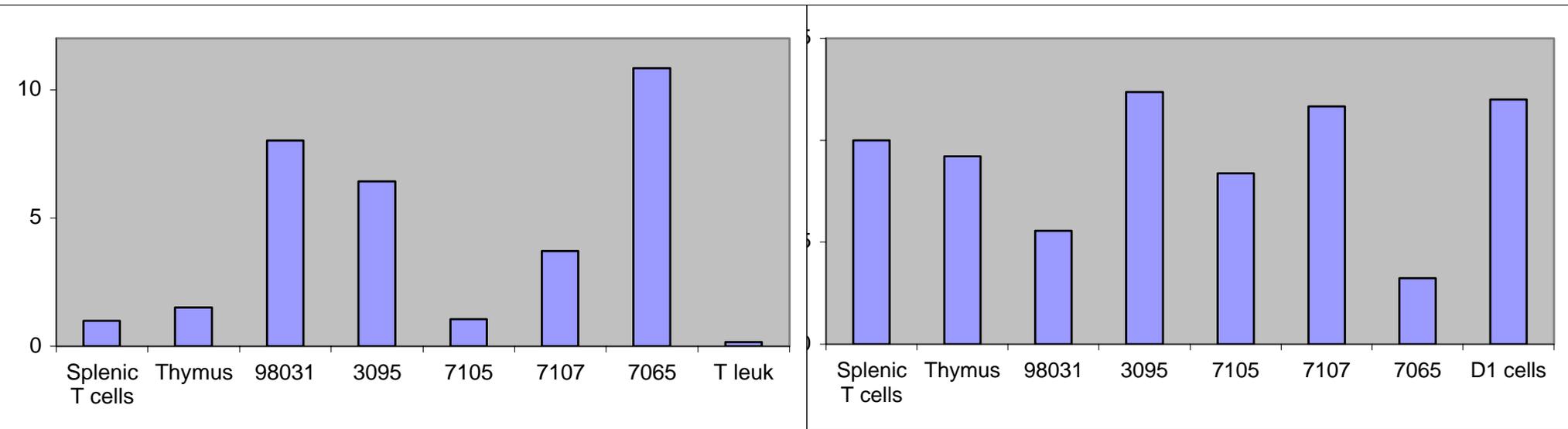
Il2rg integrations in tumors are also clonal



Real time PCR analysis of *Lmo2* and *Il2rg* expression in leukemias

Lmo2

Il2rg

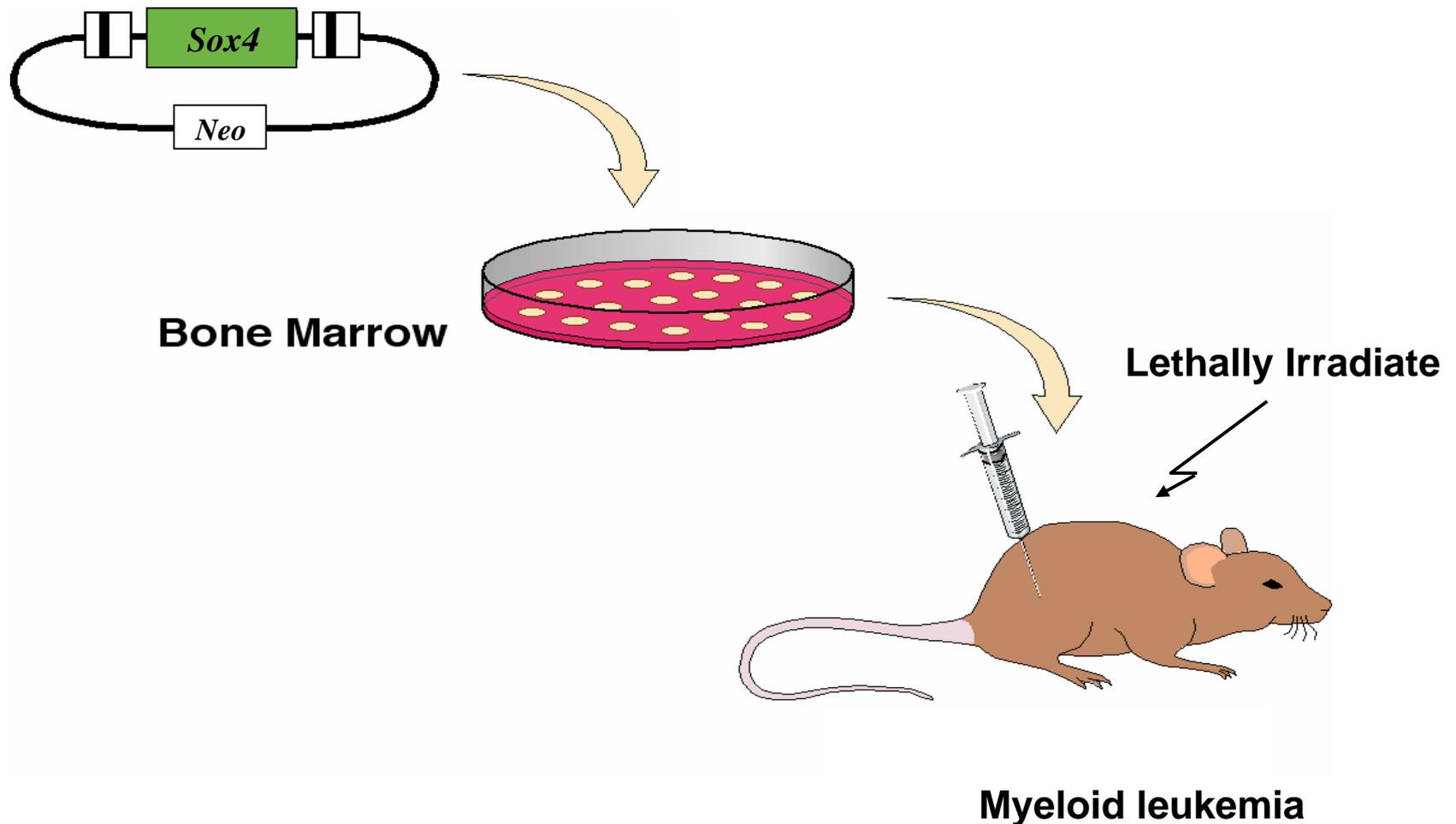


Is this problem unique to the IL2RG-containing virus?

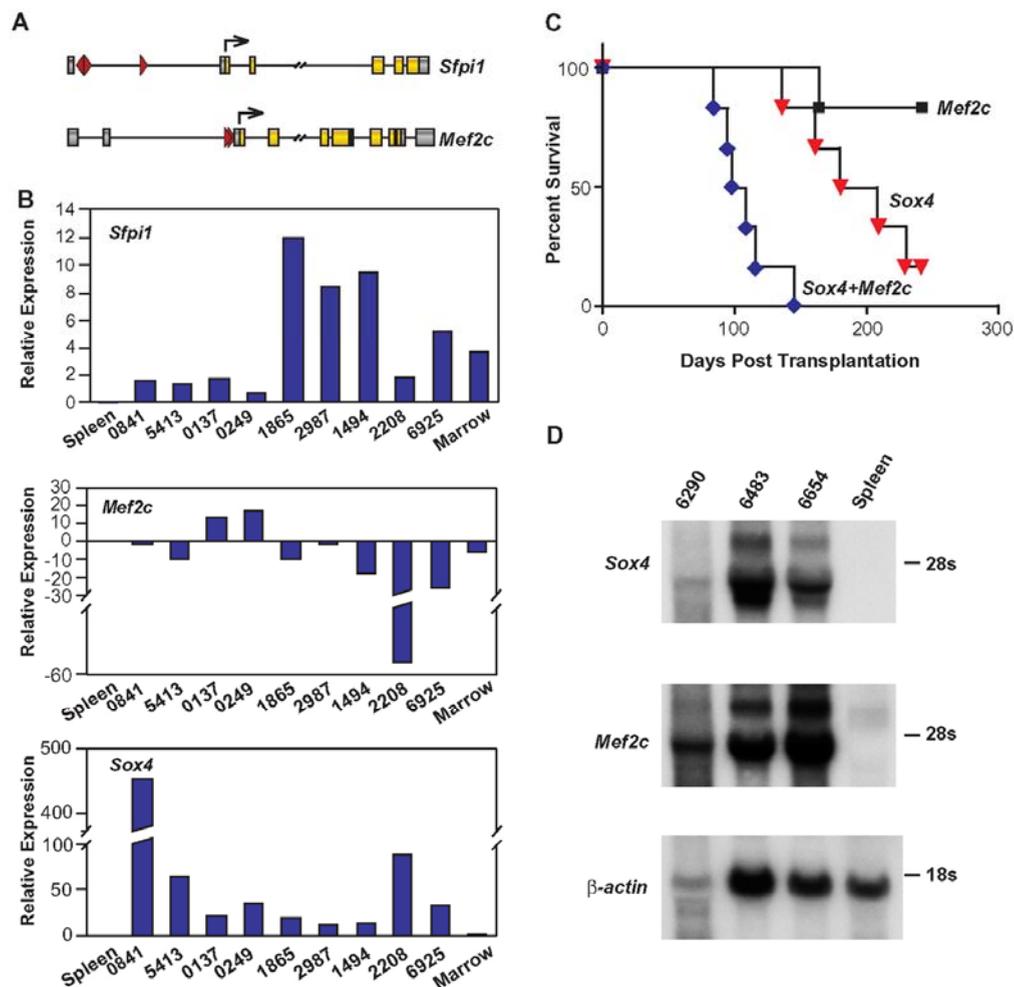
Will this happen with other oncogenic retroviruses?

Can our mouse models help answer this questions?

Does an oncogenic *Sox4* retrovirus induce leukemias in mice via insertional mutagenesis of cooperating cancer genes?



Insertional mutagenesis of cooperating cancer genes occurs in *Sox4*-virus-induced leukemias



Why didn't all SCID patients develop T cell leukemia?

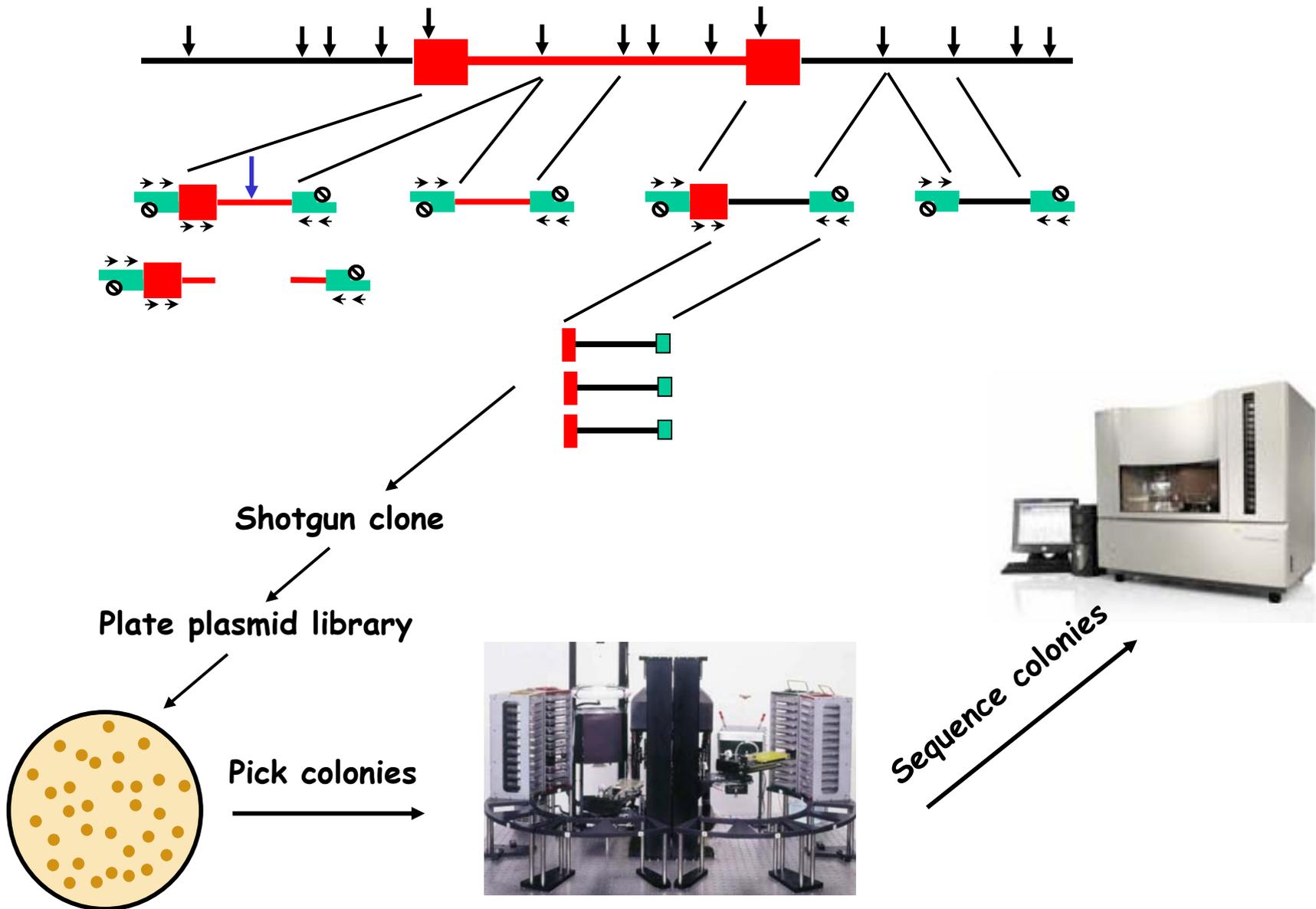
Estimated that each SCID patient received multiple cells with an *IL2RG* integration at *LMO2*

Other cooperating mutations are required for tumors to form

How many additional mutations does it take to induce a tumor with mutations in *LMO2* + *IL2RG* ?

Can our mouse models provide any insights?

Shotgun clone all viral integrations from five mouse *Lmo2* tumors using ligation-mediated PCR



Twenty-two viral integration sites cloned from tumor 7107

Tumor	Chr	Gene symbol	Protein function	CIS	Cancer	Location	Distance	Hits
7107	chr2	<i>Lmo2</i>	Transcriptional regulation	Yes	Yes	5 prime	68.386 kb	2
	chrX	<i>Il2rg</i>	Interleukin receptor	Yes		exon 1	not disrupt CDS	2
	chrX	<i>Il2rg</i>	interleukin receptor	Yes		5 prime	0.292 kb	2
	chr8	<i>Irs2</i>	Insulin receptor substrate 2	Yes		3 prime	15.147	1
	chr8	<i>Irs2</i>	Insulin receptor substrate 2	Yes		3 prime	13.399	1
	chr7	<i>Fgf3</i>	Fibroblast growth factor	Yes		5 prime	118.834 kb	2
	chr5	<i>Tacc3</i>	FGFR3-associated protein			3 prime	8.939 kb	7
	chr11	<i>Prkca</i>	Kinase		Yes	intron 2	disrupt CDS	1
	chr15	<i>Pacsin2</i>	PKC substrate			intron 1	not disrupt CDS	IPCR
	chr17	<i>Ddr1</i>	Tyrosine kinase receptor			5 prime	11.411 kb	4
	chr14	<i>Uchl3</i>	Deubiquitinating enzyme			3 prime	24.205 kb	1
	chr11	<i>Cbx1</i>	Heterochromatin stabilization			5 prime	2.931 kb	1
	chr4	<i>Prdm16</i>	Transcription factor	Yes	Yes	intron 1	disrupt CDS	12
	chr19	<i>2610041P08Rik</i>	ND			5 prime	14.016 kb	1
	chr13	<i>Mef2c</i>	Transcription factor	Yes	Yes	5 prime	394.215 kb	1
	chr16	<i>Cd47</i>	Integrin associated protein			5 prime	6.824 kb	1
	chr8	<i>AK014460</i>	ND			3 prime	269.933 kb	1
	chr14	<i>D830030K20Rik</i>	ND			intron 4	disrupt CDS	1
	chr4	<i>Dnb5</i>	Sugar transporter	Yes		3 prime	59.918kb	IPCR
	chr5	<i>0610009M14Rik</i>	TSC22-related transcription factor	Yes		N/D	N/D	IPCR
	chr4	<i>Laptm5</i>	Lysosomal protein	Yes		intron 1	disrupt CDS	IPCR
	chr11	<i>Thoc1</i>	mRNA export			exon 1	disrupt CDS	IPCR

Summary for all five *Lmo2* tumors

Total integrations cloned	75
Integrations per leukemia	15
Total integrations that are common insertions in RTCGD	35 (47%)
Common insertion sites (CIS) per leukemia	7
CIS expected by random chance	1/75

Tumor	Proviral Integrations
86277	<i>Lmo2</i> , <i>Sox4</i>
98031	<i>Lmo2</i> , <i>Il2rg</i> , <i>Sox4</i> , <i>6030413G23Rik</i> , <i>Mef2c</i>
7105	<i>Lmo2</i> , <i>Prdm16</i> , <i>Mef2c</i> , <i>Sox4</i> , <i>6030413G23Rik</i>
7107	<i>Lmo2</i> , <i>Il2rg</i> (2), <i>Prdm16</i> , <i>Irs2</i> (2)
3095	<i>Lmo2</i> , <i>Mef2c</i>

Summary of these results

Many mutations are required to produce an *Lmo2* + *Il2rg* tumor

Can cancer gene therapy complications be limited by transplanting less cells?

Are the cancer free SCID patients leukemia prone?

Are the genes we identified in mouse *Lmo2* tumors relevant to human *LMO2* tumors?

Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia

Adolfo A. Ferrando,¹ Donna S. Neuberg,² Jane Staunton,³ Mignon L. Loh,^{4,8} Christine Huard,^{3,9} Susana C. Raimondi,⁵ Fred G. Behm,⁵ Ching-Hon Pui,⁶ James R. Downing,⁵ D. Gary Gilliland,⁴ Eric S. Lander,³ Todd R. Golub,^{1,3} and A. Thomas Look^{1,7}

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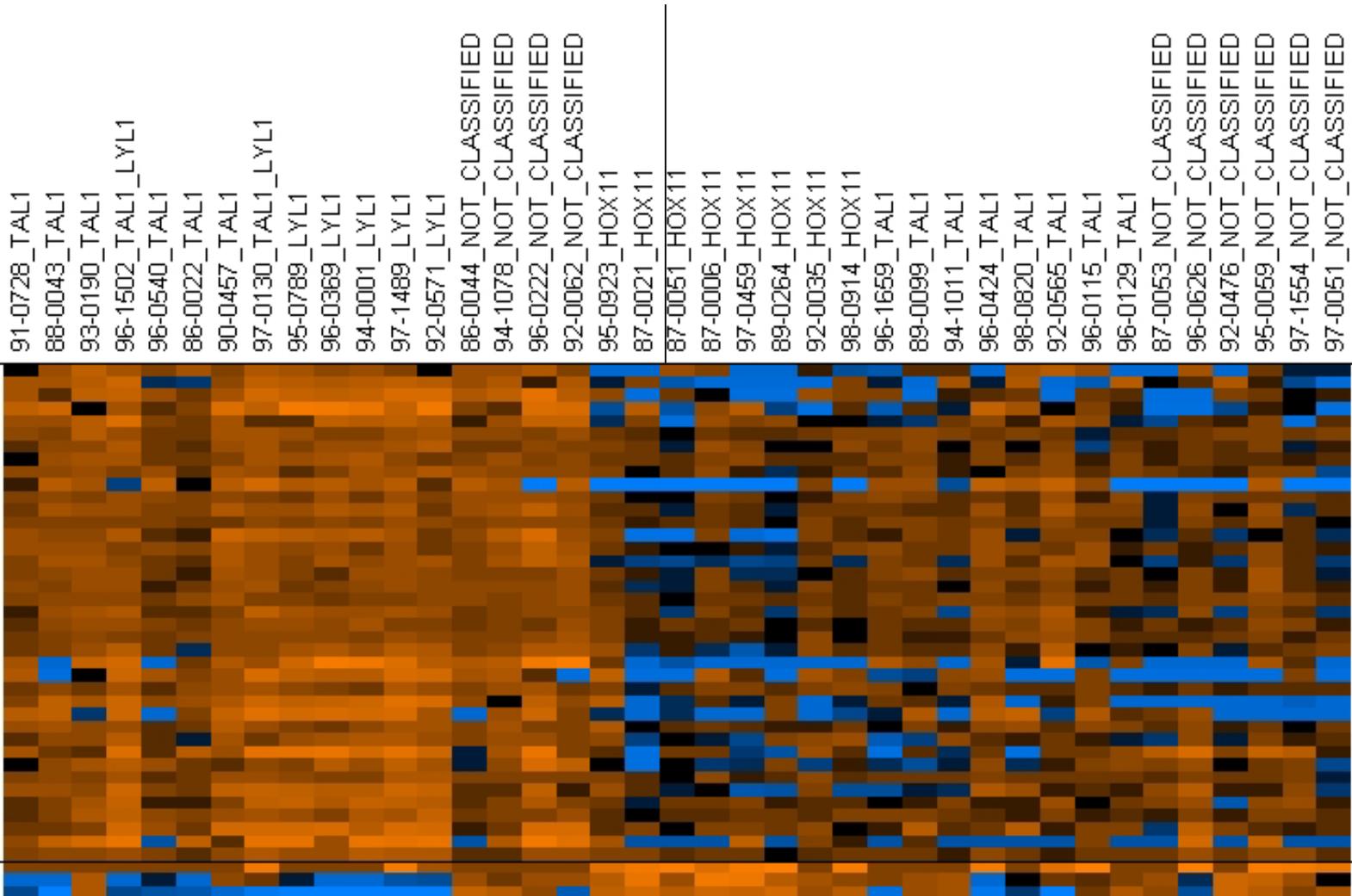
39 T cell leukemias microarrayed

Search results

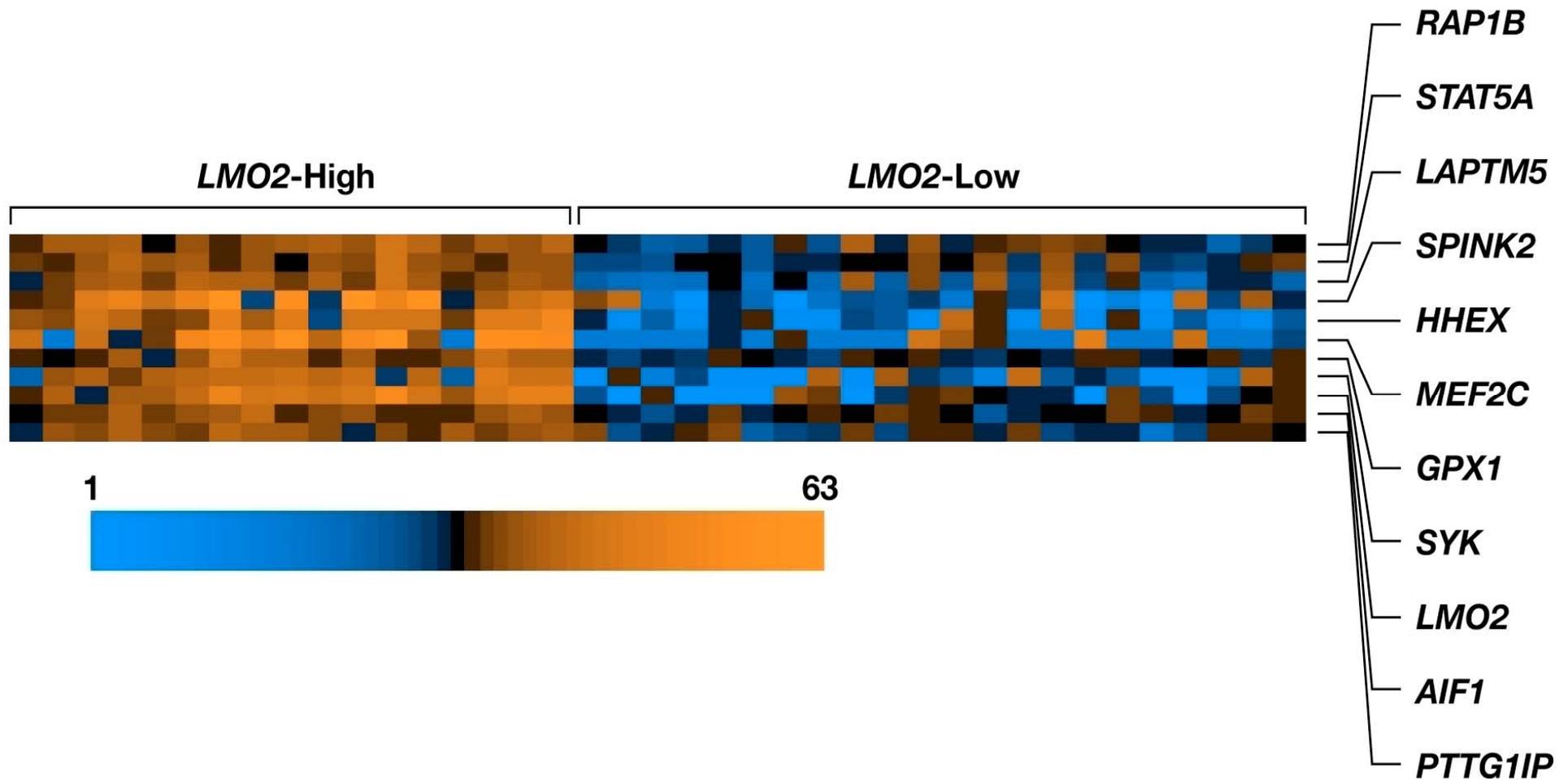
Order genes by *t*-test with $p < 0.001$

19 *LMO2*-high
expressing

20 *LMO2*-low
expressing



Ten most statistically significant genes



Excellent correlation between human and mouse genes

p value	Expression	Gene	Product	<i>Lmo2</i> tumors
4.00E-07	20.9	<i>HHEX</i>	Transcription factor	
2.20E-06	3.8	<i>LAPTM5</i>	Lysosomal protein	<i>Laptm5</i>
4.70E-06	2.1	<i>GPX1</i>	Peroxidase	
5.10E-06	1.8	<i>AIF1</i>	Interferon response	
7.20E-06	2.4	<i>RAP1B</i>	RAP1 GTPase	<i>Rap1gds</i>
8.50E-06	19.9	<i>MEF2C</i>	Transcription factor	<i>Mef2c</i>
1.00E-05	19.6	<i>SPINK2</i>	Serine protease	<i>Spink3</i>
1.20E-05	2.0	<i>STAT5A</i>	Transcription factor	<i>Il2rg</i>
2.00E-05	2.2	<i>PTTG1</i>	Chromatid segregation	
4.90E-05	10.9	<i>SYK</i>	Kinase	<i>Wbscr5</i>

Conclusions

SCID IL2RG gene therapy trial represent a special case and is not predictive of most other gene therapy trails

Changes to the *SCID IL2RG* gene therapy protocol seem warranted

- Transplant less infected cells
- Use SIN vectors and endogenous *IL2RG* promoter

Collaborators

Nancy Jenkins

Utpal Dave
Keiko Akagi

Dennis Du