Vektor Lentivirus

Lentivirus

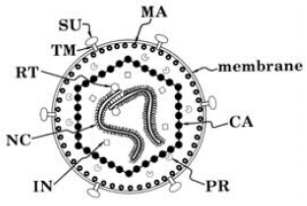
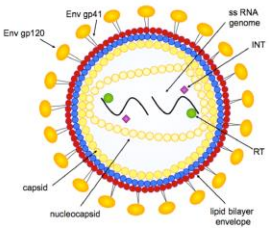


Figure 1.

- i.e. HIV
- Lentivirus-based vectors can efficiently infect nondividing slowly dividing cells
- RNA virus
  - Surrounded by Lipid bilayer + protein

TABLE 1.: Roles and Functions of retroviral proteins

Denotation	Protein Name	Function
CA	Capsid	gag gene; protects the core
IN	Integrase	pol gene; needed for integration of the provirus
MA	Matrix	gag gene; lines envelope
NC	Nuclear Capsid	gag gene; protects the genome and forms the core
PR	Protease	Essential for gag protein cleavage during maturation
RT	Reverse Transcriptase	Reverse transcribes the RNA genome
SU	Surface Glycoprotein	The outer envelope glycoprotein; major virus antigen
TM	Trans Membrane Protein	The inner component of the mature envelope glycoprotein
Accessory Proteins (HIV)	Ex: Nef, Vif, Vpu, Vpr	Play a role for infectivity and pathogenicity of HIV



Lentivirus

- Lentivirus:
  - structural proteins (matrix),
  - viral enzymes and
  - 2 copies of a single stranded RNA genome
    - *gag*, *pol* and *env* genes
      - *gag* gene →the structural matrix proteins, capsid and nucleocapsid,
      - *pol* →the reverse transcriptase (RT), integrase (INT) and protease and
      - *env* →the envelope glycoproteins (gp120 and gp41).
    - accessory genes : Tat, Rev, Vpr, Vpu Vif and Nef.
      - Tat and Rev → for viral replication
        - » Tat
          - a potent trans-activator of HIV-1 gene expression
          - "jump-starts" the HIV replication cycle.
        - » Rev
          - post-transcriptional trans-activator → accelerates mRNA transport from the nucleus to the cytoplasm via binding to the Rev-responsive element (RRE)
          - essential for the expression of the *gag*, *pol* and *env* genes
          - Essensial for the transport of the full length RNA genome.
      - Vpr, Vpu Vif and Nef → for viral pathogenicity

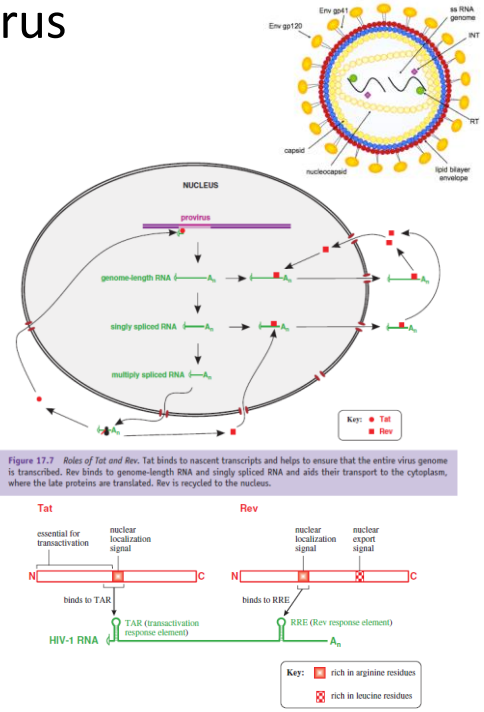


Figure 17.7 *Roles of Tat and Rev.* Tat binds to nascent transcripts and helps to ensure that the entire virus genome is transcribed. Rev binds to genome-length RNA and singly spliced RNA and aids their transport to the cytoplasm, where the late proteins are translated. Rev is recycled to the nucleus.

- Lentiviruses
  - interact with the host cell chromatin
  - do not integrate preferentially into close proximity of transcription start sites but rather
  - integrate into introns in chromosomal regions rich in expressed genes

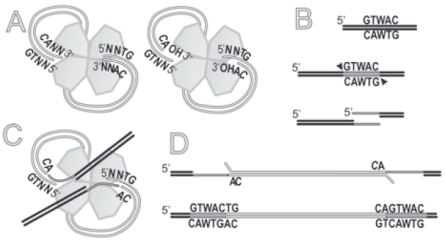
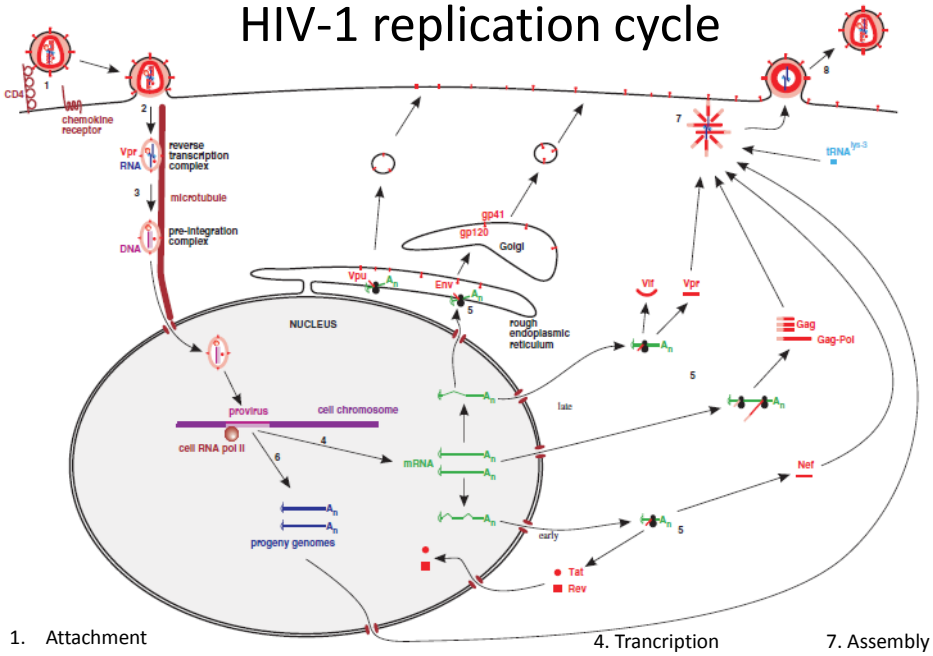


Figure 4. Scheme of provirus integration.
Integration of provirus to the host genome DNA is performed by lentiviral integrase (IN). The enzyme recognizes 5′ and 3′ short terminal fragments called *att*, binds and bends the DNA molecule, removes two nucleotides from each 3′ end (staggered cut) and exposes OH group (*3′ processing*) (**A**). IN cleaves host genomic DNA, creating five-nucleotide-long overhangs (**B**), which then are bound to the free 3′ OH groups of the provirus (*DNA strand transfer*) (**C**). The last step of integration comprises DNA repair of five-nucleotide gaps at both sides of proviral DNA by cellular enzymes (**D**). IN is shown in tetrameric form. GTWAC–HIV-1 target site consensus sequence (W means A or T).

HIV-1 replication cycle



1. Attachment
2. Entry
3. Reverse transcription
4. Trancription
5. Translation
6. Genome replication
7. Assembly
8. Exit

Lentivirus vector

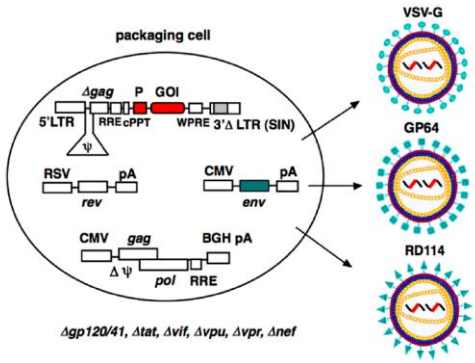
- *ex vivo* or *in vivo* gene transfer into dividing and non-dividing cells.
- transgene up to~10 kb + expressed using an internal promoter.
- Lentiviral vectors can be pseudotyped with distinct viral envelopes that influence vector tropism and transduction efficiency.
- cell-type specific targetable vectors ➔ + cell-type specific ligands or antibodies into the vector envelope.

Vector Design

- lentiviral vectors (LV) :
  - replication-deficient and
  - unable to revert back to an infective wild-type HIV-1

Packaging cells

- Packaging cells ➔ transfected with
  - a plasmid containing a modified vector genome that expresses the gene of interest (transfer vector)
  - The helper (or packaging) plasmids that encode the essential viral proteins *in trans*



Packaging cells are transfected with
- the lentiviral vector plasmid and
- 3 helper (i.e. packaging) constructs encoding Gag, Pol, Rev and Env (e.g. VSV-G, GP64, RD114).

+ Constructs encoding different envelope genes ➔ for the production of distinct LV pseudotypes which exhibit different tropisms.
- self-inactivating (SIN) LTR sequences that contain a partial deletion
- the promoter of interest (P),
- Woodchuck post-transcriptional regulatory element (WPRE), ➔ increase transgene expression
- Central polypurine tract (cPPT),
- the Rous Sarcoma Virus promoter (RSV),
- Cytomegalovirus promoter (CMV),
- Rev-responsive element (RRE) bovine growth hormone polyadenylation signal (BGHpA)

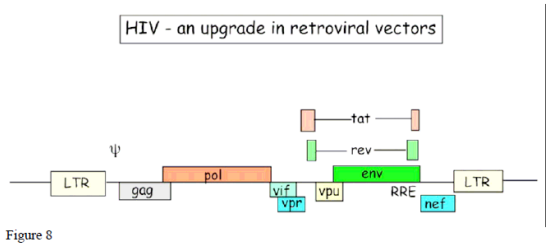


Figure 8

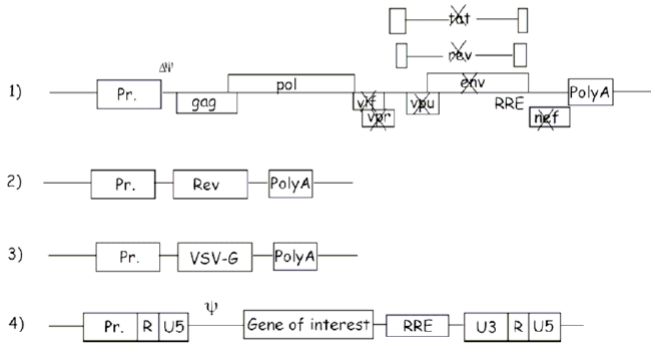
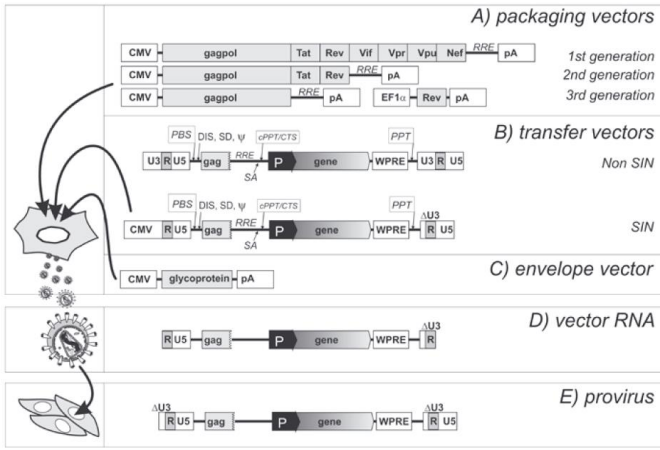


Figure 9



Packaging vector plasmids.
First-generation vector : all regulatory and accessory viral genes expressed from CMV promoter.
Second-generation : vector encodes only Tat and Rev proteins.
Third-generation : consists of two plasmids: one encoding Gag and Gag-pol polyproteins, the second — Rev protein.

Transfer vector plasmids. In non-SIN vectors: viral RNA is expressed from intact 5' LTR. SIN (self-inactivating) vector bears deletion in U3 region (ΔU3), which inactivates transcription of entire viral RNA after provirus integration.

Envelope vector. Depending on glycoprotein used, different viral pseudotypes are formed.

After provirus integration into host cell DNA, transcription from mutated 5' LTR (duplicated 3' ΔU3) is brogated.

Gene Transfer Concepts and Potential Applications

- ***Target Cells and Diseases***
  - LV can also transduce non-dividing cells
  - Stable transduction
  - Systemic administration : efficient and widespread transduction
  - LV are ideally suited to transduce various stem/progenitor cells, particularly HSCs

Gene Transfer Concepts and Potential Applications (cont)

- ***Pseudotyping***
  - Pseudotyping HIV-1 vectors → safety concerns : HIV-1 gp120 →has pathogenic consequences

viral envelope protein used for pseudotyping LV is the vesicular stomatitis virus glycoprotein (VSV-G), other envelopes including rabies, MLV-amphotropic, Ebola, baculovirus and measles virus envelopes

- pseudotyping has a dramatic impact on the biodistribution, vector tropism, and viral particle stability:
  - filovirus envelope-pseudotyped LV enhance transduction of airway epithelia or endothelial cells
  - whereas baculovirus GP64 and hepatitis C E1 and E2 pseudotyping enhances hepatic transduction.
  - RD114 pseudotyping favors transduction in lymphohematopoietic cells.
  - LV pseudotyped with the Edmonston measles virus (MV) glycoproteins H and F allowed efficient transduction through the MV receptors, SLAM and CD46, both present on blood T cells.

Gene Transfer Concepts and Potential Applications (cont)

- ***Cell Type Specific Targeting***
  - Pseudotyping also provides a means to generate cell-type specific LV → redirect the binding of the LV particles to the corresponding cellular receptor

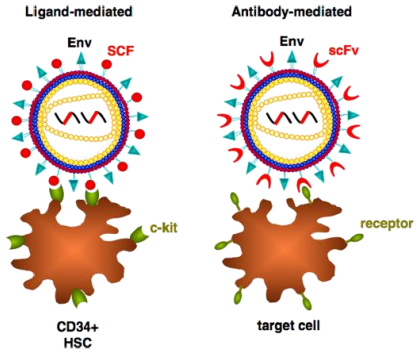
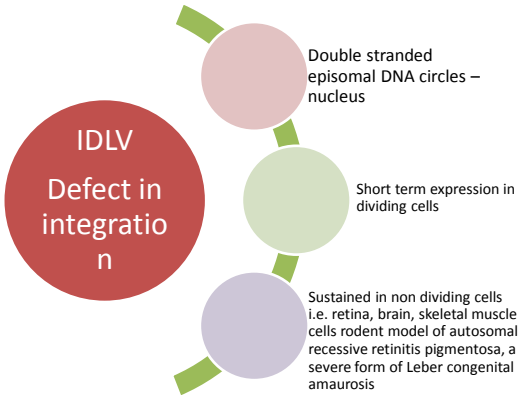


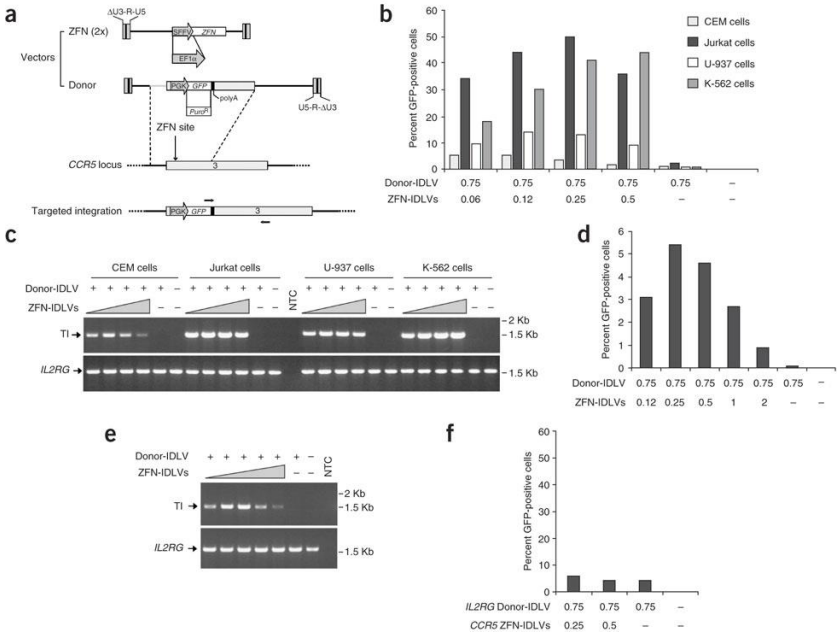
Fig. 4.3 LV targeting into specific cell types. The LV Env protein is amenable to engineering allowing the display of cell type specific ligands or single chain antibody fragment (scFv). These ligands or scFv can then bind onto cell surface receptors that are specifically expressed on the desired target cells. Some of the ligand-receptor interaction may activate the target cells and consequently enhance transduction. Some Env proteins like the amphotropic MLV Env can be engineered to display the stem cell factor (SCF) as ligand for the SCF cellular receptor (i.e. c-kit) allowing enhanced transduction of CD34+ HSC. A mutated version of the RD114 envelope is used as fusogen. An alternative retargeting paradigm is based on the display of scFv on the measles hemagluttinin envelope H protein while the native tropism of this measles Env was ablated. The measles envelope F protein acts as fusogen.

Gene Transfer Concepts and Potential Applications (cont)

- ***Integration-Defective Lentiviral Vectors***
  - functional integrase is required to achieve stable genomic lentiviral integration, it is possible to reduce this integration by mutational inactivation of the integrase protein →IDLV
- integration-defective LV (IDLV) →
  - minimize random integration and insertional oncogenesis.
  - low frequency residual integrations cannot be excluded.



- The combination of episomal lentiviral vectors with the transposon-mediated transgene integration
- The IDLVs provide an efficient gene delivery vehicle for the system
- transposase directs transgene integration away from the transcriptionally active *loci*, favored by the regular integrase-proficient lentiviral vectors
  - For gene repair or knock-down, the site-directed integration
  - chimeric zinc-finger nucleases (ZFNs) that can be engineered to target desired sites



Site-specific gene addition into *CCR5*.
Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery
Angelo Lombardo, Pietro Genovese, Christian M Beausejour, Silvia Colleoni, Ya-Li Lee, Kenneth A Kim, Dale Ando, Fyodor D Urnov, Cesare Galli, Philip D Gregory, Michael C Holmes & Luigi Naldini
Nature Biotechnology **25**, 1298 - 1306 (2007)

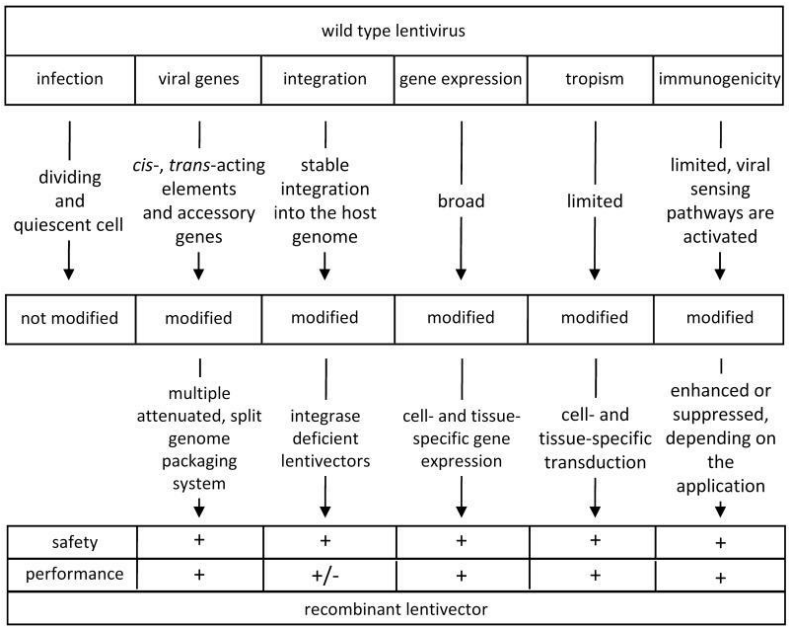
Immune Consequences

- LV can trigger innate and adaptive immune reactions directed against the vector particles, the transduced cells and/or the transgene product encoded by the vector
- vector particles trigger
  - a transient cytokine surge (e.g. interleukin-6) and IFN*αβ* response
    - animals that lack the capacity to respond to IFNß, there was a dramatic increase in hepatocyte transduction, and stable transgene expression was achieved
  - induce vector-specific antibodies →neutralize the vector particles and consequently prevent gene transfer by subsequent vector readministration
    - pre-existing antibodies to the heterologous Env protein used to pseudotype the LV may interfere with viral transduction
- The strength of these antigen-specific humoral and cellular adaptive immune reactions following LV administration, depends on
  - transgene product,
  - vector design,
  - vector dose,
  - route of vector administration,
  - target cell type and
  - genotype of the recipient animal or patient.
- LV transduction of APCs may result in ectopic expression of the transgene product. ➔ use cell types specific promoter/enhancers to restrict transgene expression to the target tissue + preventing inadvertent ectopic transgene expression in APCs

Table 2. Cell Type-Specific Promoters and Enhancers for Transcriptional Targeting in Retroviral Gene Therapy

Promoter	Target Cell/Tissue	Transgene	References
PEPCK promoter	Hepatocyte	Neo, bovine growth hormone	[99]
hAAT promoter	Hepatocyte	Alpha I antitrypsin	[100]
MMTV-LTR	Mammary gland	TNF-α	[92]
MCK promoter	Muscle	β-galactosidase, dystrophin minigene	[101]
AFP promoter	Cancer: Hepatocellular carcinomas	HSV-tk, VZV-tk	[102]
Tyrosine promoter	Cancer: Melanomas	HSV-tk, IL-2	[91]
Col1a1 promoter	Bone	β-geo (β-gal, neo fusion)	[103]
HSP70 promoter	Cancer	Dominant negative IGF-IR	[104]
WAP promoter	Cancer: Mammary	β-galactosidase	[105]
ppET1 promoter	Cancer: Endothelium	β-galactosidase	[106]
AFP enhancer; PGK promoter	Cancer: Hepatocellular carcinomas	HSV-tk	[96]
HRE, PGK-1 enhancer; E-selectin, KDR promoter	Cancer: Endothelium	TNF-α, luciferase	[95]
HRE enhancer; AFP promoter	Cancer: Hepatocellular carcinomas	HSV-tk, luciferase	[107]
Rat alpha-fetoprotein	Human hepatocarcinoma cell	HSV-tk, luciferase	[97]
HS2 of erythroid-specific GATA-1 gene; HIV-1 promoter	Mature erythroblasts	GFP	[98]

Escors &Breckpot, 2010



APPLICATIONS OF LENTIVIRAL VECTORS

- APPLICATIONS OF LENTIVIRAL VECTORS FOR GENOME-WIDE FUNCTIONAL STUDIES OF GENE EXPRESSION
  - a combination of Lentivirus-based vectors and RNAi technology has led to the establishment of libraries for high-throughput loss-of function screens in mammalian cell types, including primary and nondividing cells
  - protein tagging in mammalian cells based on lentiviral vector that harbors an artificial exon encoding EGFP tag
- LENTIVIRAL VECTORS IN ANIMAL TRANSGENESIS
  - transgenic cloned animals using lentiviruses such as domestic cats (Gómez *et al.,* 2009) and prairie voles (Donaldson *et al.,* 2009).
  - lentiviral-modified transgenic primate model of Huntington's disease
- LENTIVIRAL VECTORS FOR CELL ENGINEERING
  - Reprogramming of somatic cells-Induced pluripotent stem (iPS) cells

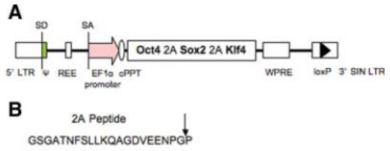


Figure 1. OSK lentiviral vector for reprogramming adult skin fibroblast to iPS cells. **(A):** Human Oct4, Sox2, and Klf4 cDNAs were linked with porcine teschovirus-1 2A sequences that function as cis-acting hydrolase elements to trigger "cleavage" and ribosome skipping. This polycistron was subcloned downstream of an EF-1α promoter in a SIN lentiviral vector containing a loxP site in the truncated 3′ LTR. **(B):** The amino acid sequence of the 2A polypeptide is listed; the arrow marks the site of "cleavage" during translation. Abbreviations: cPPT, central PolyPurine Tract; EF-1α, elongation factor 1 alpha; LTR, long terminal repeat; RRE, rev response element; SA, splice acceptor; SD, splice donor; SIN LTR, self-inactivating LTR; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element.

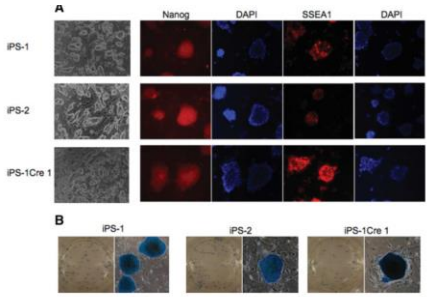


Figure 2. iPS cell colonies stained for markers of pluripotency. Following OSK lentiviral transduction of adult fibroblasts, colonies were picked, expanded, and stained for alkaline phosphatase, Nanog, and SSEA1. iPS-1 and iPS-2 are independent colonies derived from the original transduction. iPS-1 Cre1 is one of many colonies obtained after Cre recombinase-mediated deletion of the OSK lentiviral vector in iPS-1 cells. Abbreviations: DAPI, 4′-6-diamidino-2-phenylindole; iPS, induced pluripotent stem; SSEA1, stage-specific embryonic antigen-1.

Chang et al., 2009

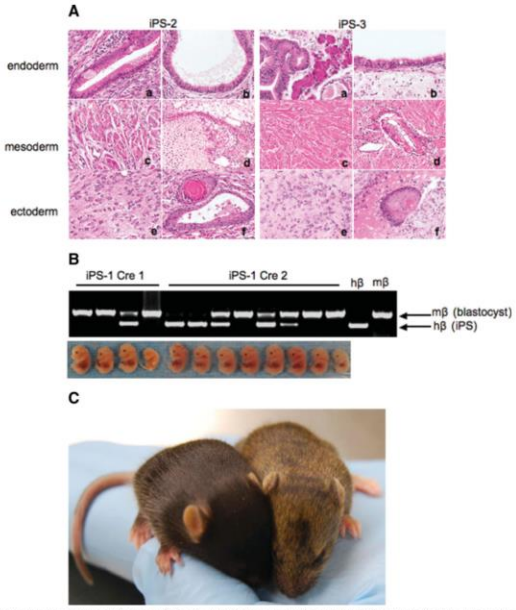


Figure 5. Teratomas and chimeras derived from iPS cells. **(A):** Teratomas containing tissue derived from all three germ layers were obtained when iPS cells were injected into the dorsal flanks of nonobese diabetic/severe combined immunodeficiency IL-2gammaR −/− mice. **(a):** Intestine-like epithelium, with pancreatic acini in iPS-3 teratoma; **(b):** respiratory epithelium; **(c):** skeletal muscle; **(d):** bone, with hyaline cartilage in iPS-2 teratoma; **(e):** nervous tissue; **(f):** skin-like stratified squamous epithelium. **(B):** Chimeric embryos were obtained following injection of iPS-1 Cre 1 and iPS-1 Cre 2 cells into wild-type blastocysts and transfer of these blastocysts into the uteri of pseudopregnant female mice. Two weeks after injection, embryos were analyzed for chimerism by polymerase chain reaction with primers specific for hβ and mβ genes. iPS cells contain only hβ genes. **(C):** Adult chimeric animal (right) obtained by injection of iPS-1 Cre 1 cells into murine blastocysts and transfer of the blastocysts into pseudopregnant recipients. Nonchimeric littermate (left). Abbreviations: hβ, human β-globin; iPS, induced pluripotent stem; mβ, mouse β-globin.

Chang et al., 2009

Journal of Reproduction and Development, Vol. 58, No 4, 2012

—Original Article—

Generation of Human β-thalassemia Induced Pluripotent Stem Cells from Amniotic Fluid Cells Using a Single Excisable Lentiviral Stem Cell Cassette

Yong FAN[1)], Yumei LUO[1)], Xinjie CHEN[1)], Qing LI[1)] and Xiaofang SUN[1)]

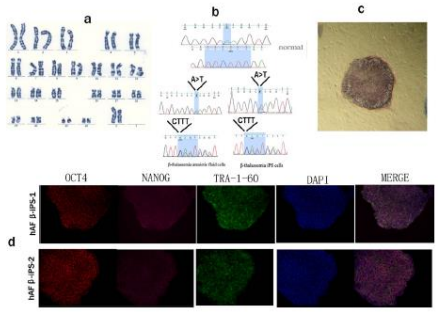


Fig. 1. Generation of β-thalassemia iPS cells from human AF cells. (a) The karyotype of β-thalassemia iPS cells is 46XX. (b) Sequence analysis of normal human fibroblasts, AF cells and iPS cells of the patient with β-thalassemia. The sequence results show that a double heterozygous codon 41-42/17 (CTTT; A>T) mutation. (c) β-thalassemia iPS cells were stained for alkaline phosphatase (AP) (10× magnification with an inverted microscope). (d) Immunostaining of β-thalassemia iPS cells. β-thalassemia iPS cells were positive for the pluripotency genes OCT4 and NANOG, and they expressed the cell surface marker TRA-1-60

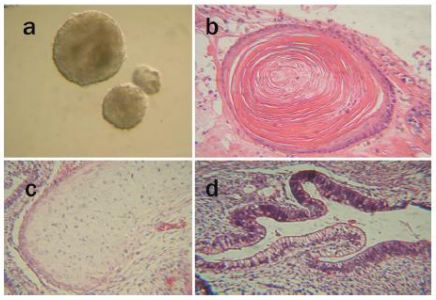


Fig. 3. Differentiation of β-thalassemia iPS cells. *In vitro* differentiation of β-thalassemia iPS cells to form embryoid bodies (EBs) for 7 days in suspension culture (10× magnification with an inverted microscope). (b–d) *In vivo* differentiation of β-thalassemia iPS cells. Teratomas that formed 8 weeks after injection of iPS cells contained tissues from all three germ layers, including (b) squamous cells (ectoderm), (c) cartilaginous tissue (mesoderm) and (d) glandular tissue (endoderm) (20× magnification with an inverted microscope).

- **LENTIVIRAL VECTORS IN CLINICAL GENE THERAPY APPLICATIONS**
  - a lentiviral vector was approved in 2001 for anti-HIV therapy → immune function improved infusion of 10^{10} *ex vivo* gene-modified autologous CD4+ T cells

Gene therapy procedures: Cells were collected from the bone marrow of a patient with β-thalassemia (Cavazzano-Calvo et al. 2010).

A.W. Nienhuis and D.A. Persons

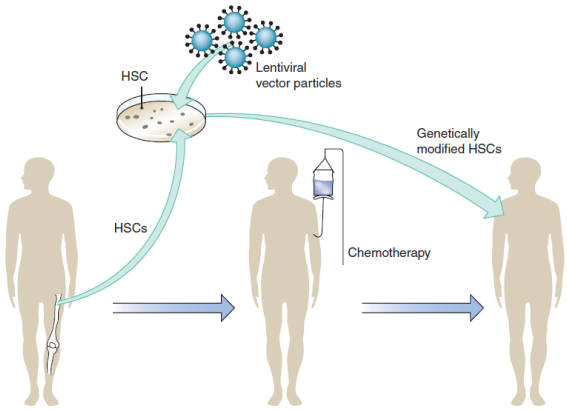
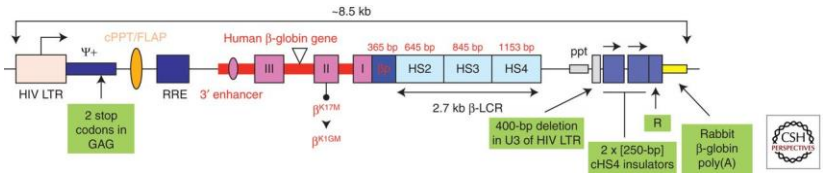


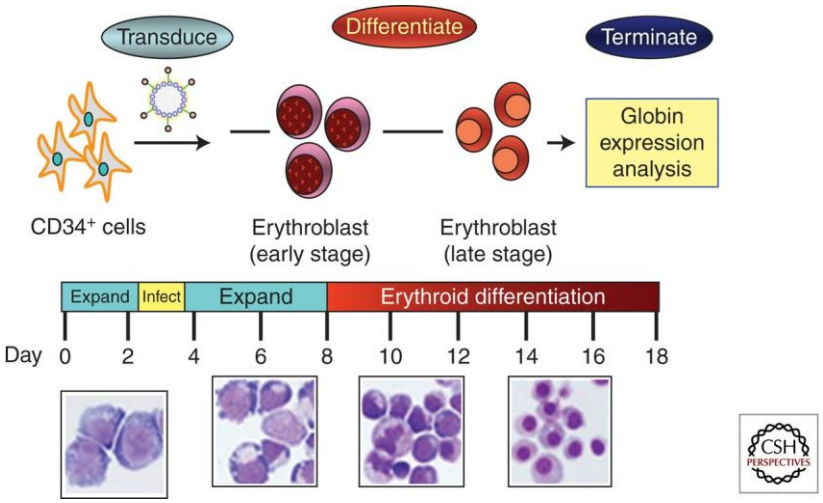
Figure 1. Gene therapy procedures: Cells were collected from the bone marrow of a patient with β-thalassemia (Cavazzano-Calvo et al. 2010). After purification, the hematopoietic stem cells (HSCs) were maintained in culture for 34 h. Lentiviral vector particles containing a functional β-globin gene were added, and culture was continued for 18 h. The patient underwent chemotherapy to eradicate the remaining HSCs and make room for the genetically modified cells. The transduced HSCs were then transplanted into the patient by infusion into a peripheral vein. (Adapted from Persons 2010; reprinted, with permission, from the author.)

Diagram of the lentiglobin vector showing the 3′ β-globin enhancer, the 372-bp IVS2 deletion, the βA-T87Q mutation [ACA(Thr) to CAG(Gln)], and the DNase I hypersensitive sites (HS) 2, HS3, and HS4 of the human β-globin locus control region (LCR).



Nienhuis A W , and Persons D A Cold Spring Harb Perspect Med 2012;2:a011833

Two-stage culture model of human erythropoiesis.



Nienhuis A W , and Persons D A Cold Spring Harb Perspect Med 2012;2:a011833

Table 1. Transduction conditions and outcome in lentiviral clinical trials

Disease	Metachromatic leukodystrophy	Adrenoleukodystrophy	β-Thalassemia
Age of patient		7 or 7.5 yr	19 yr
Source of $CD34^+$ cells	G-CSF mobilization Peripheral blood	G-CSF mobilization Peripheral blood	Bone marrow
Medium	X-vivo 15	X-vivo 20	X-vivo 20
Cell density/viral density	1×10^6 mL	2.0×10^6 mL^{-1}/ 5.0×10^7 mL^{-1}	2.0×10^6 mL^{-1} / 1.1×10^8 mL^{-1}
Cytokines (ng/mL)	SCF, 300 TPO, 100 FlT3L, 300 IL3, 60	SCF, 100 TPO, 100 FlT-3, 100 IL3, 60	SCF, 300 TPO, 10 FlT3L, 300 IL3, 100
Protamine/ RetroNectin		4 μg/mL / 50 μg/mL	4 μg/mL / 50 μg/mL
Prestimulation	24 h	19 h/no retro	34 h
Transduction	12 h wash/rest 12 h/12 h proteasome inhibitor MG132	16 h	18 h
Cells recovered		4.6×10^6 or 7.2×10^6 cells/kg	3×10^8 total
Labeling—bulk—CFU		50% or 33%	0.6 vg/cell
In vivo labeling	>50%	23%–13%–10%[a] 25%–17%–10%[a]	70% RBCs 11% Erythroid 19% granulocyte 9% B-lymphoid
Myeloablation		Cytoxan + busulfan	Busulfan
Clinical outcome	Polyclonal/prevention of neurological deterioration	Sustained polyclonal hematopoiesis/clinical improvement	Polyclonal with dominant clone/transfusion independent

G-CSF, granulocyte-colony stimulating factor; CFU, colony-forming unit; RBCs, red blood cells.
[a]Values obtained over 16 mo following gene transfer procedure.

Nienhuis A W , and Persons D A Cold Spring Harb Perspect Med 2012;2:a011833

Safety issues

- SIN vector configuration, heterologous envelopes and Tat independent vector production has significantly improved the overall vector safety.
- homologous overlap between vector and packaging constructs is minimized to reduce the risk of generating replication competent lentiviruses (RCL)