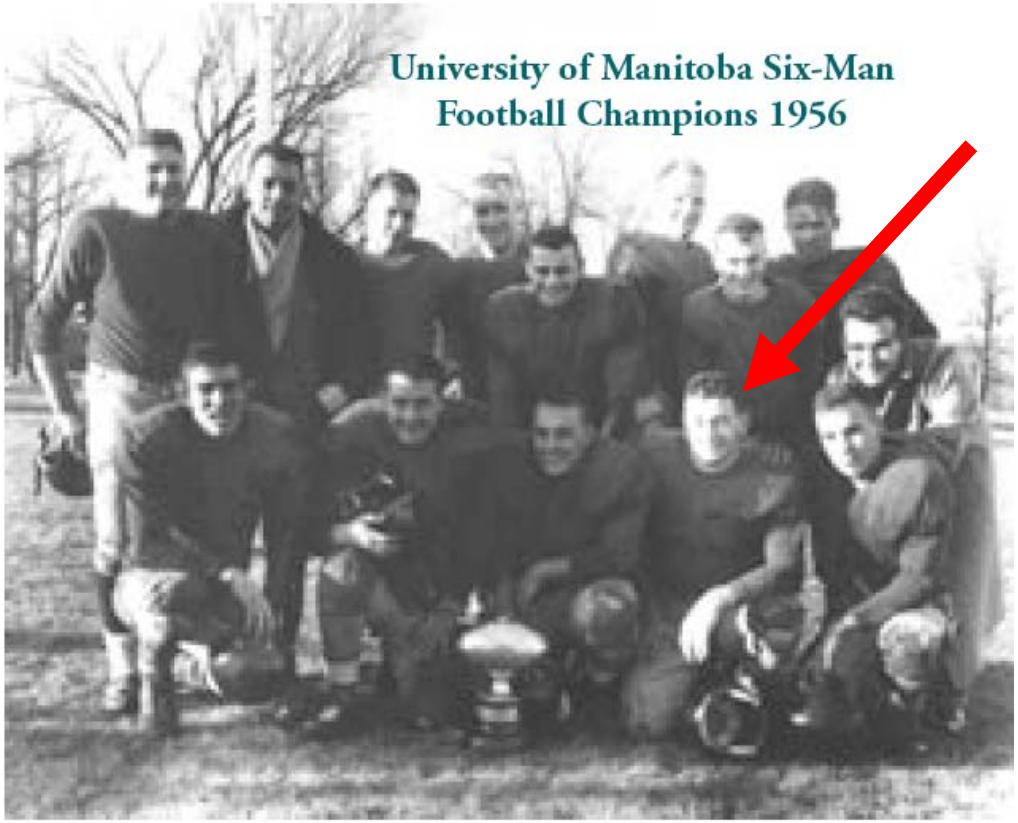
Molecules and mechanisms of the graft versus leukemia (GVL) effect

Back row
(from left to right):
David Brown
Arnold Naimark
Robert Hoeschen
Colin Sinclair
Earl Herschfield
Gordon Watters
Monty Hart
Tom Casey
Larry Kussin
Front Row:
Sherman Hershfield
George Yee
Martin Hollenberg
Gerald Goldenberg
Rod McPherson



(photo from Faculty of Medicine Archives)

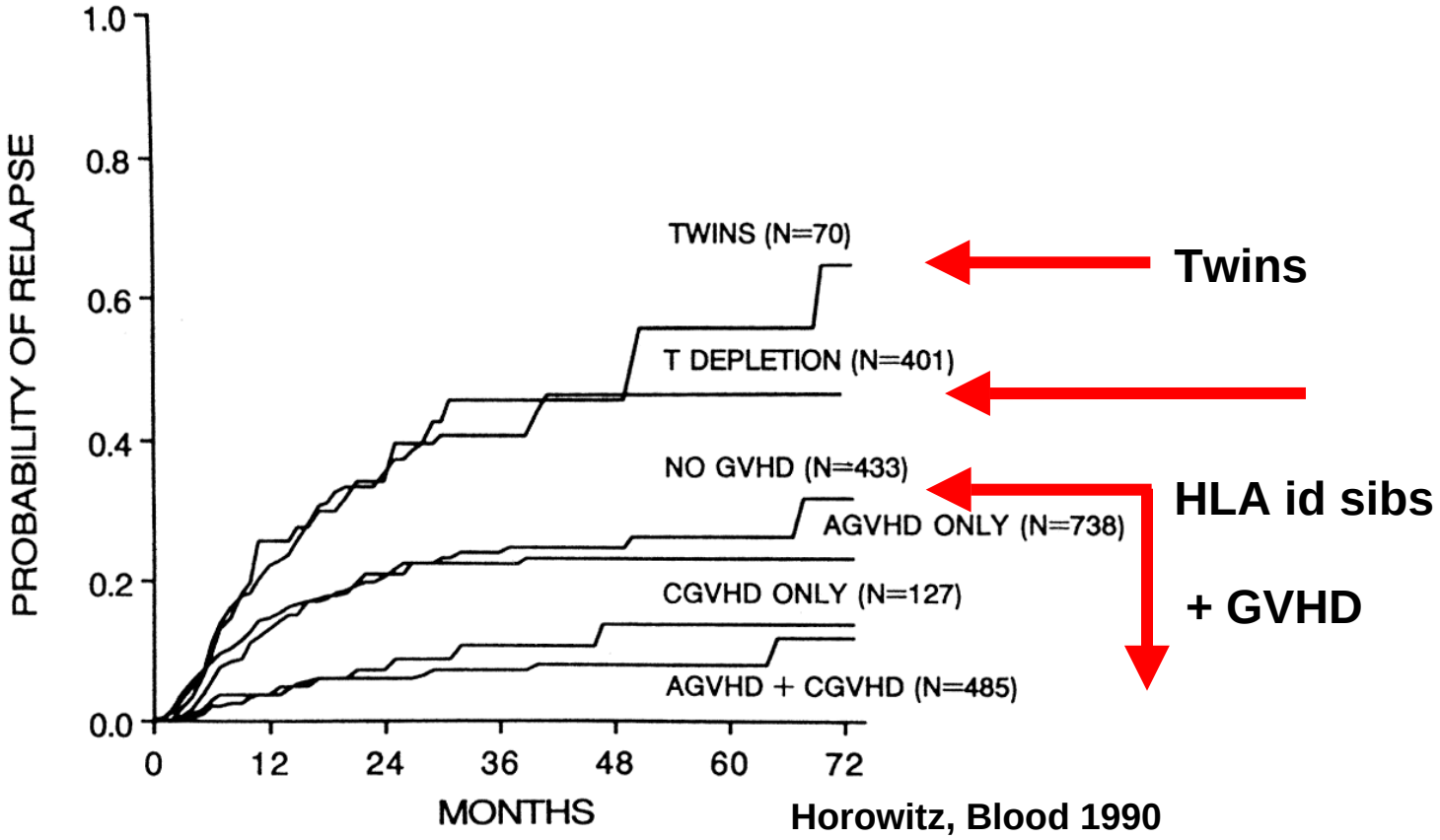
- **University of Manitoba Faculty of Medicine Archives**

The “Goldenberg” Rules

- **We are fortunate to have the opportunity to participate in research. Do not squander the opportunity -- commit yourself to a life-time of learning**

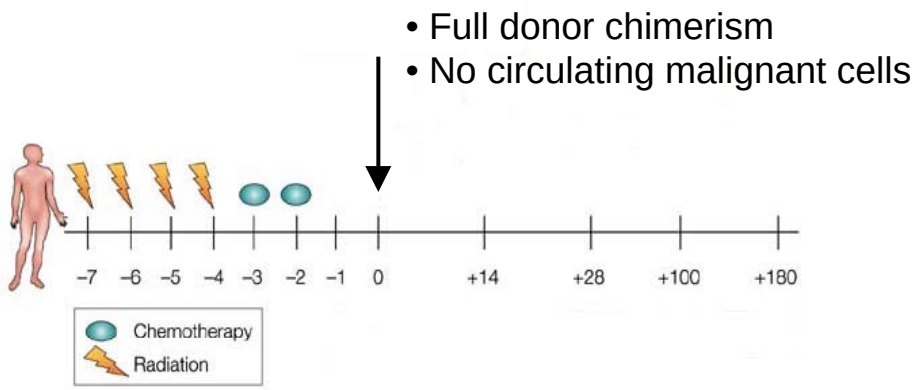
2. **Patients and professors can teach us much, if we pay attention**
3. **Treasure the time you have for research, it is special**
4. **Use correct grammar!!**

Immunologic Non Identity Contributes To The Efficacy Of Allogeneic HCT



CML > CLL, low grade lymphoma, myeloma > AML, ALL

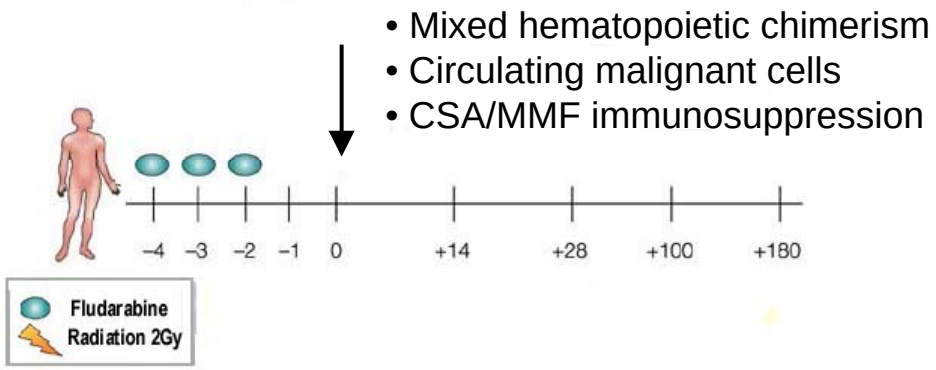
Myeloablative Conditioning (Age <50)



- **Toxicity**
- **GVHD**
- **Relapse**

Success rate ~ 30 - 60% in AML/ALL/MDS, 80% CML

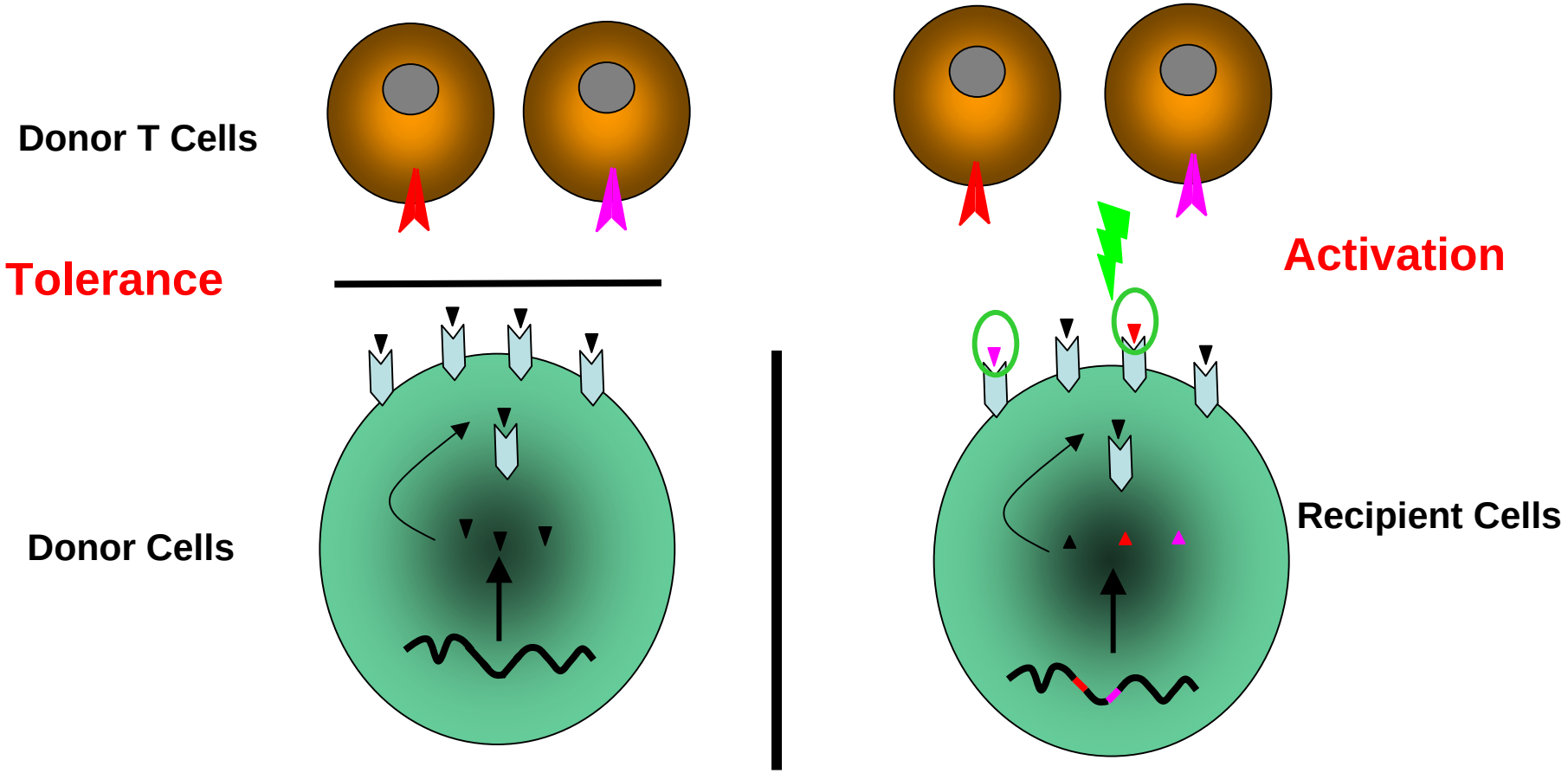
Nonmyeloablative Conditioning (Age up to 70)



- **GVHD**
- **Relapse**

Success rate ? (~50% for indolent leukemias (CLL), follicular lymphoma

Genetic Polymorphism Results in the Display of Unique Peptides by Cell Surface MHC Molecules



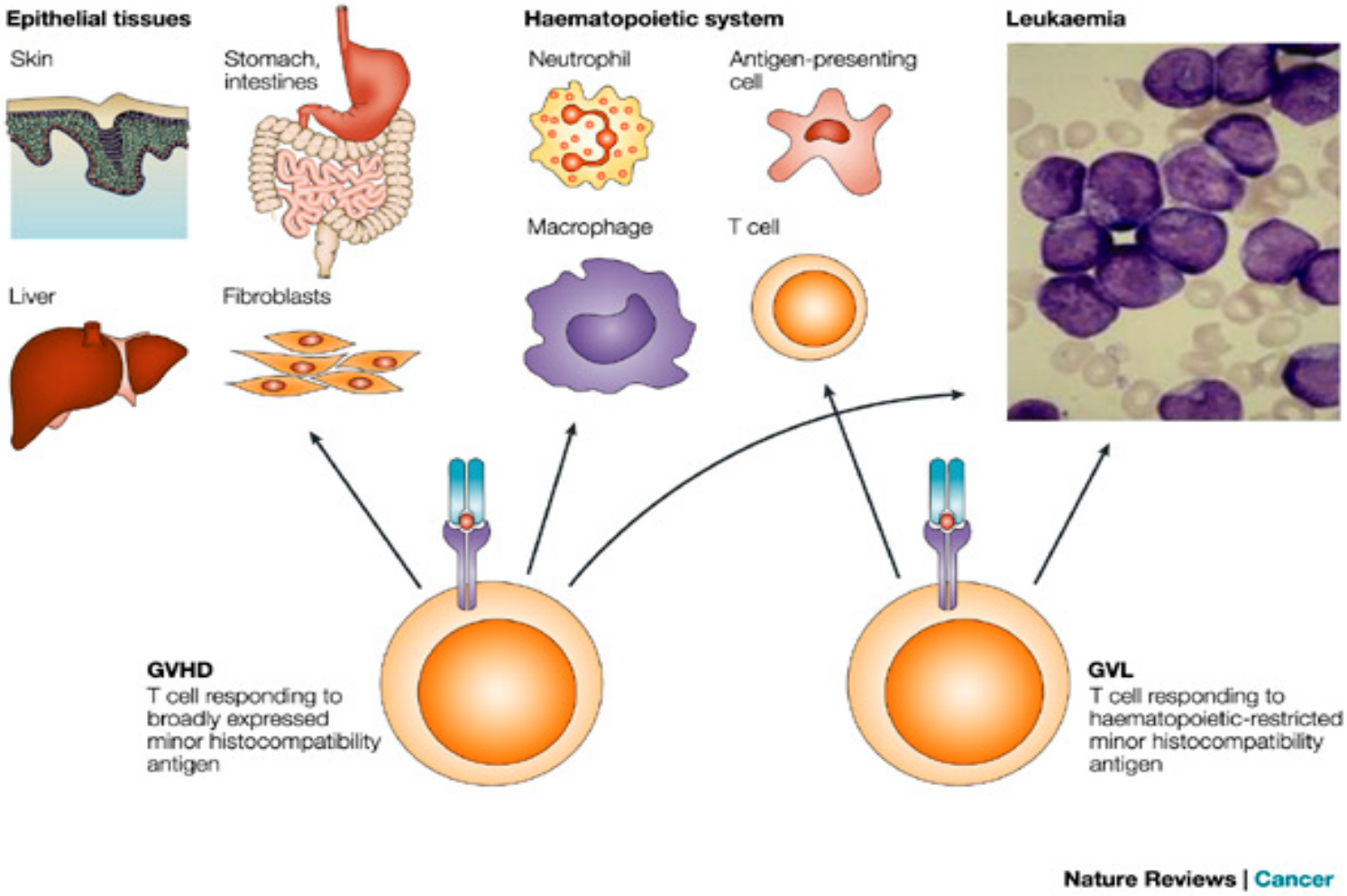
- nonsynonymous SNPs
- 5' "untranslated regions"
- differential gene expression
- peptide splicing/reassortment

Minor H Antigens - Characteristics of Effective Targets for Cancer Immunotherapy

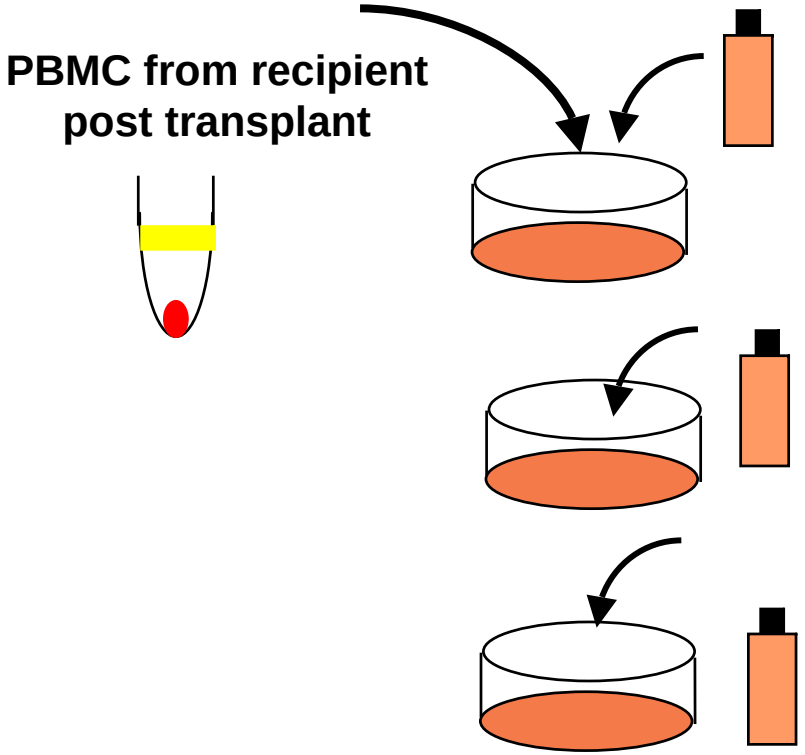
- Antigens are “foreign” to donor T cells
- T cell responses are of high avidity
- T cell responses may be multivalent
- Responses typically involve both CD8 and CD4 T cells

Can the GVL effect for acute leukemias be augmented and separated from GVHD?

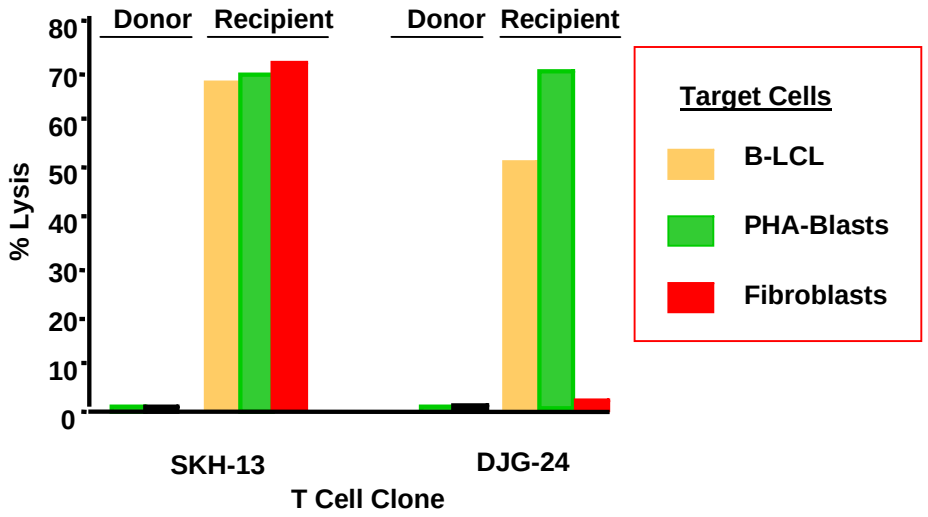
Tissue Expression of Minor H Antigens May Provide A Basis for Segregating GVHD and GVL Responses



Isolation of T Cells Specific For Recipient Minor H Antigens After In Vivo Priming

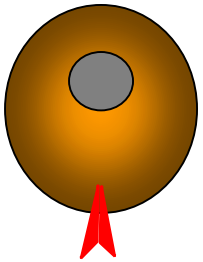


γ-irradiated APC (PBMC or leukemia)
from the recipient pre transplant



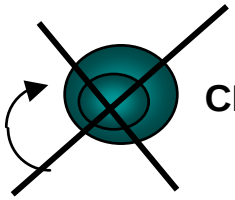
Assay for cytolytic activity
Clone reactive T cell lines and characterize T cell clones
for phenotype, HLA restricting allele, tissue expression

Part 1. Lost in Translation



"Hematopoietic Lineage"-Restricted Minor H Antigen

Self Renewal

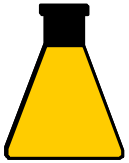


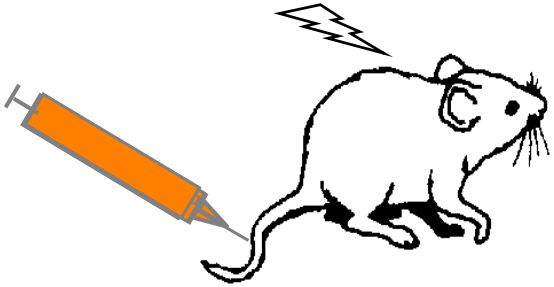
$CD34^{+}CD38^{-}$

Leukemic Stem Cell

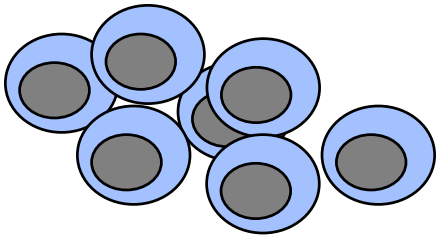
AML +/- CTL

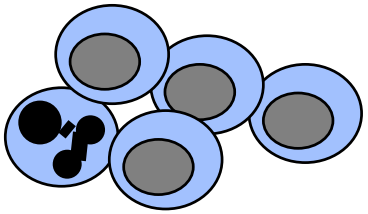
375 cGY

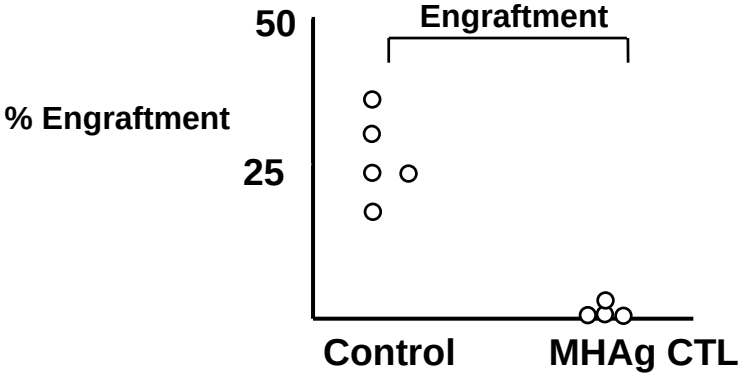




Clonogenic Leukemic Progenitors



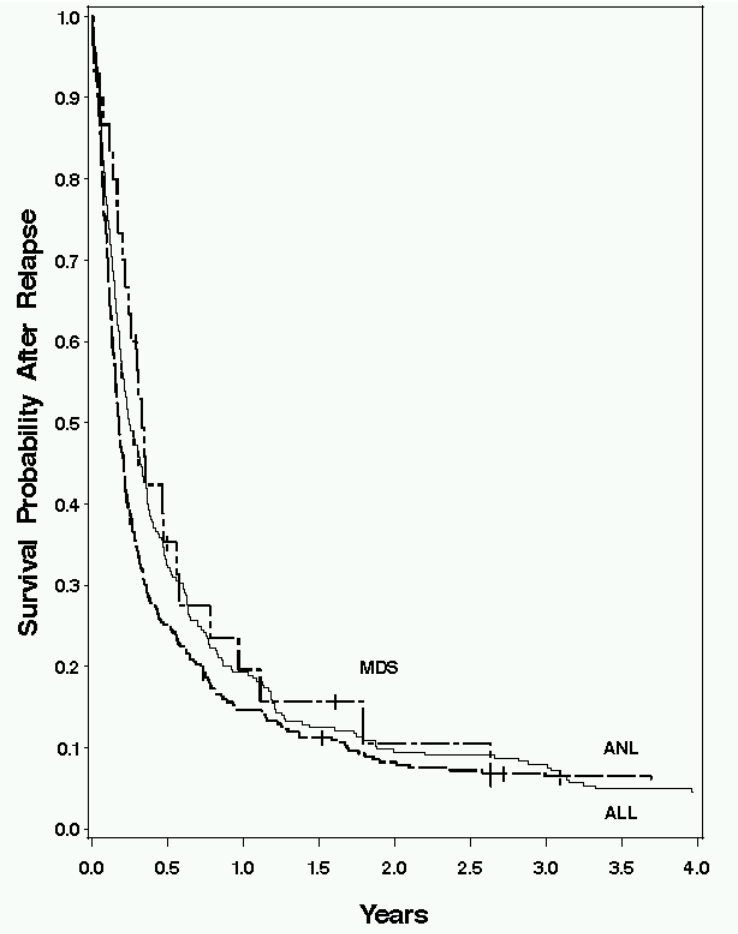




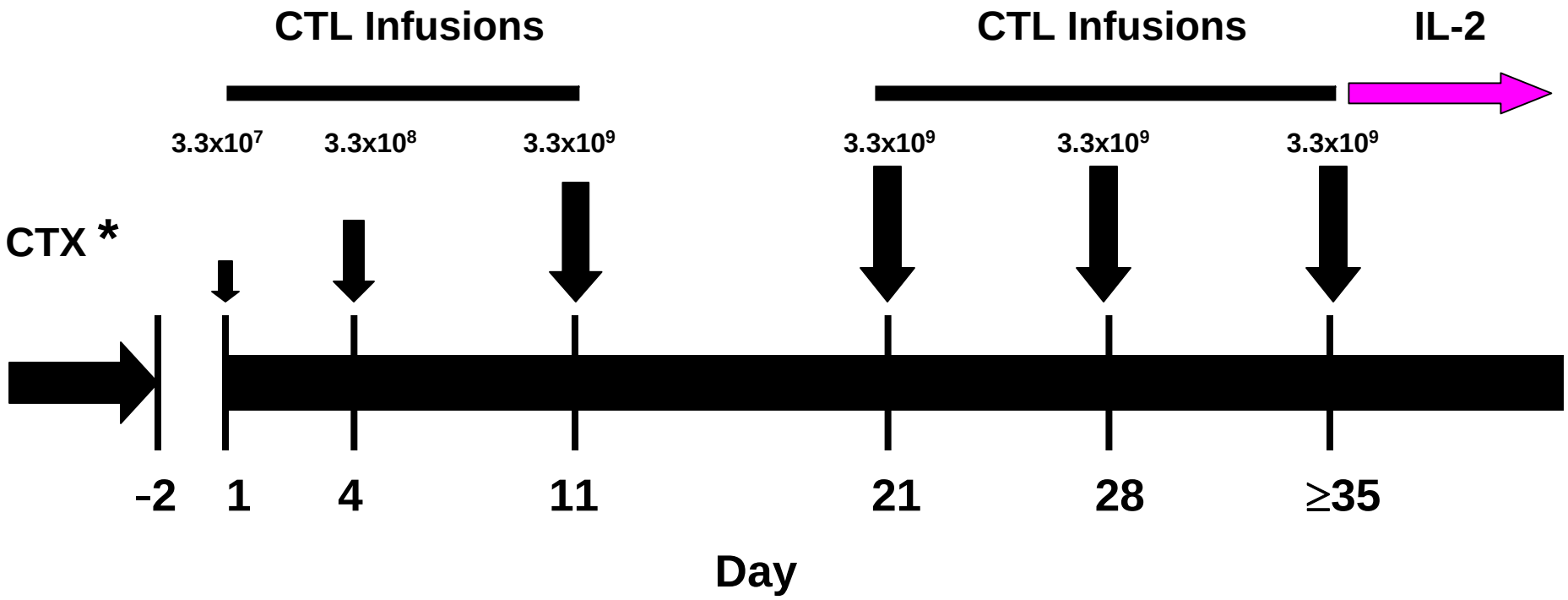
• Bonnet D. et al. Proc Natl Acad Sci 1999

Adoptive Transfer of Minor H Antigen-Specific T Cell Clones To Treat Post Transplant Leukemic Relapse

Outcome - Post transplant Relapse



- **Enroll patients with advanced leukemia (>2nd CR, refractory disease)**
- **Isolate $CD8^+$ T cell clones from recipients early post transplant**
- **Select clones that lyse recipient hematopoietic cells (including leukemia) BUT NOT skin fibroblasts**
- **QC testing & cryopreservation**
- **Treat patients at relapse with escalating doses of T cells**
- **Evaluate toxicity, T cell migration and persistence, and antitumor activity**

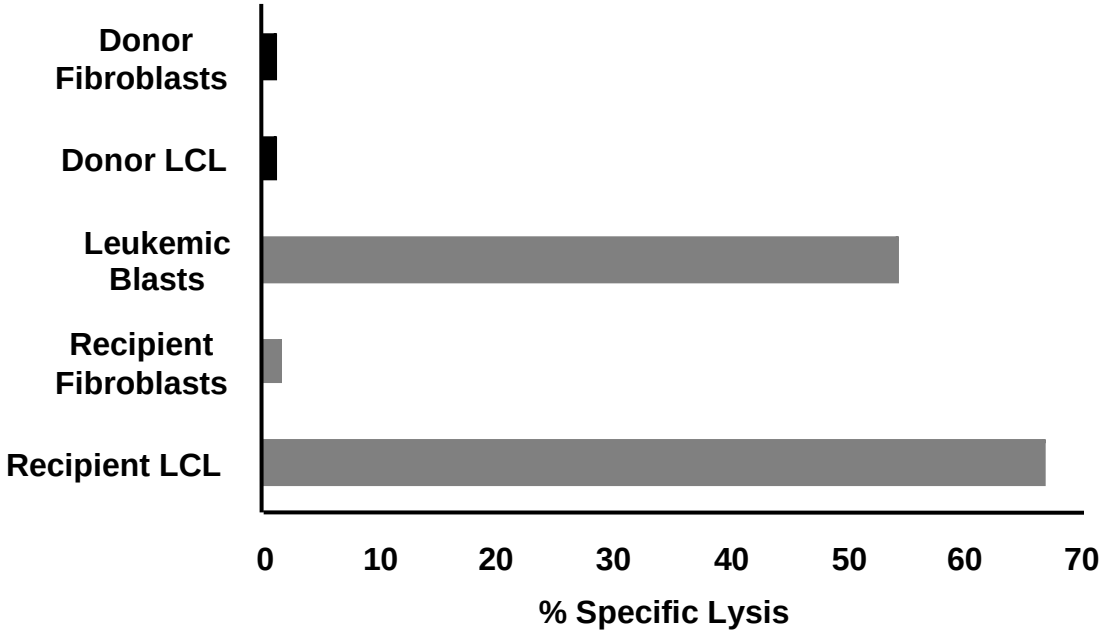


- **Early TRM or relapse -- 32% of enrolled patients**
- **7 patients relapsed and received T cell infusions**
- **3 patients relapsed and had T cells available - not treated**
- **6 patients have T cells available and remain at risk**

UPN-15652

- primary refractory Ph$^+$ ALL
- MRD SCT with Cy/TBI
- Isolated minor H antigen-specific CTL clones
- Relapse 7 months post SCT (>90% blasts)
- Cytoreduction with Mitoxantrone/VP16

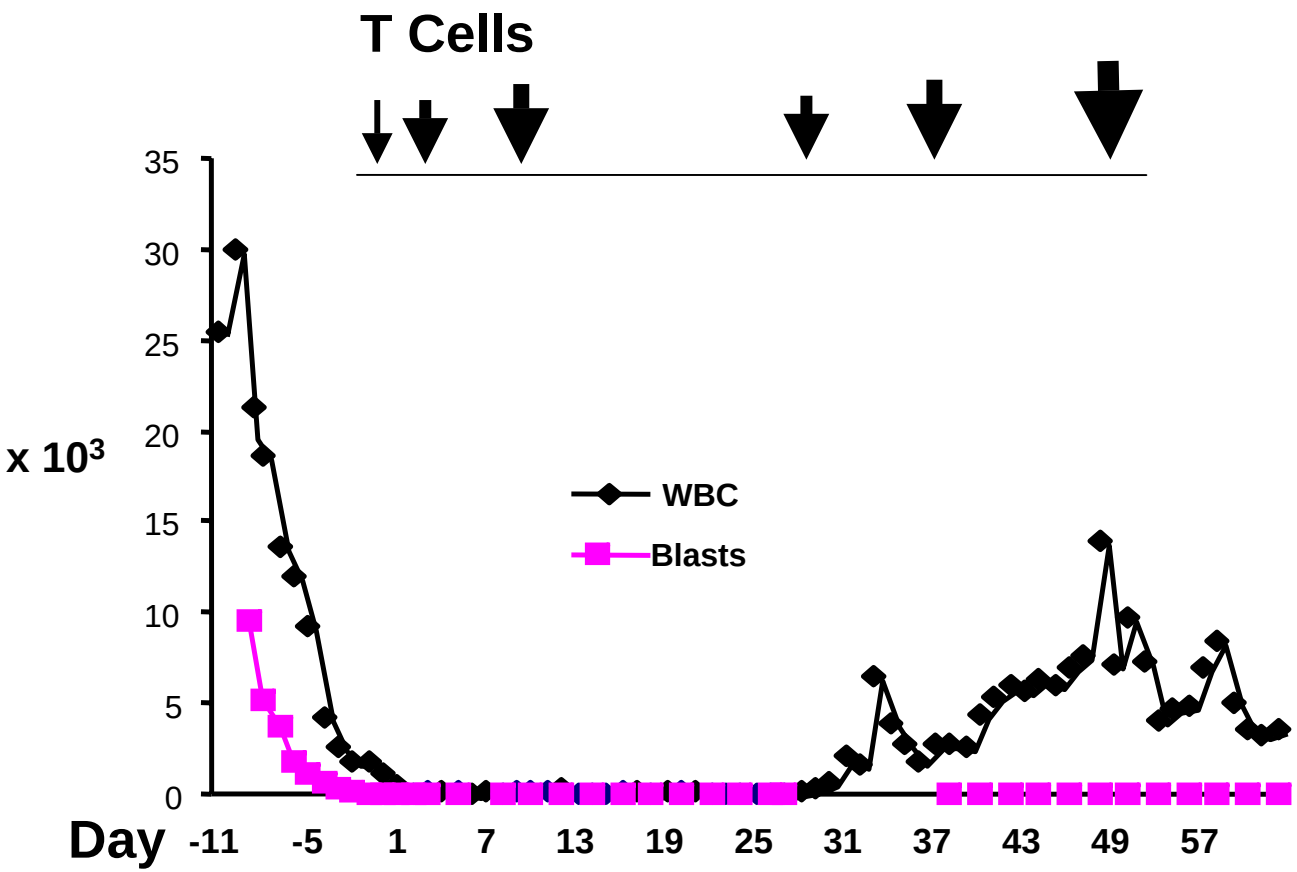
CTL Clone 11C6



Toxicity:

* Skin GVHD (PCR -ve for CTL)

* Lung Toxicity



Blasts in BM	90%	>15%

Transferred CTL Localize At Site of Tissue Injury and Modulate TcR Expression

Pre-Inf

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

4 hr Post T Cells

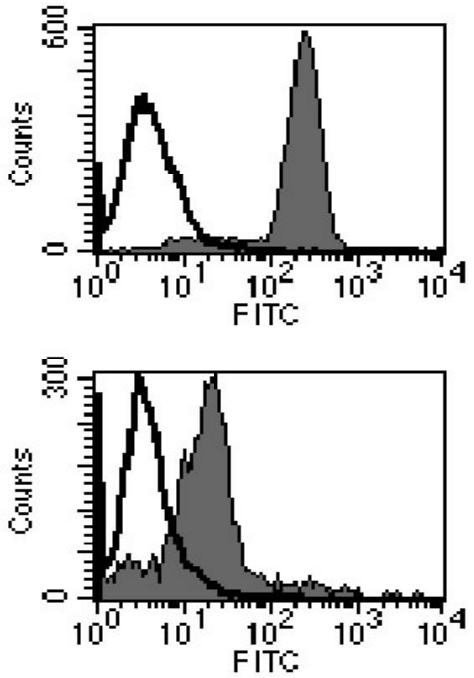
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

MP

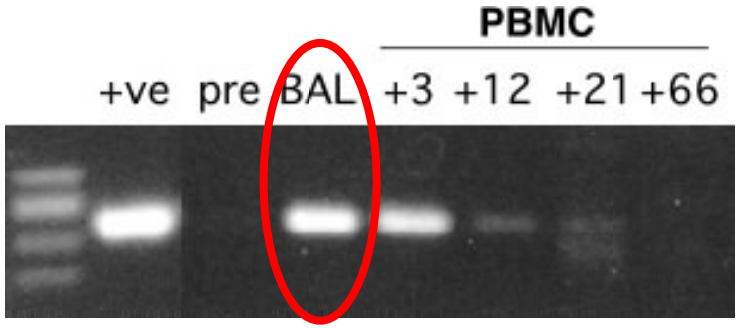
2 days Post T Cells

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Modulation of Vβ 13 TcR Expression in bronchoalveolar lavage fluid



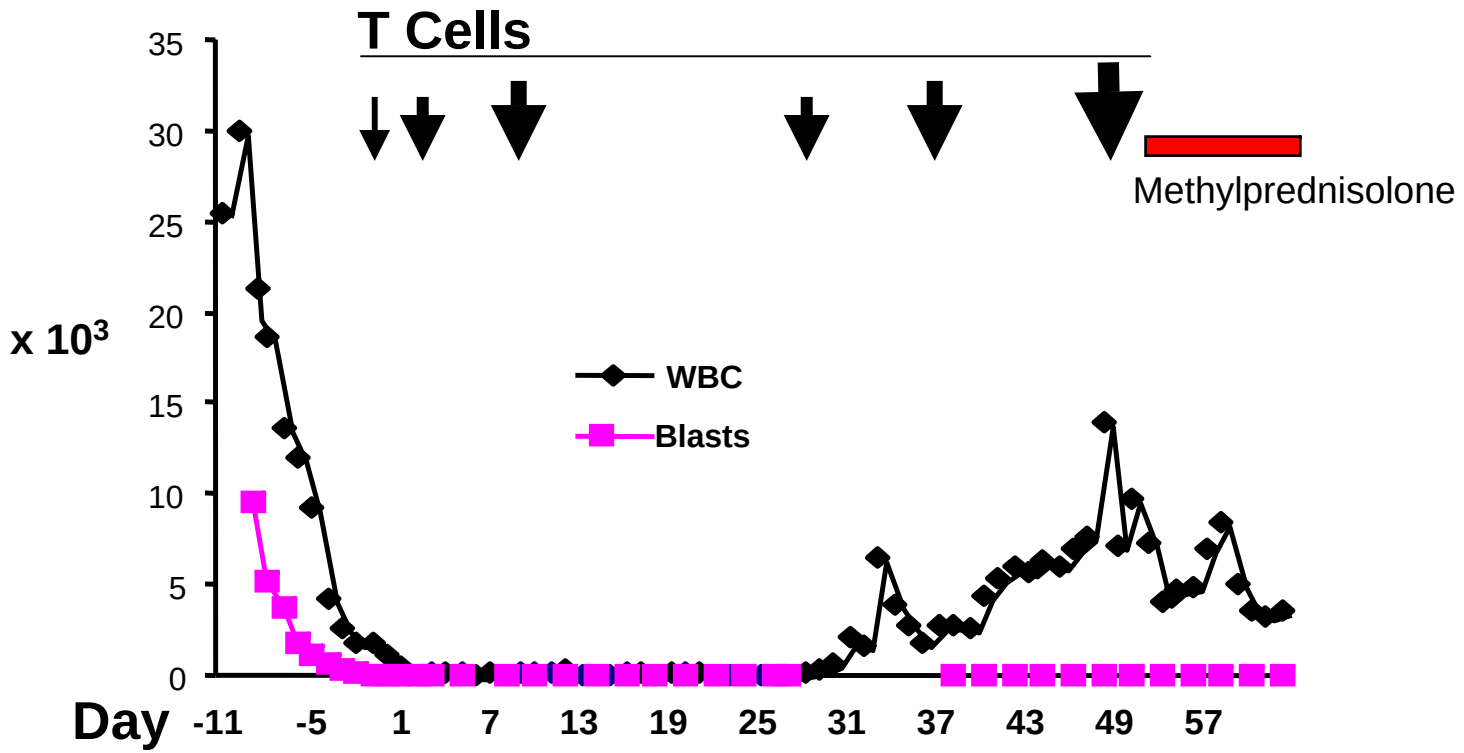
Detection of CTL by clone-specific PCR



Toxicity:

* Skin GVHD (PCR -ve for CTL)

* Lung Toxicity

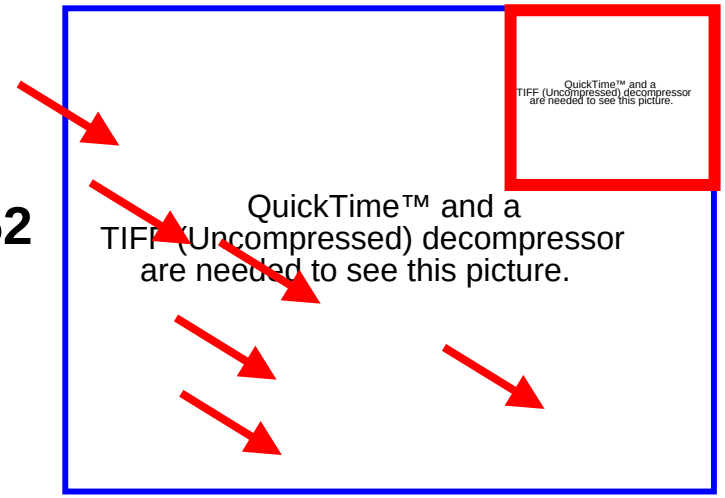


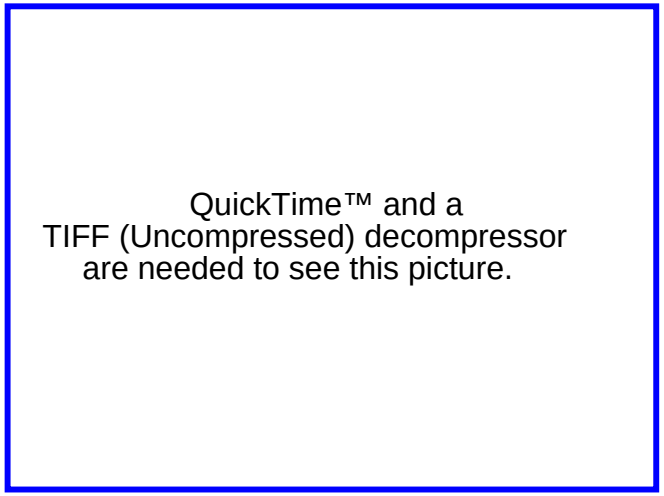
Blasts in BM	90%	>15%	<5%

Post chemotherapy - Relapse

Post T cell infusions - Remission

UPN15652





UPN 19492

Bone Marrow Blasts

Patient	Pre	Post CTX	Post T Cells	Outcome
#3 ALL	**90%**	**80%**	**<5% CR**	**No GVHD, toxicity**

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

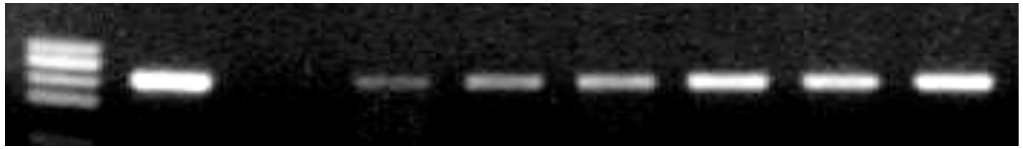
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Infusion #

3 **4** **6**

+ **–** **PBL** **M** **PBL** **M** **PBL** **M**

Clone-specific TcR V beta PCR



Results

- **Toxicity**
  - **3/7 patients (2 lung, 1 GVHD)**
- **Transfer efficiency and migration**
  - **high levels of transferred T cells in the blood and bone marrow (>8% of PBMC), t1/2 of transferred cells in the blood ~ 7 days**
- ***Efficacy**
  - **4/6 evaluable patients achieved CR, including 2 with persistent disease after chemotherapy.**
  - **6/7 patients subsequently progressed 4 -15 months after therapy, 1 patient remains alive >39 months**

- Isolation of minor H antigen specific T cells from patients transplanted for advanced leukemia is feasible but problematic

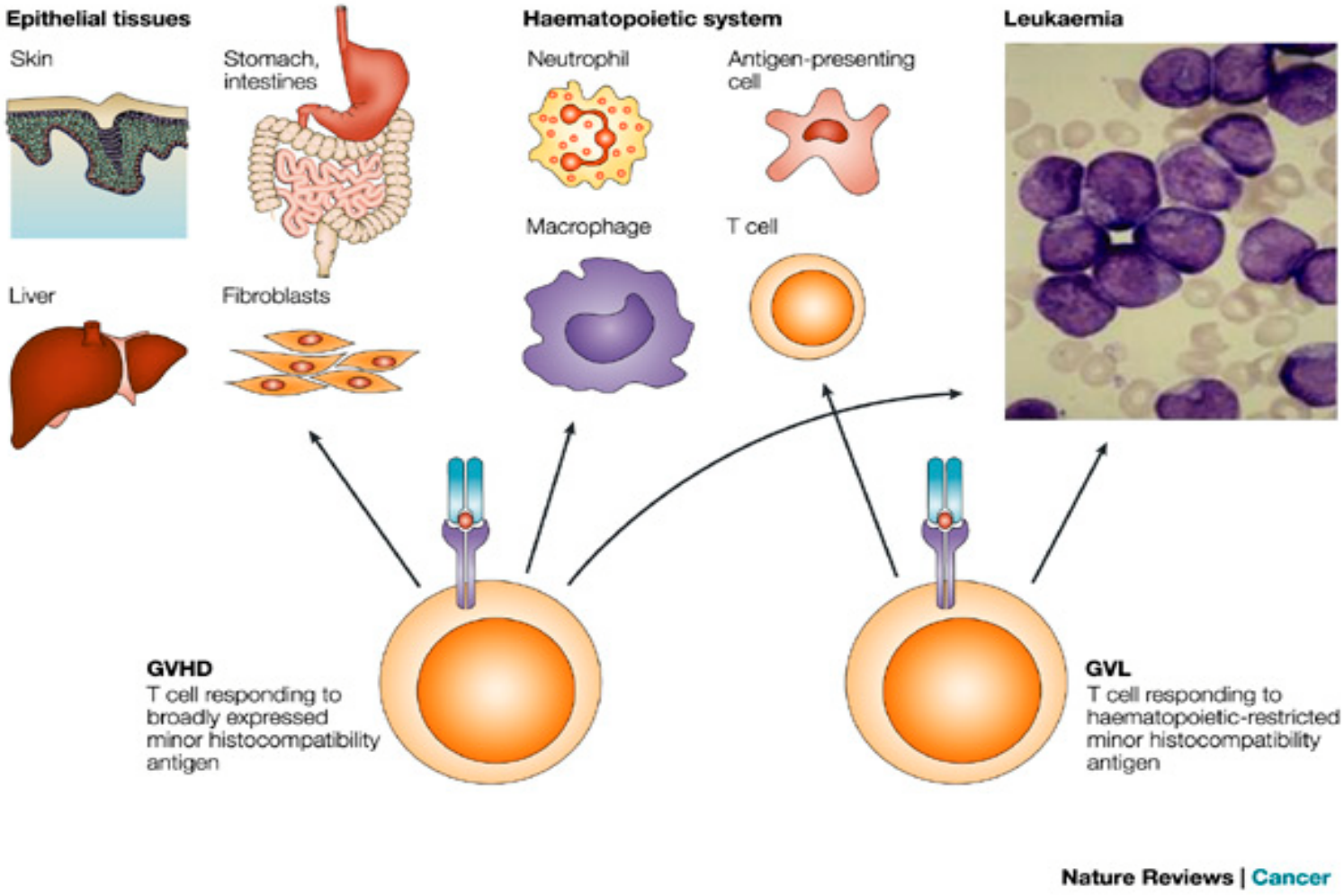
- Separation of GVL effect from toxicity cannot be achieved by selecting clones based on in vitro cytotoxicity against non-hematopoietic targets

3. Adoptively transferred $CD8^+$ T cell clones exhibit limited persistence in vivo

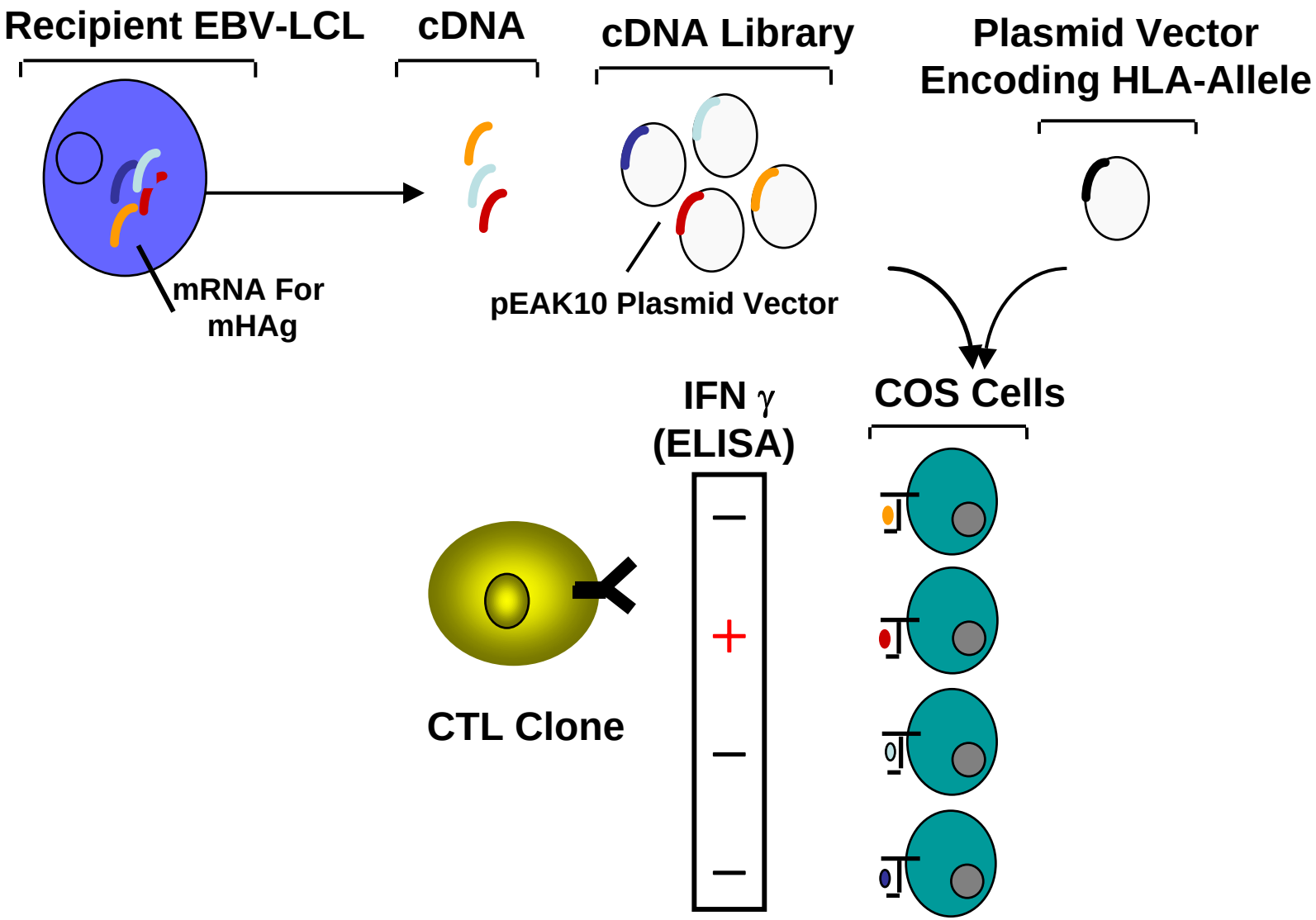
Mechanisms responsible may include:

- high antigen load - activation induced cell death
- inadequate T helper responses/prosurvival cytokines
- intrinsic defect due to culture and/or differentiation
- other

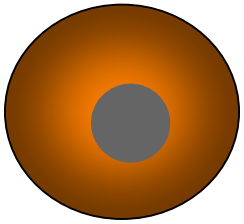
Part 2. Back to Basics



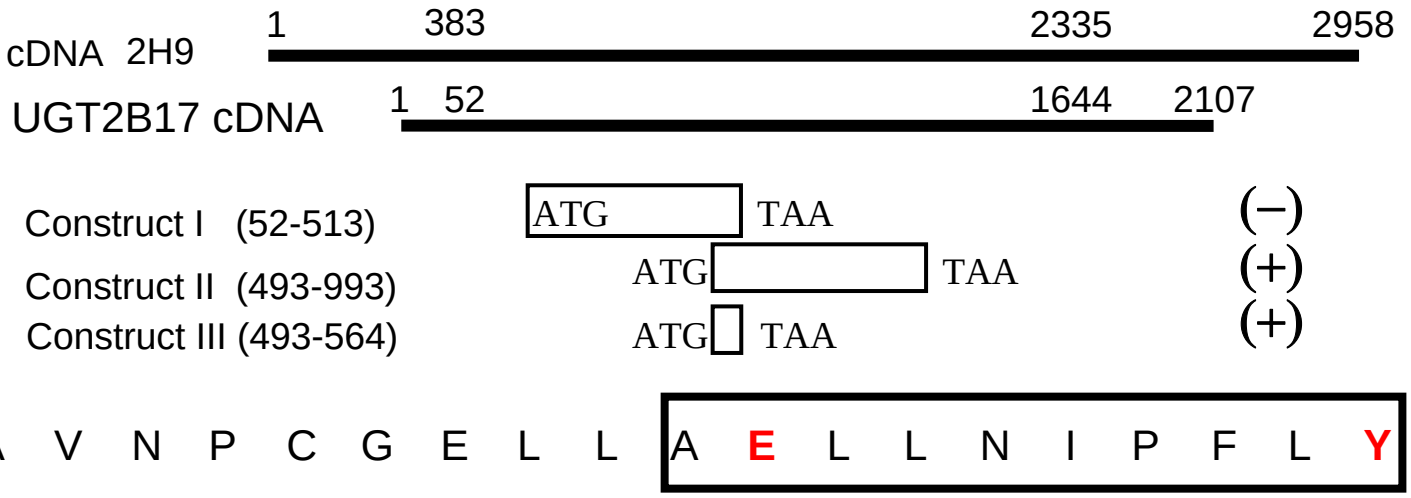
Identification of the Genes that Encode Minor H Antigens



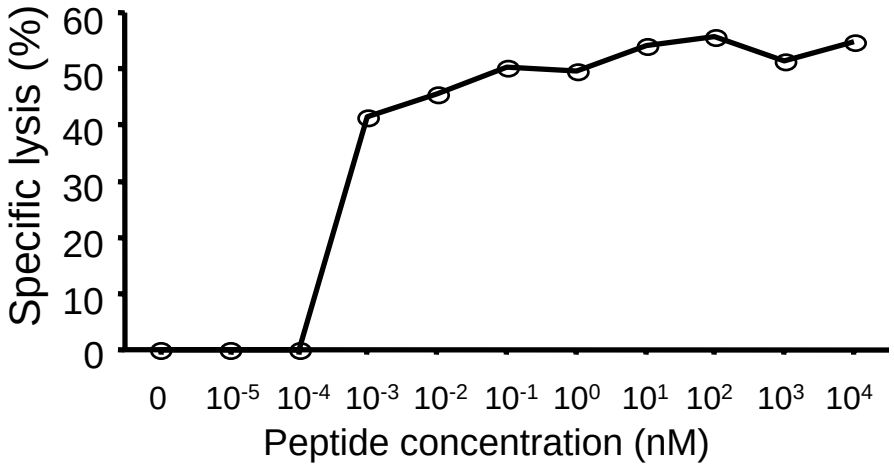
UDP Glycosyltransferase 2 Family, Polypeptide B17 (UGT2B17) Encodes a Minor H Antigen Presented by HLA A29



CTL Clone PL-8



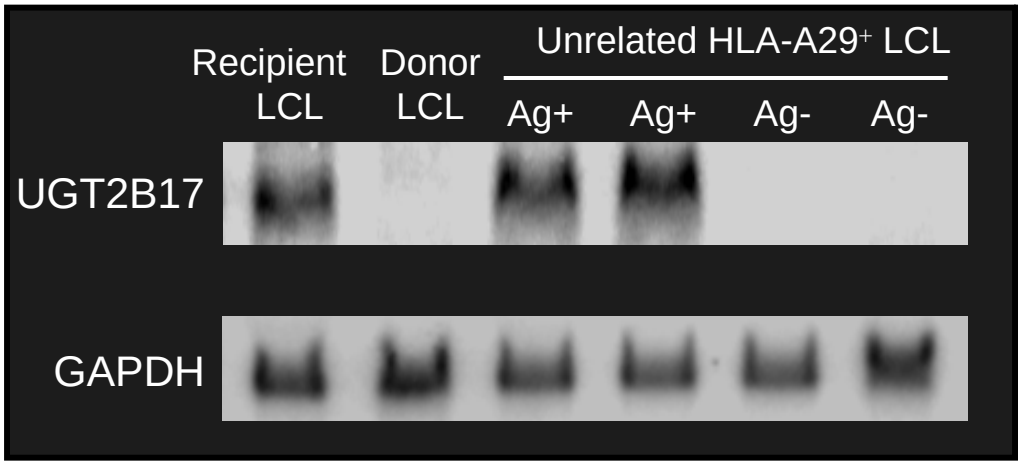
V L L A D A V N P C G E L L A **E** L L N I P F L **Y**

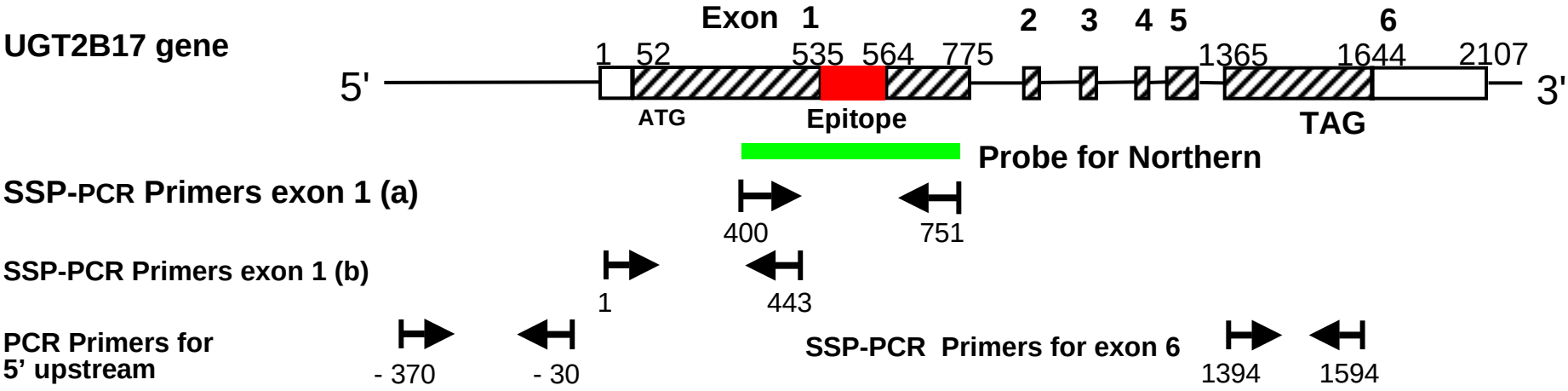


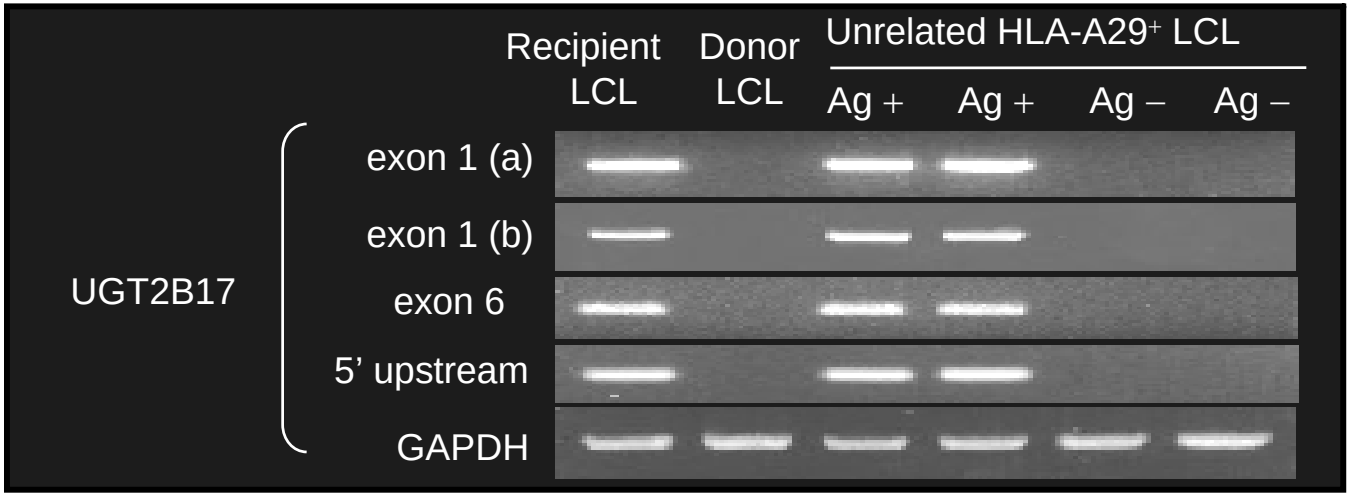
HLA-A29 Anchors									
1	2	3	4	5	6	7	8	9
E								**Y**

Immunogenicity of UGT2B17 Results from Differential Transcription in Recipient versus Donor Cells

Northern Blot Analysis of Total RNA



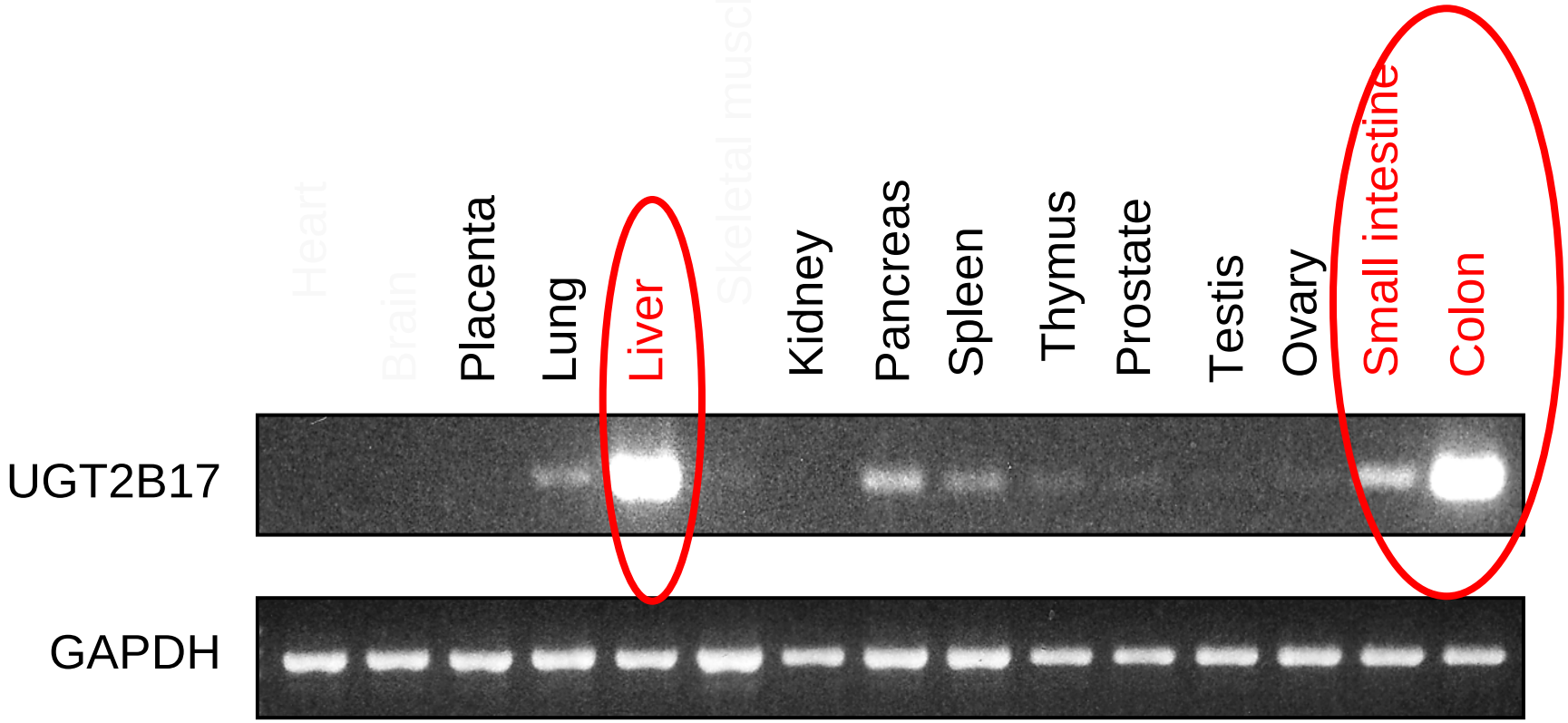




- **11% of Caucasian donors are deficient in UGT2B17**

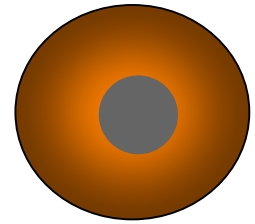
Tissue Expression Matters

SSP-PCR for UGT2B17 cDNA from Human Tissues



- **the recipient had acute GI/liver GVHD and cGVHD involving the GI tract**

DRN-7 CTL Recognize A Minor H Antigen Presented By HLA -A3 And Encoded by the SP110 gene



CTL Clone DRN-7

241 336

Donor SP110 - G

MPHSPLGSMPEIRDNSPEPNDPEEPQEVSSTPSDKKGKKRKRGIWSTPKRRHKKKSLPRGTASSRHGIQKKLKRVDQVPQKKDDSTCNSTVETRAQ +

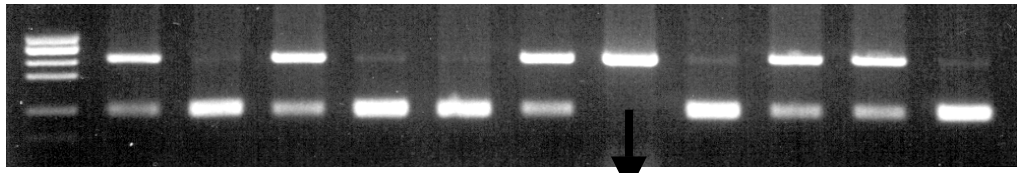
MSTPKRRHKKKSLPRGTASSRHGIQKKLKRVDQVPQKKDDSTCNSTVETRAQ +

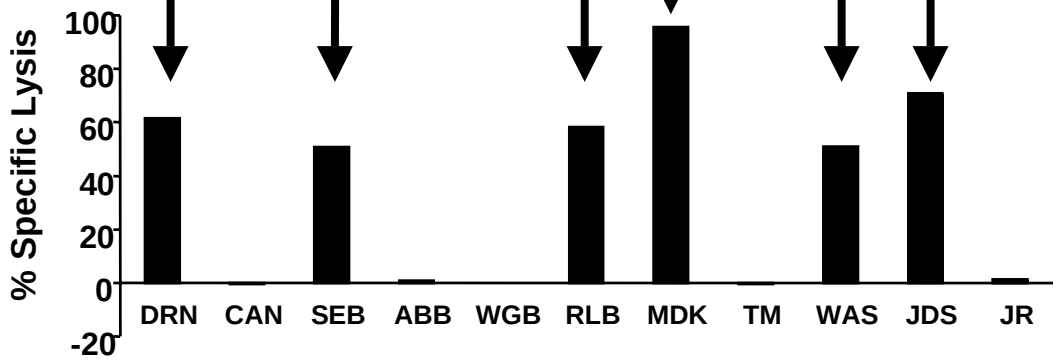
MPHSPLGSMPEIRDNSPEPNDPEEPQEVSSTPSDKKGKKRKRGIWSTPKRRHKKKSLPRGTASSR +

STPKRRHKKKSLPRGTASSR

Genotype A/G G/G A/G G/G G/G A/G A/A G/G A/G A/G G/G

PCR-RFLP

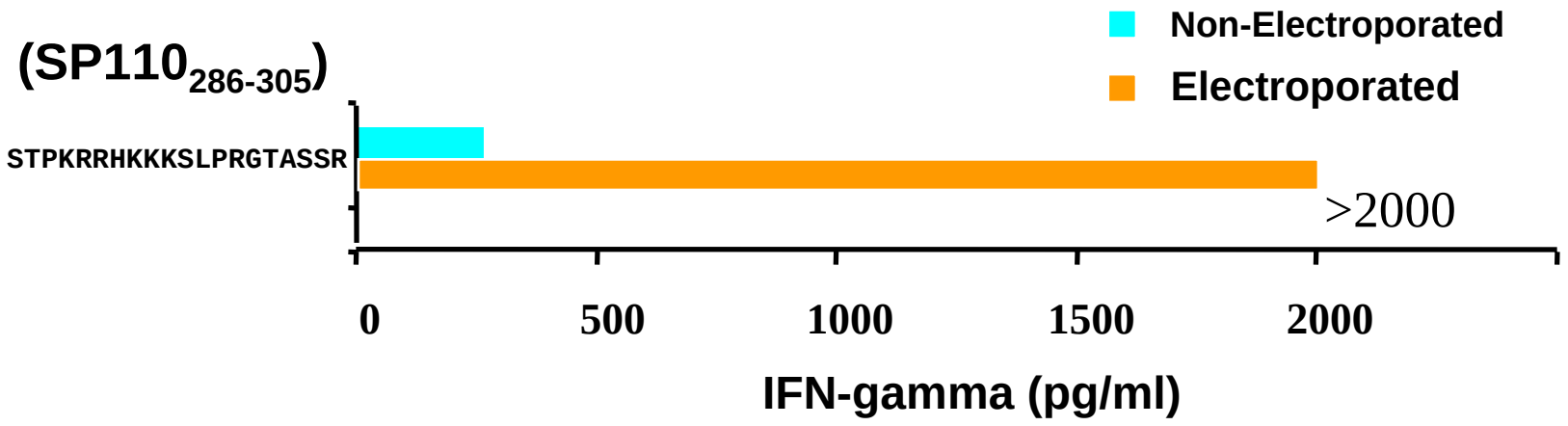




What is the SP110 epitope?

- 20 mer peptide containing G-->R substitution sensitized donor LCL for recognition by CTL but only at high concentrations (>10 μg/ml)

- No synthetic 8 - 12 mer peptide comprised within the 20 mer sequence could be identified that sensitized target cells for recognition by the CTL clone



- Consistent with a requirement for a posttranslational modification for epitope generation

Immune recognition of a human renal cancer antigen through post-translational protein splicing

Ken-ichi Hanada[1], **Jonathan W. Yewdell**[2] **& James C. Yang**[1]

[1]*Surgery Branch, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Building10, Room 2B42, Bethesda, Maryland 20892, USA*
[2]*Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*

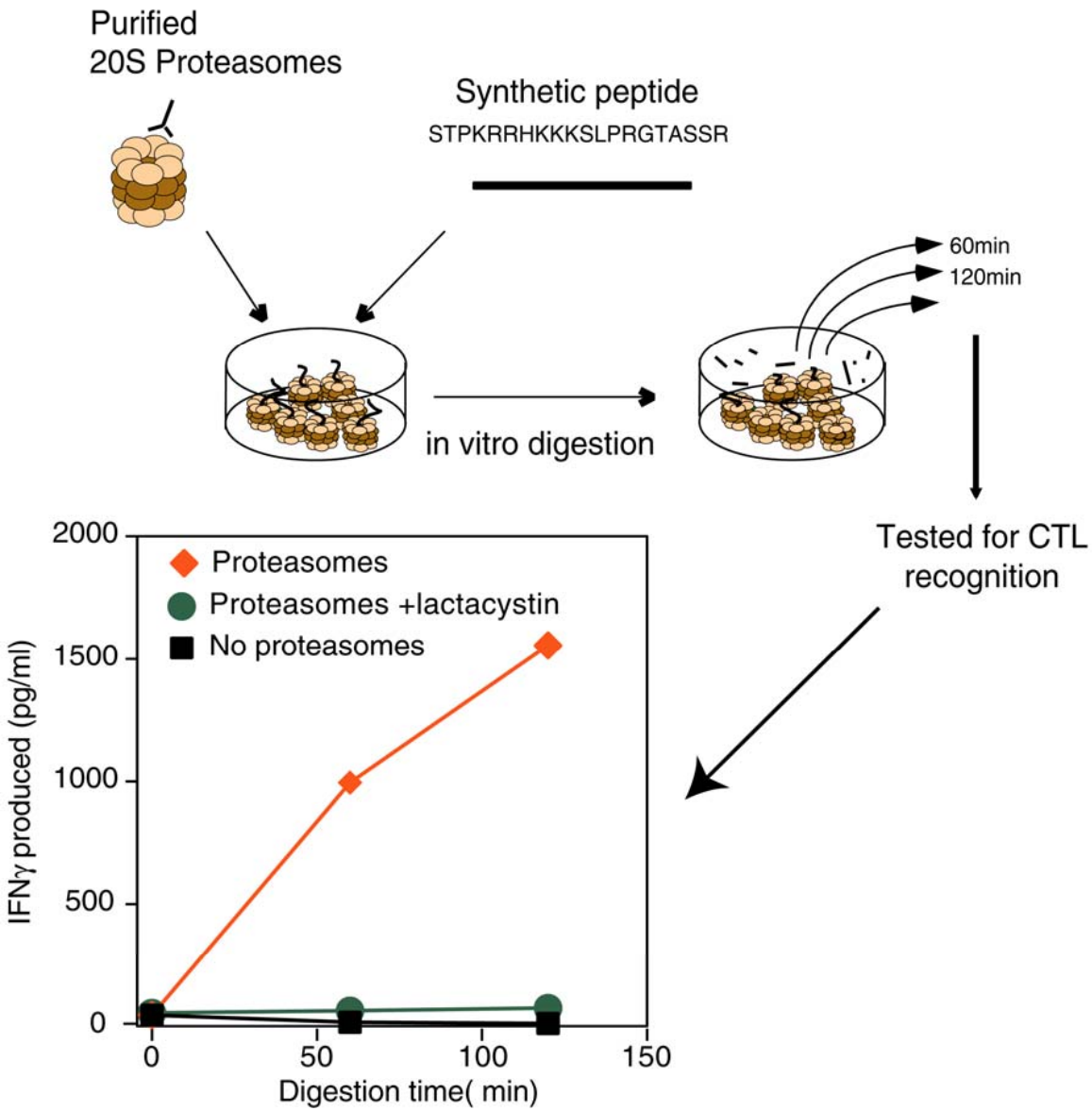
NATURE | VOL 427 | 15 JANUARY 2004 | www.nature.com/nature

An Antigenic Peptide Produced by Peptide Splicing in the Proteasome

Nathalie Vigneron,[1]* Vincent Stroobant,[1]* Jacques Chapiro,[1] Annie Ooms,[2] Gérard Degiovanni,[2] Sandra Morel,[1]† Pierre van der Bruggen,[1] Thierry Boon,[1] Benoît J. Van den Eynde[1]‡

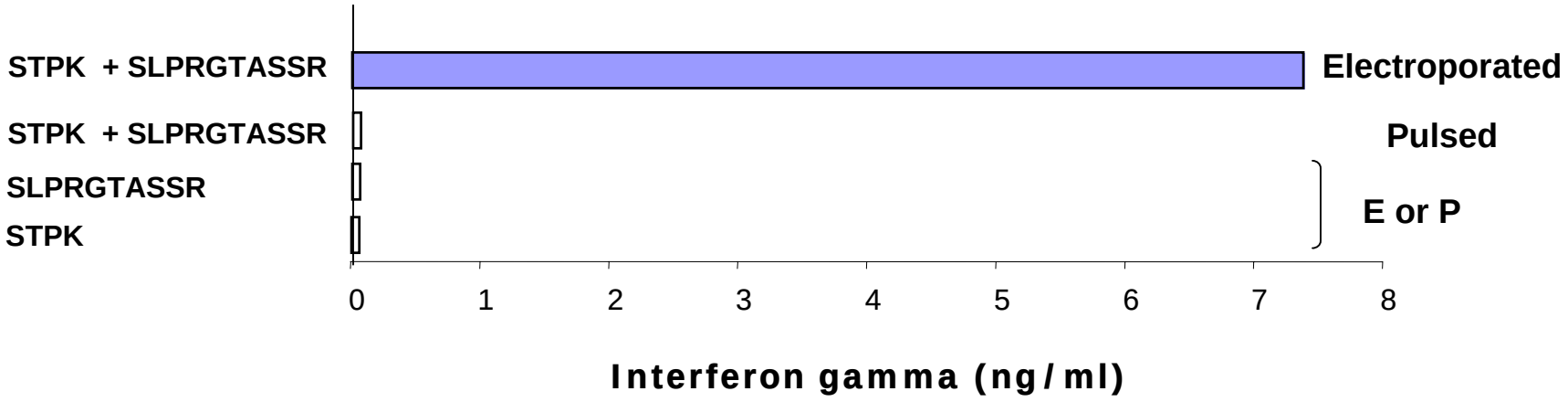
www.sciencemag.org SCIENCE VOL 304 23 APRIL 2004

Does the Proteasome Generate The SP110 Epitope?



Is the SP110 epitope a product of peptide splicing?

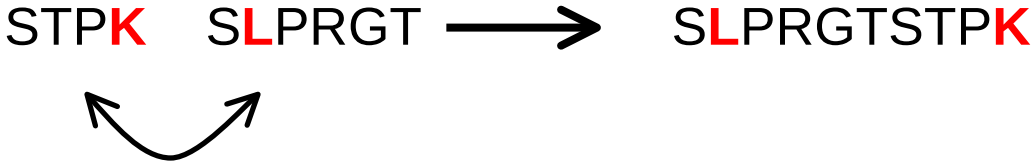
STPKRRHKKKSLPRGTASSR



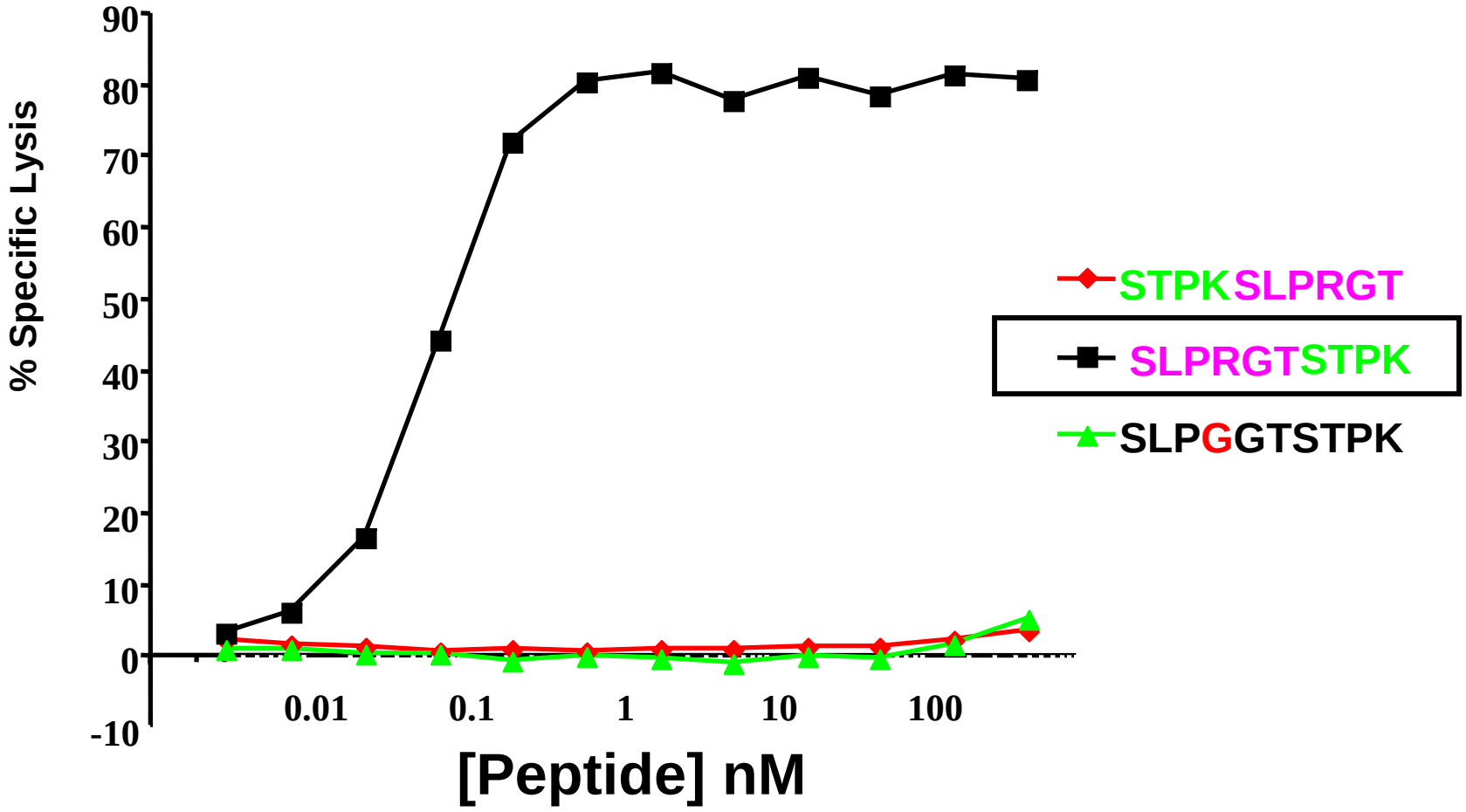
But ---- STPKSLPRGT did not senstitize target cells for recognition

HLA A3 binding motif

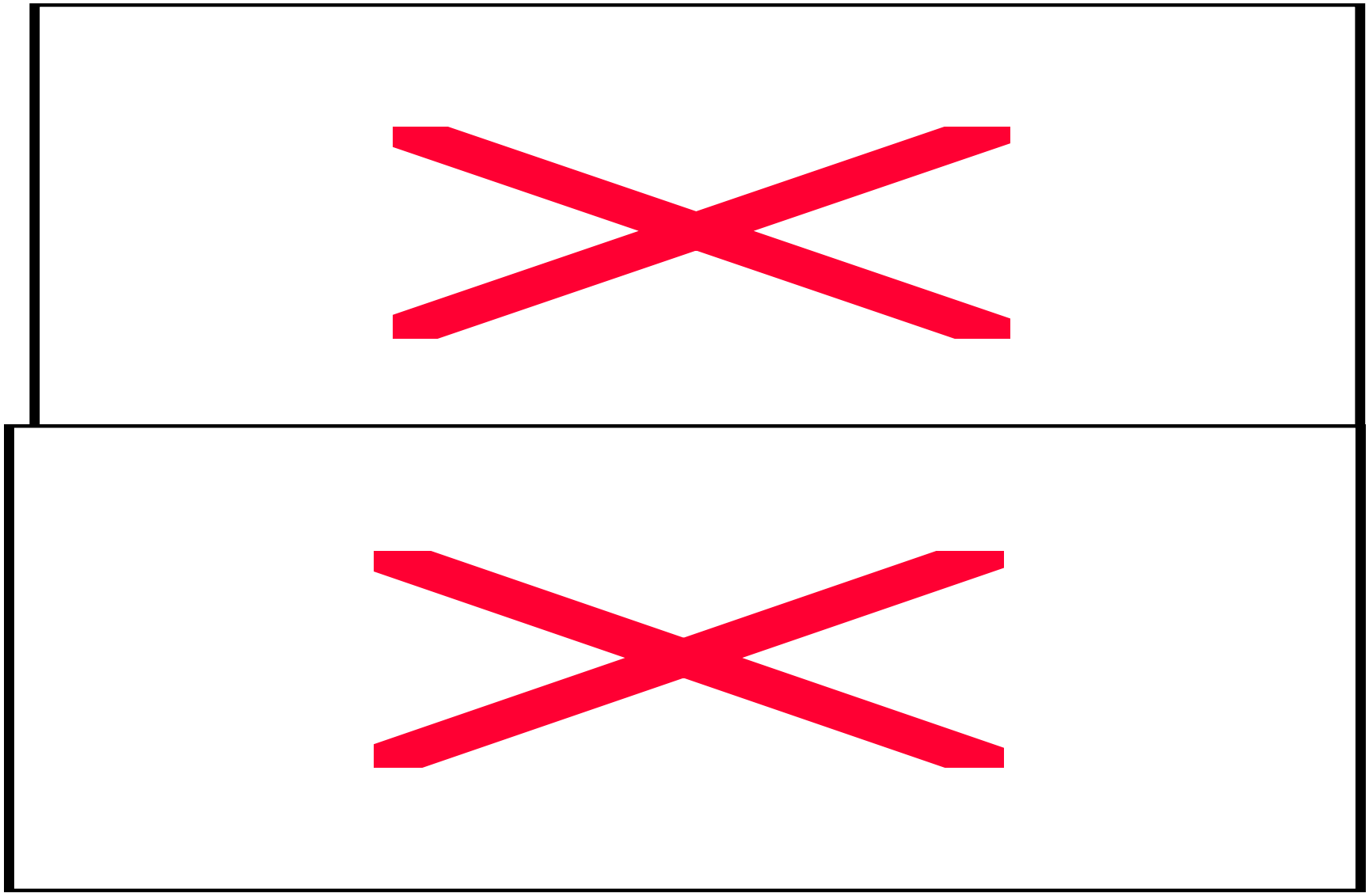
_ L _ _ _ _ _ _ _ K



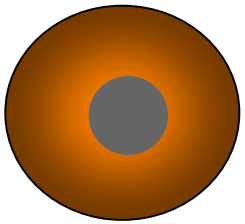
Peptide Splicing With A Twist -- Rearrangement of SP110 Encoded Peptides Creates The DRN-7 Epitope



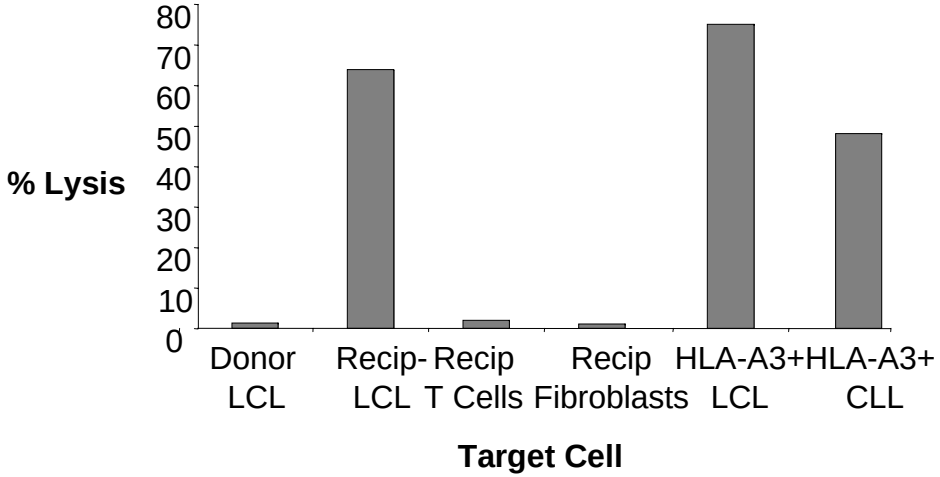
The Putative DRN-7 Epitope SLPRGTSTPK Co-elutes With The Naturally Processed Epitope



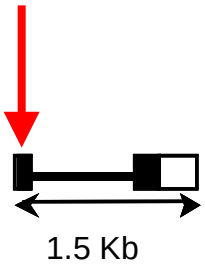
PANE-1 transcript k -- a B-lineage minor H antigen

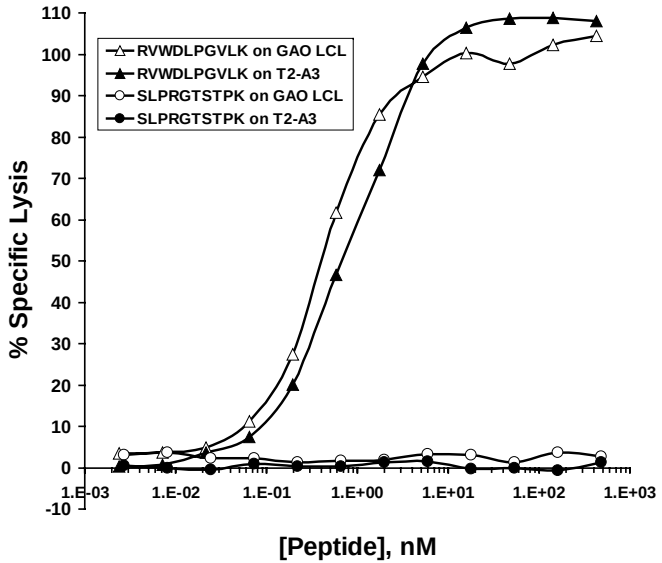


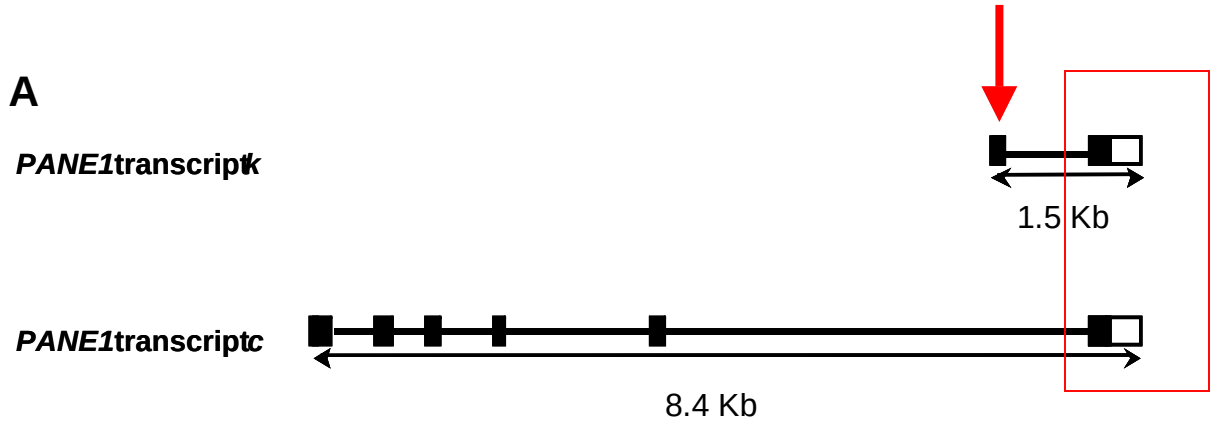
Clone KSN-7A7



PANE-1 transcript k

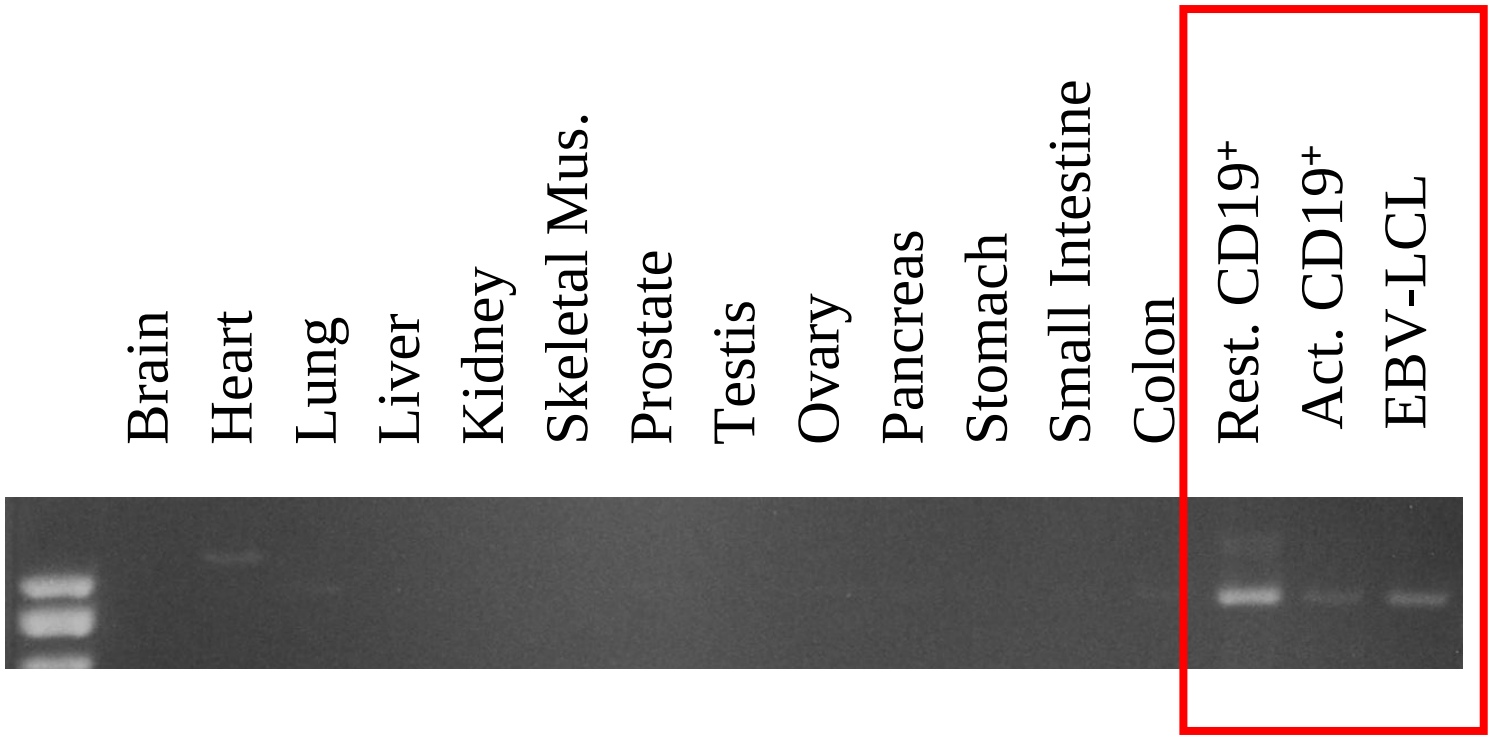


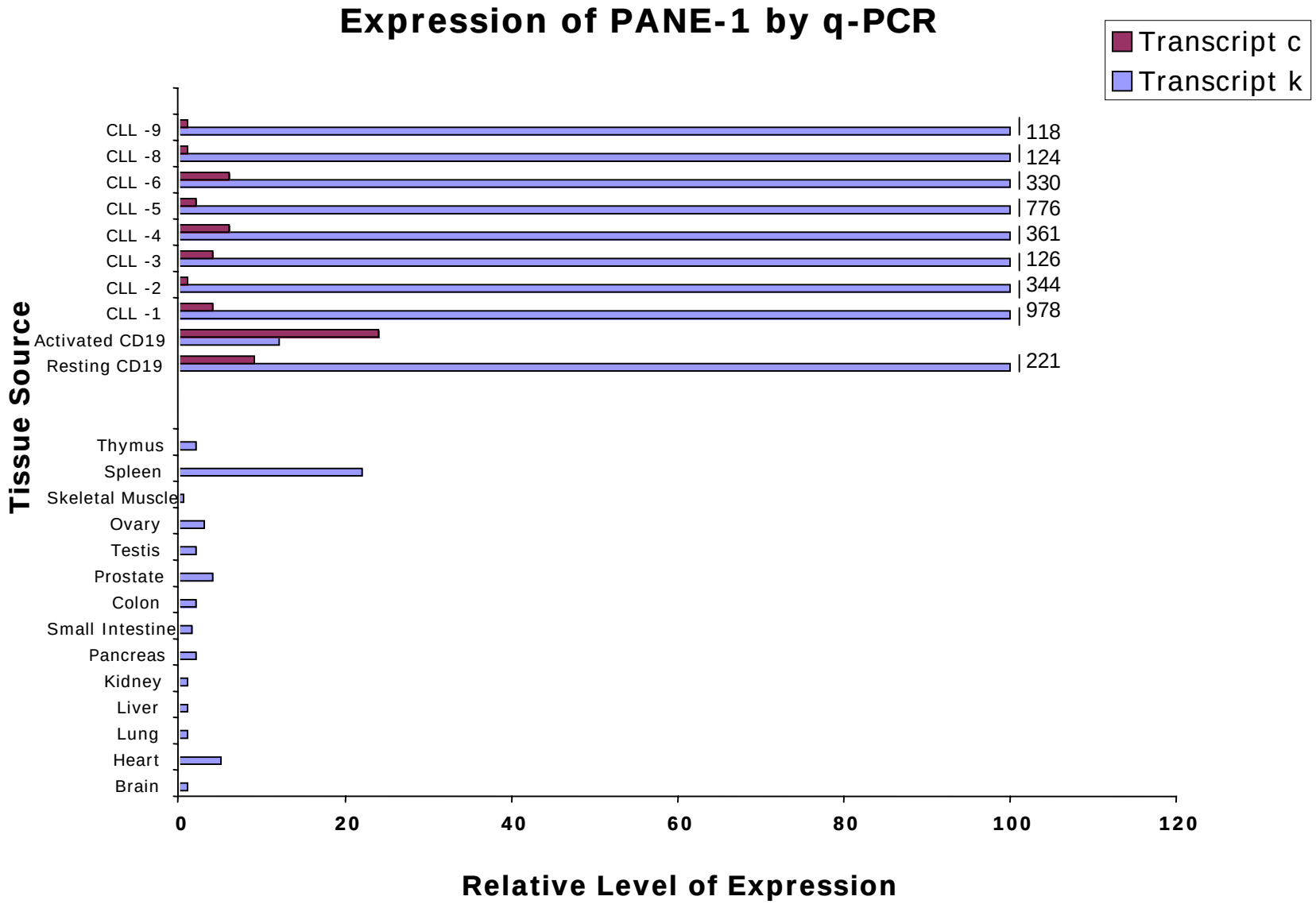





```
                                                        M  G  R  V  W  D  L  P  G
aggcaggccacggagatacctcgtggaaggaggaacagcaagtgcATGGGCCGAGTGTGGGACTTGCCTGGT
 V  L  K                                                 T
GTGCTCAAGgtctggcaggcaggcctgggtggctgcagcacaatagcaaagggcgggttggggtggagaaat
caggaatggtggggctggatcagtggagcctggtggggagctgctggagggcttggggccagggagggatgt
ggcctgacttgggttttaaatggacccctctggctgccgggaggagagtggactgaagggaaactagggcag
aaatagagagacaggtctggaggctctttctttctttctttctttctttctttttctttctttctttctttc
tttctttctttctttctttttctttctttctttctttctttttctttctttttctttctttttctttctttt
tctttctttttgagacagacactgggcagcatcgtgttcttccagcggacgtgtctagaacatctgtgccc
gggcctagagcagtcttgtccctctctggccccacagctcccctgggcccattccttttgatggcaccttc
ttatctgtggtgagggccacccgctgcccctccctccttcctatccctgtatcttctgctccctggtcttga
gcatctggggcccgagctgcctgggcattggttggaagtagccctgattcagagctcagctctggagtcact
agctgggtgaccctagacagtttgttcgacctagccagccttgttaagccttcatttcctcttgagcaggag
gtcaggctctggatgtctccggaggttgtttgaaggattgggcaagctgaggcagttggaagctactctgag
gtgtggcatgaatgagggcgtggctgtggtgggcagtggtgacctgagctacccccaagtatccgcccccag
ccctgctctggcagcccctgcagctgctctgggtaagggagcagggccagcattctatctcactgctgttct
                V  E  G  F  R  A  T  M  A  Q  R  L  V  R  V  L  Q  I  C
ctgctctccccgcaggtGGAAGGCTTTAGGGCCACCATGGCGCAGCGCCTGGTGCGCGTGCTGCAGATCTGT
 A  G  H  V  P  G  V  S  A  L  N  L  L  S  L  L  R  S  S  E  G  P  S  L
GCTGGCCACGTGCCCGGTGTCTCAGCTCTGAACCTGCTGTCCCTGCTGAGAAGCTCTGAGGGCCCCTCCCTG
 E  D  L  *
GAGGACCTGTGAGGGTGGCTGGCCCCTGGGCTGCCCCTTCTCATGGCTTCGTGCTGACTCCATAAACATTCT
CTGTTGAGGATGTCCAGTCAGGGCTTGACAGGCCCAGGCTCAGCCCGCCGTGGCTGGGAAGGTTCCCTGCAG
TGCCAGTGCTGCAGCAGGGAGAGCTGGGCAGAAGCAGCGAGGGGGCCCAGCTGGCGAGACTGTAGCCCCCTC
CCACTCCCACACTCACTCTTGCAGAGCCTGTGTCTTTAAGCAGCTGGCGTGTTACATCTCCATTTAAGGTTT
CCTTTGAACAAAAGGTCTGTGGCTAAAAAAAGTTTAAAAATCaaaaaaaaaaaaaaaaaaaaaaaaaa
```

PANE-1 is selectively expressed in B-lineage cells



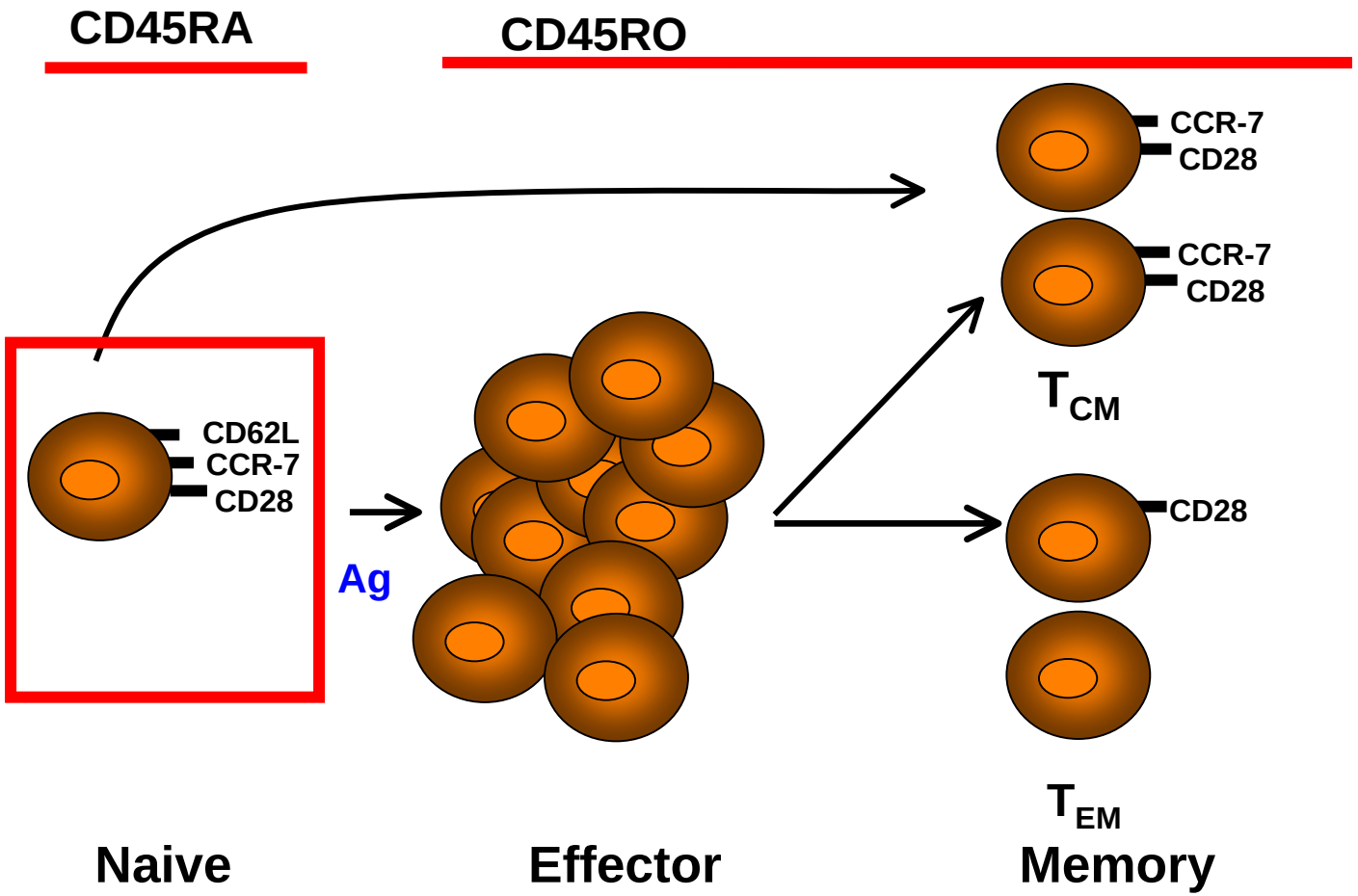

Expression of PANE-1 by q-PCR
Transcript c
Transcript k
Tissue Source
CLL -9
CLL -8
CLL -6
CLL -5
CLL -4
CLL -3
CLL -2
CLL -1
Activated CD19
Resting CD19
Thymus
Spleen
Skeletal Muscle
Ovary
Testis
Prostate
Colon
Small Intestine
Pancreas
Kidney
Liver
Lung
Heart
Brain
118
124
330
776
361
126
344
978
221
0
20
40
60
80
100
120
Relative Level of Expression

Molecularly Characterized Minor Histocompatibility Antigens

Minor H antigen	HLA restriction	Gene/chromosome	Peptide sequence	Tissue distribution	Identification technique [1]
HA-1[25]	HLA A201	KIAA0223/19 p13	VLHDDLLEA	Hematopoietic	HPLC with mass spectometry
HA-1[26]	HLA B60	KIAA0223/19 p13 Chromosome	KECVLHDDL	Hematopoietic	Polymorphic peptide screening
HA-2[27,28]	HLA A201	MYOG1/7	YIGEVLSV	Hematopoietic	HPLC with mass spectometry
HA-3[29]	HLA A1	Lbc oncogene/15q24-25	VTEPGTAQY	Ubiquitous	HPLC with mass spectometry
HA-8[30]	HLA A201	KIAA0020/9	RTLDKVLEV	Ubiquitous	HPLC with mass spectometry
HB-1[31,32]	HLA B44	Chromosome 5q32	EEKRGSLHVW	Hematopoietic esp B cell leukemias	cDNA expression cloning
UGT2B17[33]	HLA 2902 HLA B4403	UGT2B17/4q13	AELLNIPFLY	Ubiquitous	cDNA expression cloning
BCL2A1[34]	HLA A24	BCL2A1/15q2 4.3	DYLQYVKQI	Hematopoietic	Genetic linkage analysis
BCL2A1[34]	HLA B4403	BCL2A1/15q2 4.3	KEFEDDIINW	Hematopoietic	Genetic linkage analysis
HY B7 (SMCY)[35]	HLA B702	SMCY	SPSVDKARAEL	Ubiquitous	HPLC with mass spectometry
HY A2 (SMCY)[36]	HLA A201	SMCY	FIDSYICQV	Ubiquitous	HPLC with mass spectometry
HY A1 (DFFRY)[37]	HLA A101	DFFRY	IVDCLTEMY	Ubiquitous	HPLC with mass spectometry
HY B60 (UTY)[38]	HLA B60	UTY	RESEESVSL	Ubiquitous	cDNA expression cloning
HY B8 (UTY)[39] HY A2 (UTY)	HLA B8 HLA A2 HLA A2	UTY UTY UTY	LPHNHTDL YLQQNTHTL LLIADNPQL	Ubiquitous, High levels in AML	cDNA expression cloning
HY DQ5 (DBY)[40]	HLA DQ5	DBY	HIENFSDIDMGE	Ubiquitous	cDNA expression cloning
HY DRB3[41]	HLA DRB3	RPS4Y	VIKVNDTVQI	Not reported	cDNA expression cloning
C22orf18	HLA A3	C22orf18	RDWDLPGVLK	B-cell (CLL)	HPLC/mass spectrometry
SP110	HLA A3	SP110	SLPRGTSTPK**	Hematopoieitic, ifn inducible	CDNA expression cloning
LYSE 95-4- F4	HLA A2	LYSE 95-4- F4	SNP (L→H)	Not determined (Renal cell Ca)	Genetic linkage analysis

- **GVHD is common after unmodified allogeneic HCT requiring the administration of immunosuppression, poor platform for immunotherapy to augment GVL effect**

Part 3 - A Naïve Answer (?)



Memory CD4$^+$ T cells do not induce graft-versus-host disease

See the related Commentary beginning on page 25.

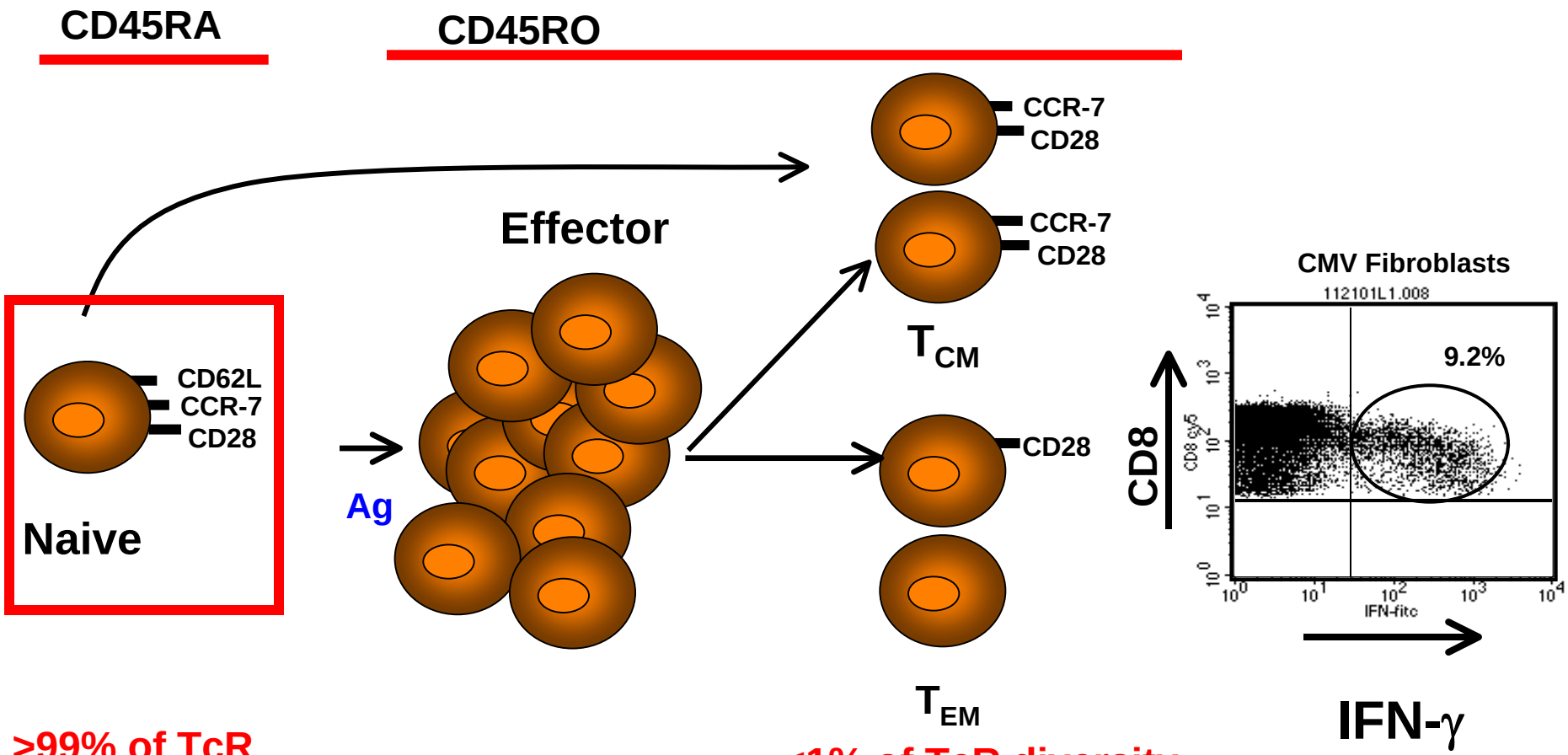
Britt E. Anderson,[1] Jennifer McNiff,[2] Jun Yan,[3] Hester Doyle,[3] Mark Mamula,[3] Mark J. Shlomchik,[1,4] and Warren D. Shlomchik[1,5]

[1]Section of Immunobiology,
[2]Department of Dermatology,
[3]Section of Rheumatology,
[4]Department of Laboratory Medicine, and
[5]Section of Medical Oncology, Yale University School of Medicine, New Haven, Connecticut, USA

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic stem cell transplantation (alloSCT). Donor T cells that accompany stem cell grafts cause GVHD by attacking recipient tissues; therefore, all patients receive GVHD prophylaxis by depletion of T cells from the allograft or through immunosuppressant drugs. In addition to providing a graft-versus-leukemia effect, donor T cells are critical for reconstituting T cell–mediated immunity. Ideally, immunity to infectious agents would be transferred from donor to host without GVHD. Most donors have been exposed to common pathogens and have an increased precursor frequency of memory T cells against pathogenic antigens. We therefore asked whether memory CD62L$^-$CD44$^+$ CD4$^+$ T cells would induce less GVHD than unfractionated or naive CD4$^+$ T cells. Strikingly, we found that memory CD4 cells induced neither clinical nor histologic GVHD. This effect was not due to the increased number of CD4$^+$CD25$^+$ regulatory T cells found in the CD62L$^-$CD44$^+$ fraction because memory T cells depletion of these cells did not cause GVHD. Memory CD4 cells engrafted and responded to antigen both in vivo and in vitro. If these murine results are applicable to human alloSCT, selective administration of memory T cells could greatly improve post-transplant immune reconstitution.

J. Clin. Invest. **112**:101–108 (2003). doi:10.1172/JCI200317601.

Chen B et al. Blood 2004
Xystrakis E. et al Eur J Immunol 2004
Zhang Y et al J Immunol 2005



>99% of TcR diversity (Arstila et al. Science, 1999)

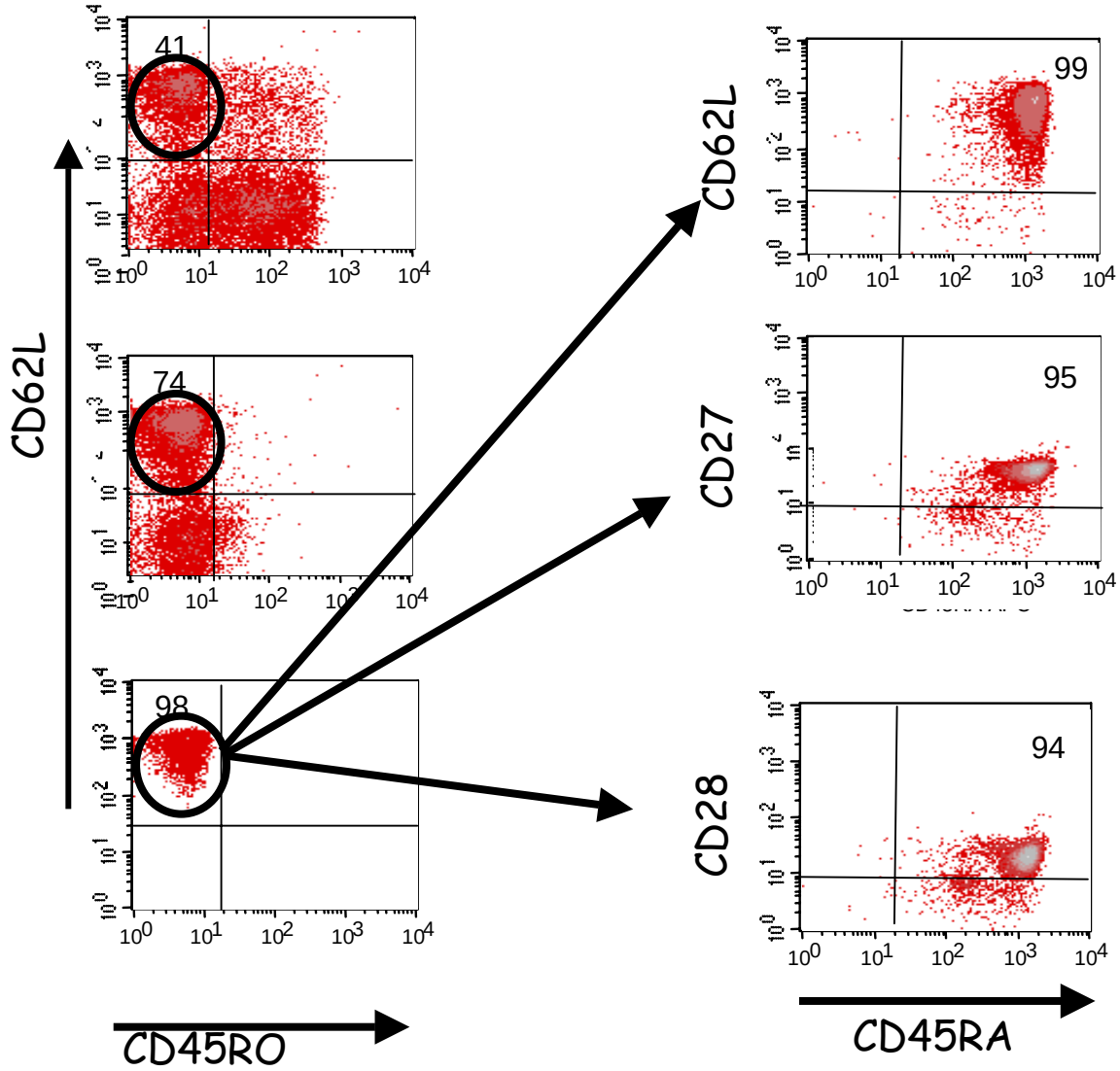
<1% of TcR diversity pathogen-specific (CMV, EBV, HSV)

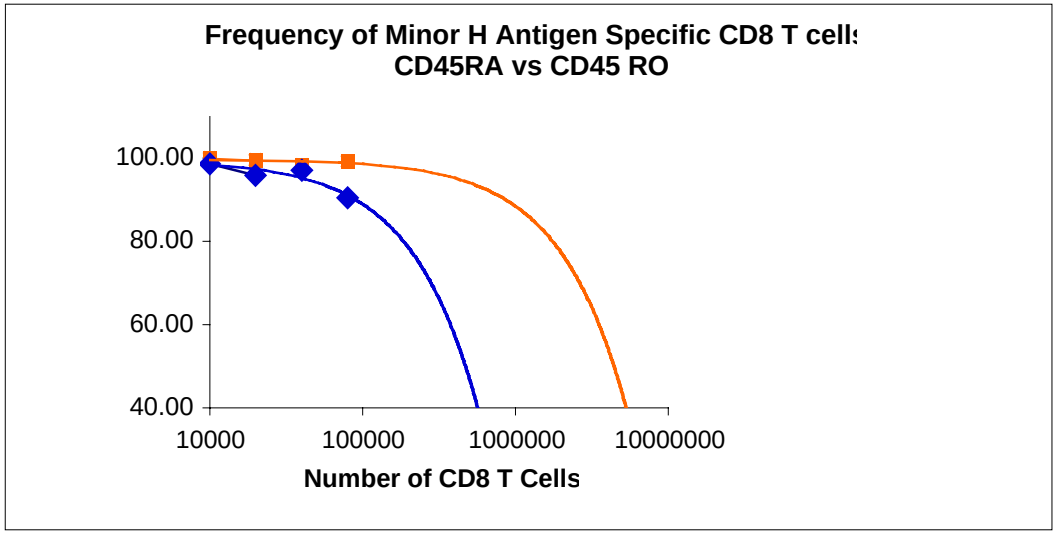
In the absence of priming of the donor to alloantigens, the overwhelming majority of alloreactivity should reside in the naïve T cell pool

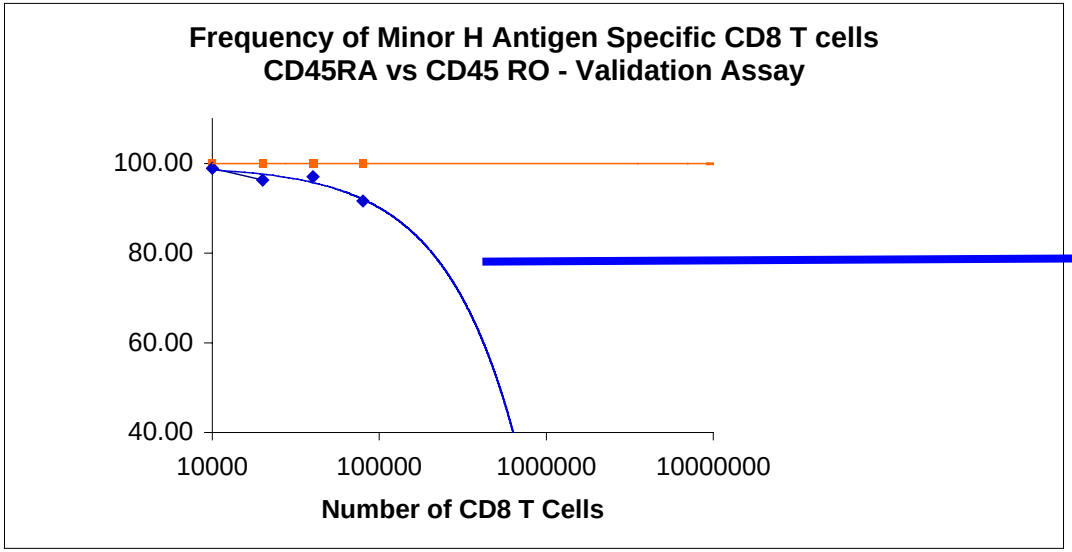
Direct Analysis of Minor H Antigen Alloreactivity In Naïve and Memory Subsets of $CD8^+$ T Cells

- **5 HLA identical sibling pairs**
- **Purified naïve (Tn -- CD45RA, CD62L+) and memory (Tm -- CD45RO) T cells**
- **Limiting dilution assay using purified Tn and Tm cells as responder cells, recipient dendritic cells as the APC, and IL12/IL15**
- **Recipient and donor DC or CD40L B cells as target cells, validation of positive wells**

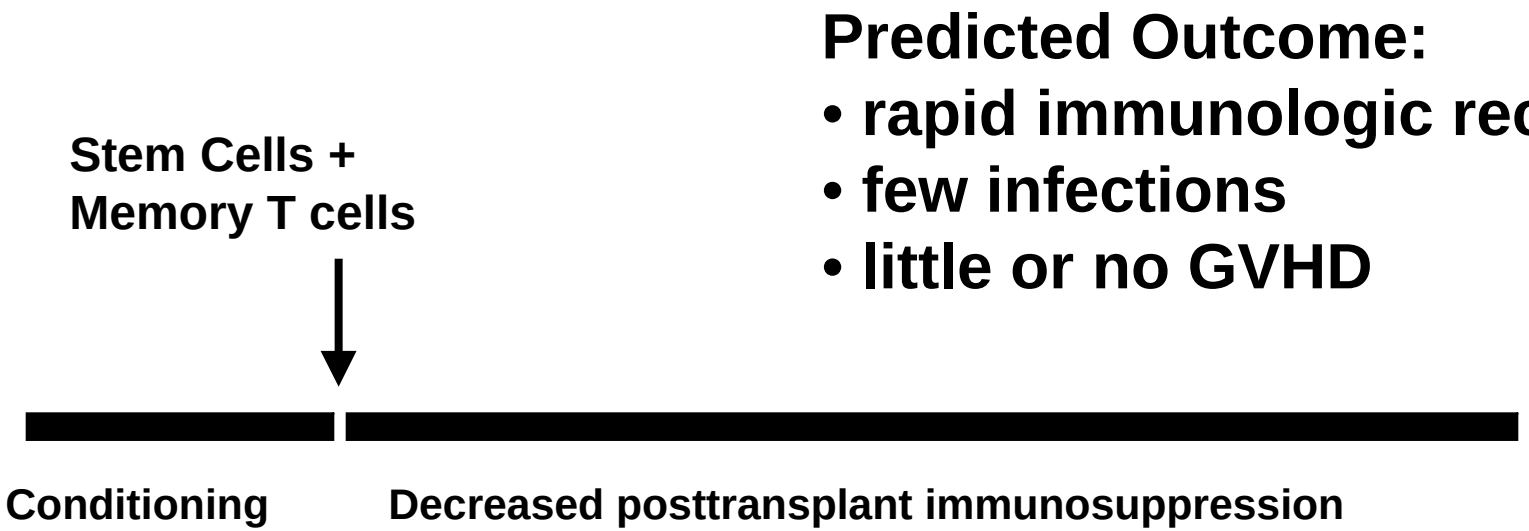
Naïve T Cell Purification







- **T cell clones specific for broadly expressed and lineage restricted minor H antigens**



How to restore the GVL effect?

- improved platform for immunotherapy
- vaccinate donors to elicit lineage restricted minor H antigen specific memory T cells

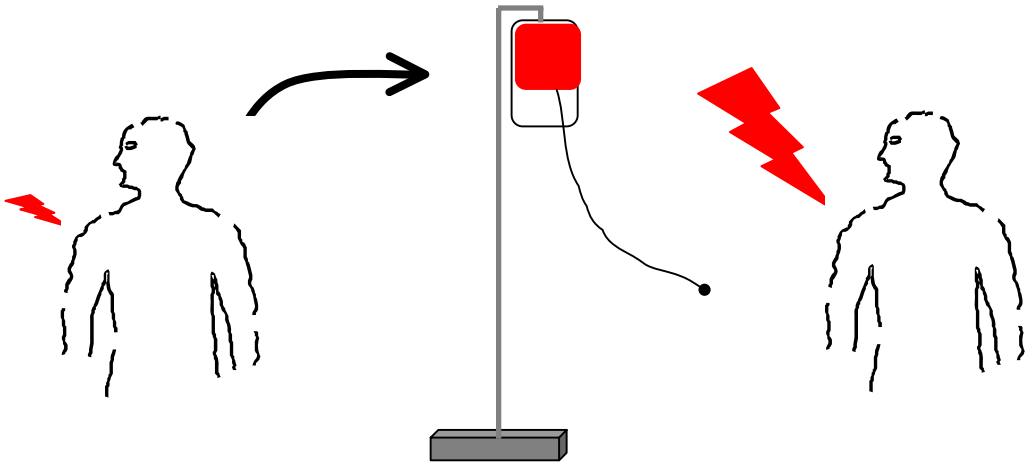
OR

- T cells specific for leukemia associated or minor H antigens isolated from naïve donor T cells could be adoptively transferred

Pilot trial of naïve T cell depletion to reduce GvHD

- HLA id siblings (acute leukemia, donor parity/transfusion hx)
- high stem cell dose
- CD34 selection, CD62L depletion (removes all naive and central memory cells) -- administer defined dose of memory T cells
- FK506/MTX initially, if reduction in GvHD, test FK506 alone (MTX may inhibit homeostatic proliferation of memory T cells and delay immune reconsititution to pathogens)
- End points
  - **engraftment**
  - relapse
  - infections
  - **GVHD**
  - immune reconstitution (CMV, EBV, HSV, aspergillus)

- Genotyping for relevant minor H antigens based on HLA type
- Vaccination of the donor to one or more antigens
- Preparation of stem cell graft (naïve T cell depletion)
- Vaccination of recipient to boost leukemia associated minor H antigen-specific T cell responses



Donor

Recipient

Edus H. Warren
Marc Gavin
Jeff Mito
Michele Brown

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Nathalie Vigneron
Benoit van den Eynde
(Ludwig Institute, Brussels)

Tony Brickner
Vic Engelhard
(University of Virginia)

Marie Bleakley
Audrey Mollerup
Thomas Manley
Larry Anderson
Tory Yamamoto

Warren Shlomchik
(Yale University)

Tetsuya Nishida