# The Clonal Repertoire of Insertional Vector Systems

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**Issues:**

Where do our vectors go in the genome?

- What do we know so far?
- How can we improve (genomic) access?
- Why?

New vectors, the same (old) questions

(e.g. ZFN, Transposases)

- On Site
- Off Site
- Cell Fate

New Applications

- Cancer Clonality
- Pretransplantation Diagnosis

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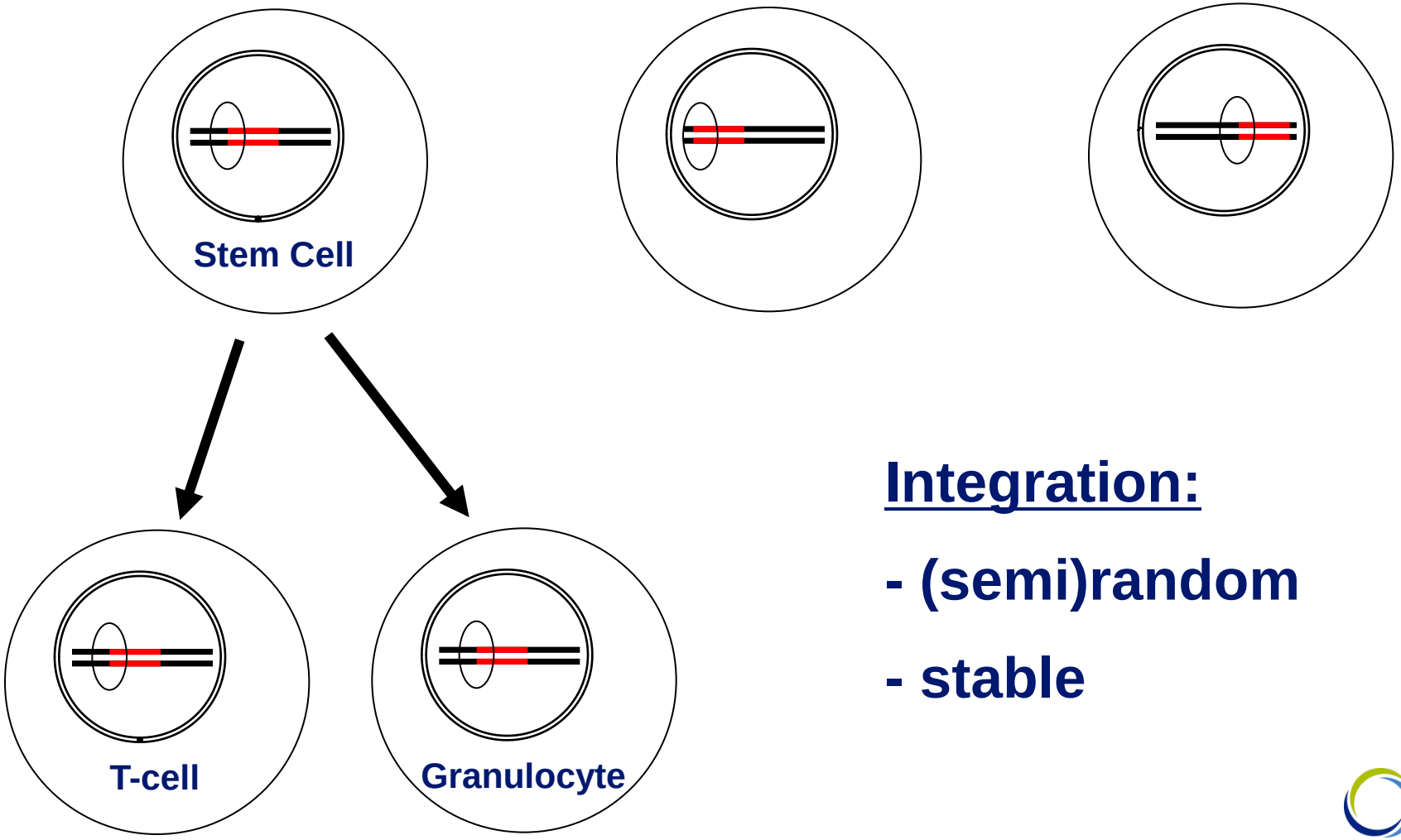
# Clonal Marking by Integrating Vectors

## - Dissect the Clonal Composition in Gene-Modified Hematopoiesis and Follow Cellular Fate -




**<u>Integration:</u>**

**- (semi)random**

**- stable**

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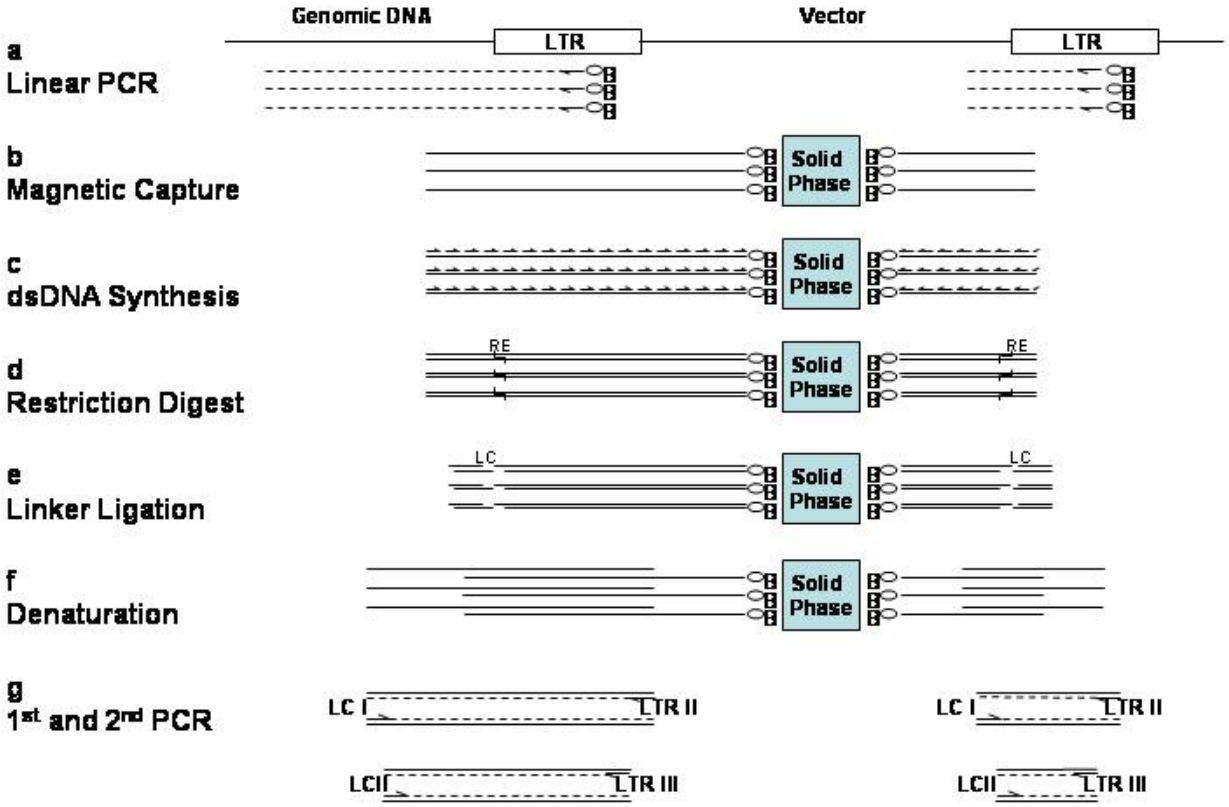
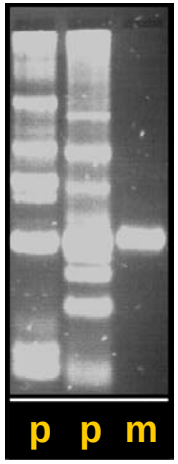
# Integration Site Analysis by LAM-PCR



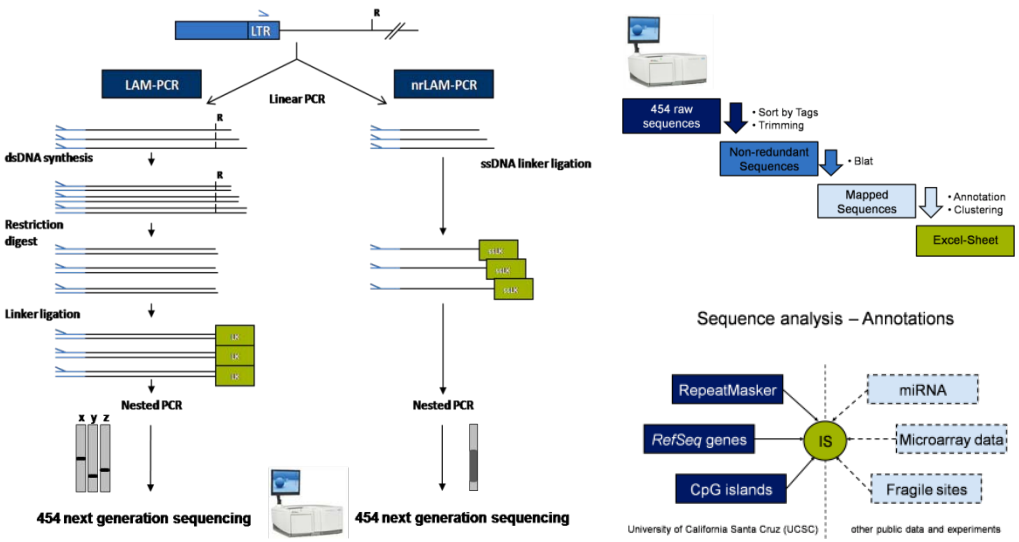

Schmidt *et al*., Nat Methods 2007

# High Throughput Sequencing for Cancer Diagnosis and Genome Wide Mutation Analysis

- Retrovirus and Lentivirus Vectors
- Hematopoietic Clonality Analysis
- Site Specific Modification by Zinc Finger Vectors
- Mechanism of Radiation Toxicity
- Chemotherapeutic Damage and Repair
- Genetic Instability in Cancer

## NCT – Mutagenesis Screening

LAM-PCR, 454 Pyrosequencing and Bioinformatics




## Kalle Lab selected Publications – Genome wide Mutation Analysis


Botzug, K *et al.,*
**N Engl J Med**. 2010 Nov 11;363(20):1918-1927.

Paruzynski, A *et al.,*
**Nature Protocols** 2010 Aug;5(8):1379-95.

International network of cancer genome projects.
**Nature**. 2010 Apr 15;464(7291):993-8.

Stein, M *et al.*,
**Nature Medicine** 2010 Feb;16(2):198-204.

Cartier N *et al.*,
**Science**. 2009 Nov 6;326(5954):818-23.

Gabriel R* *et al.*,
**Nature Medicine** 2009 Dec;15(12):1431-6.

Mitsuyasu RT *et al.*,
**Nature Medicine** Mar;15(3):285-92.

Howe SJ *et al.*,
**J Clin Invest**. 2008 Sep;118(9):3143-50.

Schmidt M *et al.*,
**Nature Methods.** 2007 Dec;4(12):1051-7.

Schwarzwaelder K, *et al.*,
**J Clin Invest.** 2007 Aug;117(8):2241-9.

Deichmann A, *et al.*,
**J Clin Invest.** 2007 Aug;117(8):2225-32.

Montini E, *et al.*,
**Nature Biotechnol**. 2006 Jun;24(6):687-96.

Hajitou A, *et al.*,
**Cell**. 2006 Apr 21;125(2):385-98.

Ott MG*, *et al.*,
**Nature Med**. 2006 Apr;12(4):401-9.

Yáñez-Muñoz RJ, *et al.*,
**Nature Med**. 2006 Mar;12(3):348-53.

Hacein-Bey-Abina S*, *et al.*,
**Science**. 2003 Oct 17; 302(5644):415-9.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# Oncoretroviral Clinical Gene Therapy

## Integration Site Patterns could/can Define

- Clonal Distribution in Clinical Gene Therapy

- Side Effects

  - No (or Subtle) Side Effects

    Gaspar *et al.*, Lancet 2004; Deichmann *et al.,* JCI 2007
    Aiuti *et al.,* JCI 2007; Schwarzwaelder *et al.,* JCI 2007
    Aiuti *et al.,* NEJM 2009; Cartier *et al.,* Science 2009

  - Clonal Dominance

    Schmidt *et al*., Nat Medicine 2003; Ott *et al.,* Nat Med 2006;
    Cavazzana-Calvo *et al.,* Nature 2010

  - Leukemia/Malignancies

    Hacein-Bey-Abina *et al.,* Science 2003; Howe *et al.,* JCI 2008
    Hacein-Bey-Abina *et al.,* JCI 2008; Stein *et al.,* Nat Med 2010

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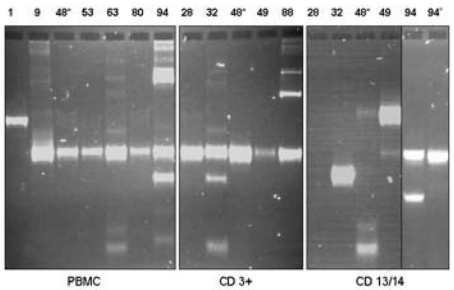
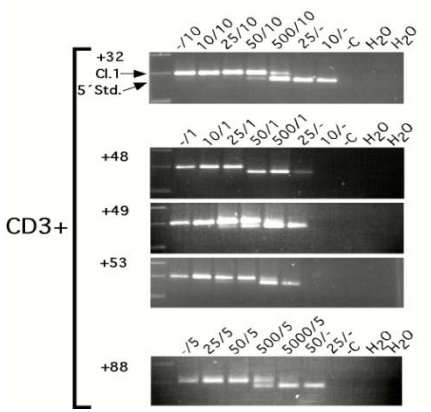
# Limited Clone Numbers Constitute Hematopoiesis in Oncoretroviral ADA-SCID Gene Therapy

**Kohn et al., 1998: Clinical T-cell function not restored**




## Integration Site Analysis

- oligoclonal hematopoietic repopulation in 2 patients
- long-term lymphopoiesis for over 8 years




## QC-PCR

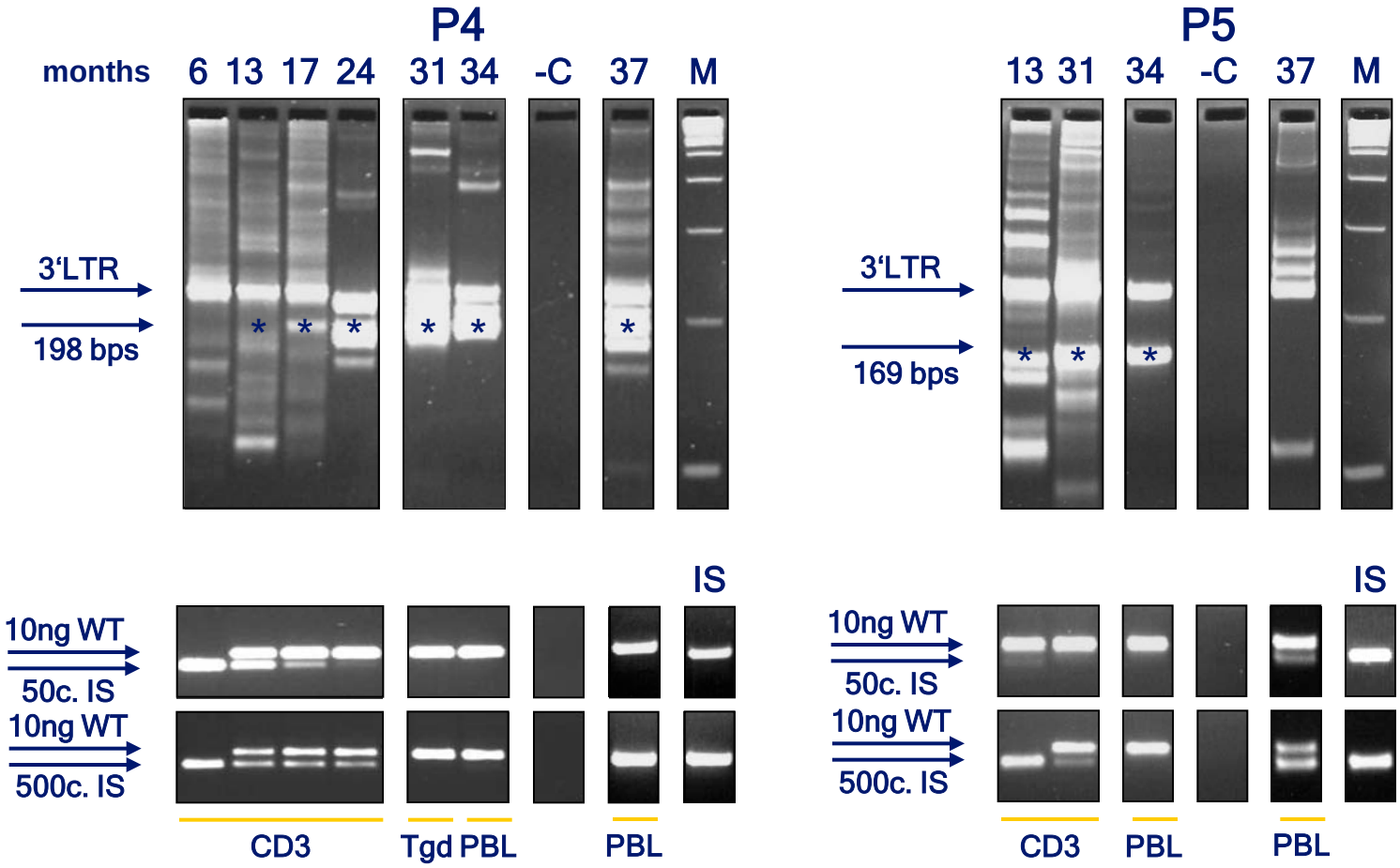
- 1 predominant clone (30 - 100% of transduced T-cell pool, 3-30% of total T cell pool)

➢ Clonal Dominance

Schmidt *et al*., Nat Med 2003

# LMO2-Activation in Oncoretroviral SCID-X1 Gene Therapy

**Cavazzana-Calvo et al., 2000: Clinical T-cell function restored**




➢ Leukemia

Hacein-Bey-Abina *et al*., Science 2003

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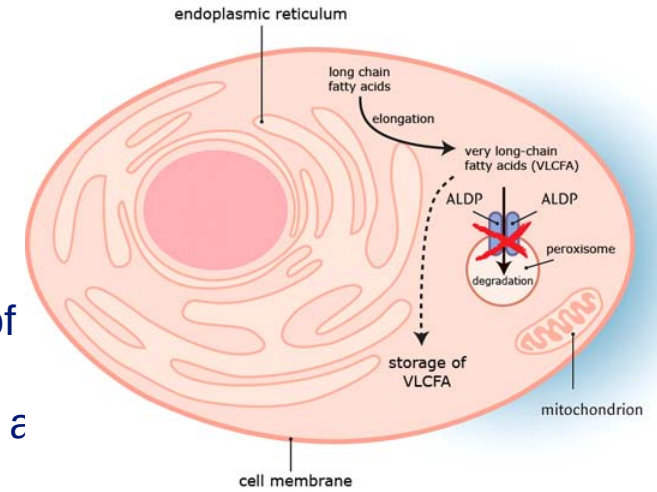
# Lentiviral Integration Site Analysis in two Patients of the X-Adrenoleukodystrophy (X-ALD) Gene Therapy Trial

(Hôpita Saint-Vincent de Paul and INSERM U745, Paris)

Gene mutation: *ABCD1*
ATP-binding cassette transporter
in peroxisomal membrane

Disease: Peroxisomal disorder
Progressive demyelinating disorder of
Adrenal insufficiency
Accumulation of very long chain fatty a




Therapy: Allogeneic hematopoietic stem cell transplantation (HCT) can arrest or even reverse cerebral demyelination

HSC gene therapy: a potential alternative to HCT
No growth selective advantage of normal or corrected cells

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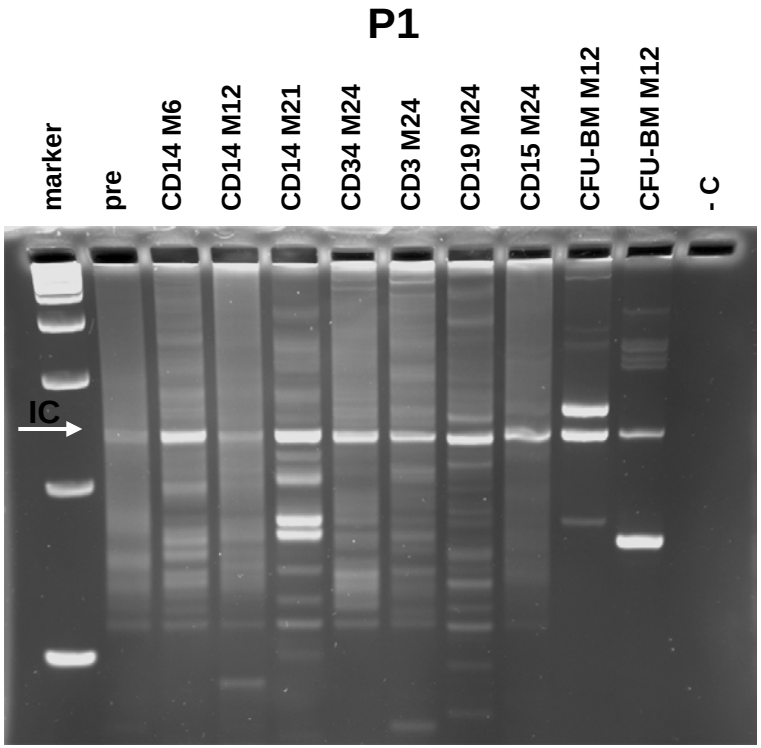
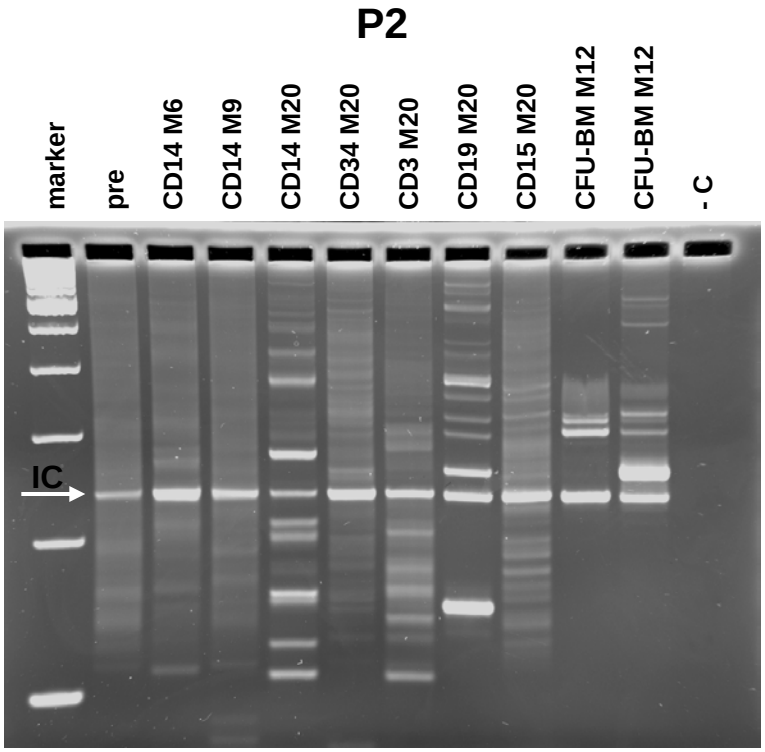
# Polyclonal Hematopoietic Repopulation in Lentiviral ALD Gene Therapy




→ Polyclonal Repopulation: >4000 unique IS

Cartier *et al*., Science 2009

# Identical IS in Myeloid and Lymphoid Lineages

## P1 (86 IS in both lineages)

| Next RefSeq Gene | M6 156 | M9 374 | M12 379 | M17 498 | M21 218 | M24 221 |
|---|---|---|---|---|---|---|
| ADRBK2 | | | | | | |
| ARHGEF3 | | | | | | |
| ARID3A | | | | | | |
| BLID | | | | | | |
| BRE | | | | | | |
| BUB1B | | | | | | |
| C11orf49 | | | | | | |
| C17orf57 | | | | | | |
| C1orf112 | | | | | | |
| C2CD3 | | | | | | |
| CCDC21 | | | | | | |
| CDC25A | | | | | | |
| CFH | | | | | | |
| CNTN5 | | | | | | |
| CRADD | | | | | | |
| ERLIN1 | | | | | | |
| FCHSD2 | | | | | | |
| FRMD8 | | | | | | |
| GLCE | | | | | | |
| GP6 | | | | | | |
| GTF2A1 | | | | | | |
| HIST1H2BC | | | | | | |
| HLA-DMB | | | | | | |
| HOOK1 | | | | | | |
| INPP5A | | | | | | |
| KIAA0776 | | | | | | |
| KIAA1128 | | | | | | |
| KIAA1267 | | | | | | |
| KIAA1303 | | | | | | |
| KIFC1 | | | | | | |
| LOC93349 | | | | | | |
| LRRK2 | | | | | | |
| LYL1 | | | | | | |
| LYPLAL1 | | | | | | |
| MAPK1 | | | | | | |
| MBOAT5 | | | | | | |
| MBTD1 | | | | | | |
| MECP2 | | | | | | |
| MEIS1 | | | | | | |
| MLZE | | | | | | |
| M-RIP | | | | | | |
| MTHFD2L | | | | | | |
| MYO9A | | | | | | |

| Next RefSeq Gene | M6 156 | M9 374 | M12 379 | M17 498 | M21 218 | M24 221 |
|---|---|---|---|---|---|---|
| NKTR | | | | | | |
| NRXN1 | | | | | | |
| NSD1 | | | | | | |
| P4HB | | | | | | |
| PACS1 | | | | | | |
| PKN1 | | | | | | |
| POU2F1 | | | | | | |
| PTPN13 | | | | | | |
| QSOX2 | | | | | | |
| RBM4 | | | | | | |
| RFX1 | | | | | | |
| ROCK2 | | | | | | |
| RPL12 | | | | | | |
| SEC14L1 | | | | | | |
| SLITRK3 | | | | | | |
| SORCS1 | | | | | | |
| SP100 | | | | | | |
| SRP68 | | | | | | |
| STAG2 | | | | | | |
| STAU1 | | | | | | |
| STK17A | | | | | | |
| STXBP5 | | | | | | |
| TACR3 | | | | | | |
| TBC1D16 | | | | | | |
| TEKT1 | | | | | | |
| TMEM87B | | | | | | |
| TNRC6C | | | | | | |
| TRIM39 | | | | | | |
| TYROBP | | | | | | |
| UGT2A3 | | | | | | |
| USP4 | | | | | | |
| USP4 | | | | | | |
| WDR17 | | | | | | |
| WDTC1 | | | | | | |
| XYLT1 | | | | | | |
| ZFAND3 | | | | | | |
| ZFP3 | | | | | | |
| ZNF462 | | | | | | |
| ZNF556 | | | | | | |
| ZNF562 | | | | | | |
| ZNF586 | | | | | | |
| ZNF711 | | | | | | |
| ZNRD1 | | | | | | |

## P2 (12 IS in both lineages)

| Next RefSeq Gene | M6 143 | M9 176 | M12 189 | M16 191 | M20 118 |
|---|---|---|---|---|---|
| ACO1 | | | | | |
| DENND4A | | | | | |
| KIAA1303 | | | | | |
| LRRFIP2 | | | | | |
| MME | | | | | |
| NUCB1 | | | | | |
| OPTN | | | | | |
| SBNO2 | | | | | |
| SEC61A2 | | | | | |
| SREBF2 | | | | | |
| XKR3 | | | | | |
| ZNF780A | | | | | |

→ **Transduction of early progenitor or stem cells**

| | |
|---|---|
| | light blue: IS identified in myeloid cells (CD14 and/or CD15 and/or CFU) |
| | mid blue: IS identified in myeloid and lymphoid cells at the same timepoint |
| | dark blue: IS identified in lymphoid cells (CD3 and/or CD19) |

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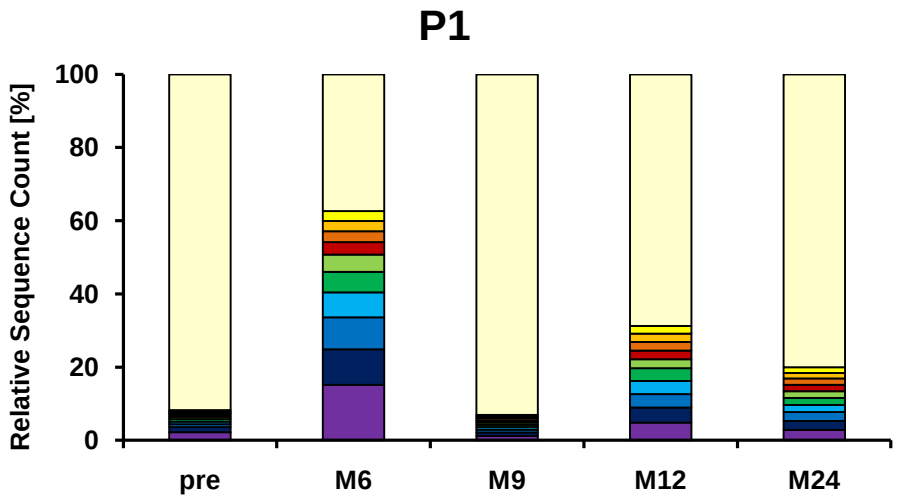
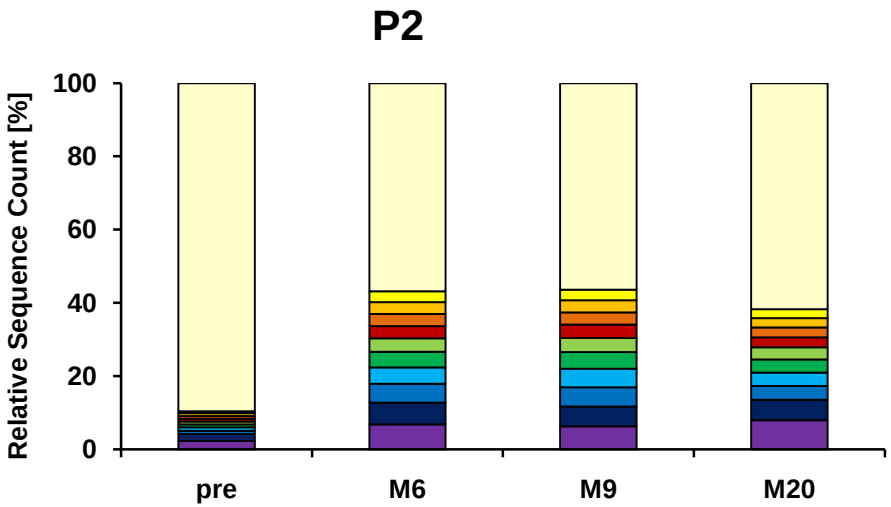
Cartier *et al*., Science 2009

# Heterogenic Hematopoietic Repopulation in ALD Calculating Sequence Counts (Retrieval Frequency)




| | | | | | |
|---|---|---|---|---|---|
| Top 1 | FBXL4 | CAT | C21orf34 | ENSA | TIPIN |
| Top 2 | **P4HB** | CPSF6 | SPPL3 | BTBD2 | TNRC6C (b) |
| Top 3 | TOX | ARHGEF3 | ZNF804A | ICEBERG (b) | ZNF681 |
| Top 4 | WDR25 | EFNA5 | ICEBERG (a) | SNX15 | SLC29A2 |
| Top 5 | GRB14 | PTK2 | KNTC1 | TNRC6B | ZNF586 |
| Top 6 | KLHL1 | NUDCD3 | CDC42 | ARHGEF18 | ZKSCAN3 |
| Top 7 | CDK8 | MED30 | KIAA1394 | GALNT17 | UBAP2L |
| Top 8 | EGFL11 | **MARK2** | **C6orf10** | DYM | VPS13C |
| Top 9 | ERCC6 | AHI1 | **TNRC6C (a)** | SLITRK3 | GABRG1 |
| Top 10 | FLJ25778 | **FBXL11** | TP53 | TBC1D22A | ENOX1 |
| All others* | 451 IS | 84 IS | 349 IS | 156 IS | 229 IS |

| | | | | |
|---|---|---|---|---|
| Top 1 | CCM2 | BCHE | DEFB108B | FBF1 |
| Top 2 | LCMT1 | XPO7 | PBRM1 | C15orf53 |
| Top 3 | GNAI3 | HORMAD2 | TYK2 | PCSK5 |
| Top 4 | SLC12A6 | NFKB1 | **EYA3** | METT5D1 |
| Top 5 | TGFBR2 | C16orf45 | FBXL17 | OGT |
| Top 6 | ERC1 | CSNK1G1 | GALNS | **ANKRD13D** |
| Top 7 | TESK2 | OR4E2 | MME | ZNF527 |
| Top 8 | ZMYM2 | FLJ13611 | CEACAM21 | LINGO2 |
| Top 9 | MARCH3 | GNG7 | CSNK1G1 | MYST2 |
| Top 10 | RAD51L1 | SUFU | UTX | FBXO7 |
| All others* | 443 IS | 65 IS | 128 IS | 107 IS |

IS detected at more than one time point

IS detected in lymphoid and myeloid lineages

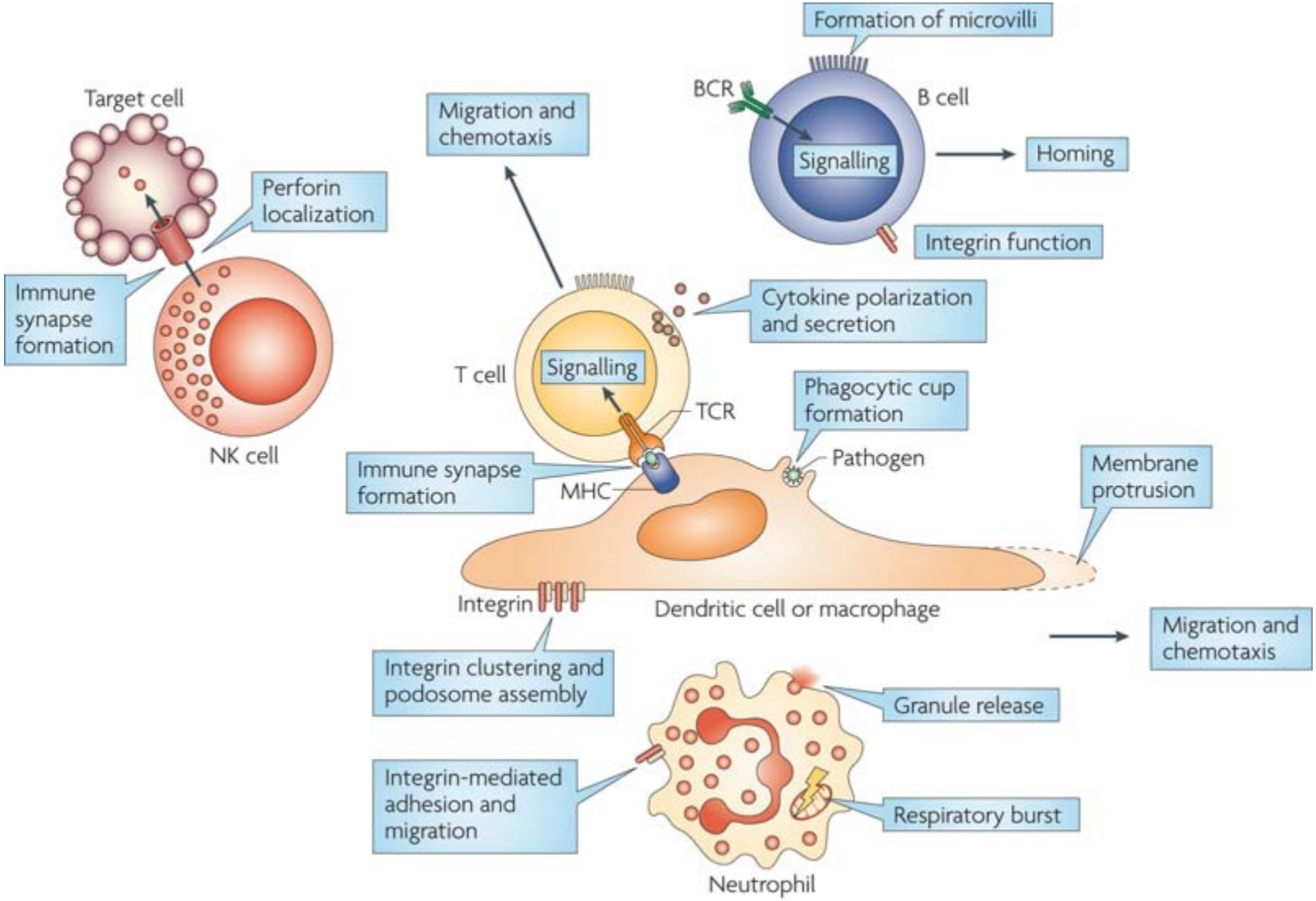
**CIS Gene in both patients**

* clonal contribution < 3.0 %

No Side Effects

Cartier *et al*., Science 2009

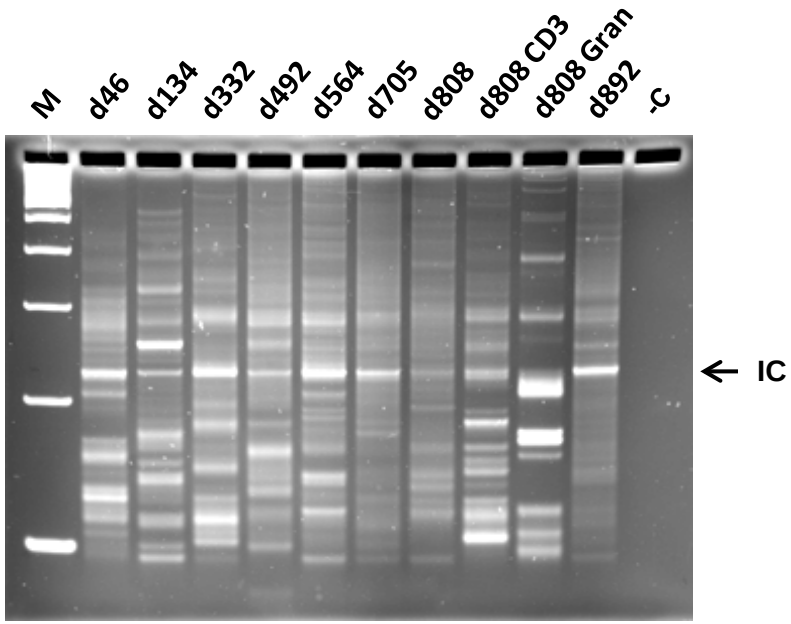
# Wiskott-Aldrich Syndrom (WAS)



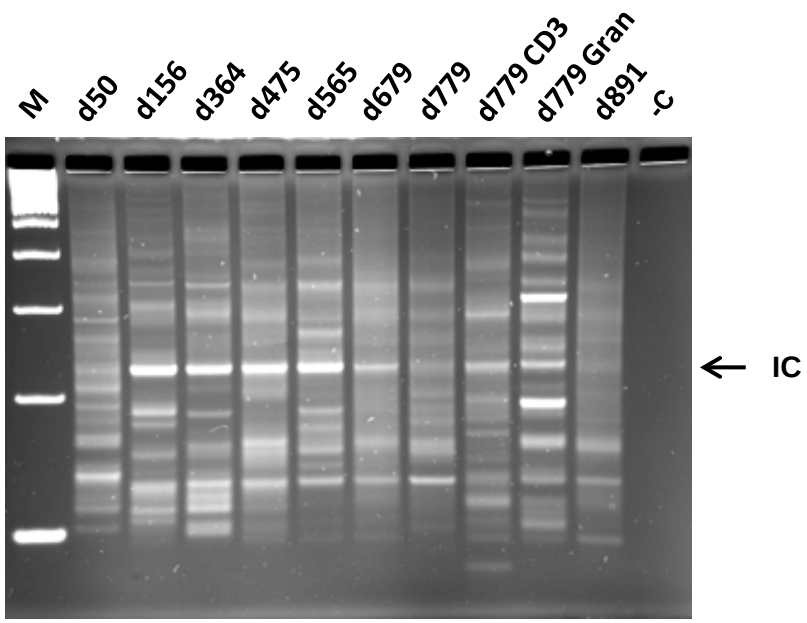

Thrasher and Burns , Nat Rev Immunol. 10, 182-192 (March 2010)

# Polyclonal Reconstitution of the Hematopoetic System in Gammaretroviral WAS Clinical Gene Therapy Trial

**Patient 1**




**Patient 2**




P1: 5709 unique IS

P2: 9538 unique IS

CIS: MDS1-EVI1, PRDM16, SETBP1, CCND2, LMO2

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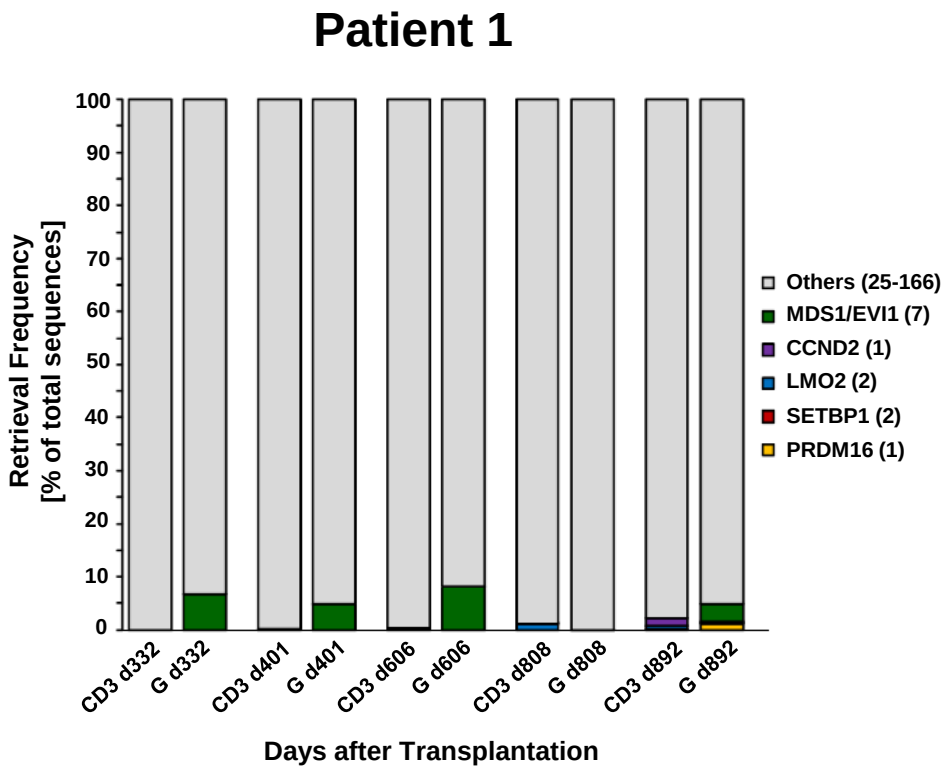
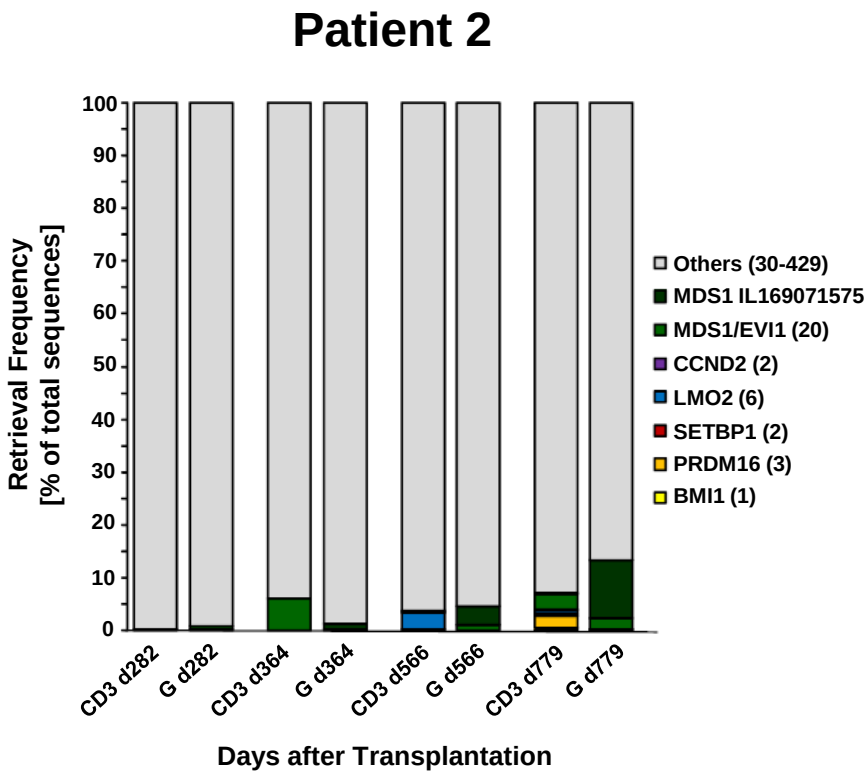
Boztug *et al*., N Engl J Med 2010

# Analyses of Sorted Fractions Revealed a Distinct Integration Site Distribution




*MDS1-EVI1*-, *PRDM16*- and *SETBP1* clones occur predominantly in the myeloid fraction, *LMO2* and *CCND2* clones in the lymphoid fraction.

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Boztug *et al*., N Engl J Med 2010

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Reinhard Seger

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