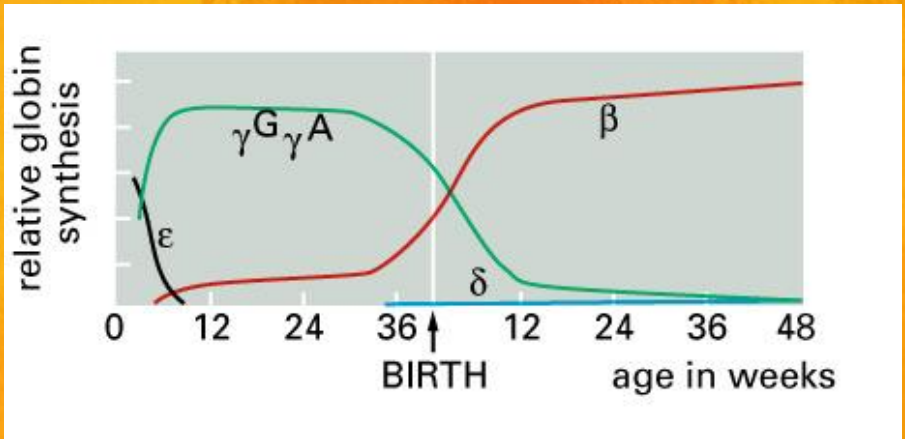
Innovation for Thalassemia; Gene and Cell Therapy

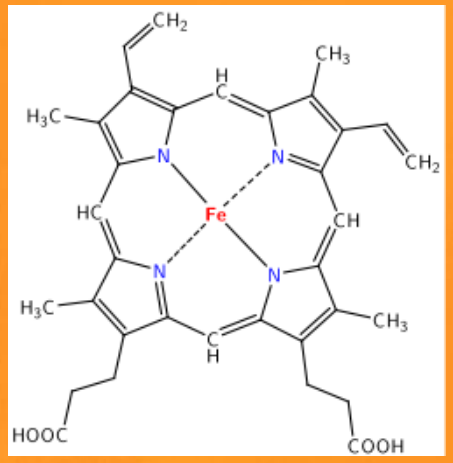


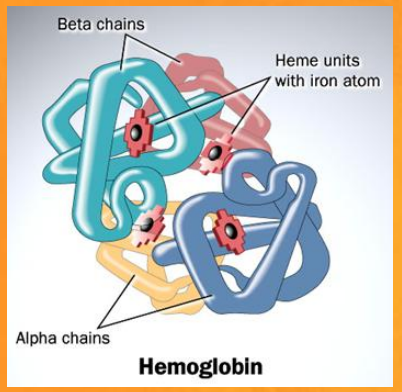
กฤษณพงศ์ มโนธรรม

นายแพทย์ทรงคุณวุฒิ โรงพยาบาลเลิดสิน กรมการแพทย์

Hemoprotein; an ancient protein

O_2 trap

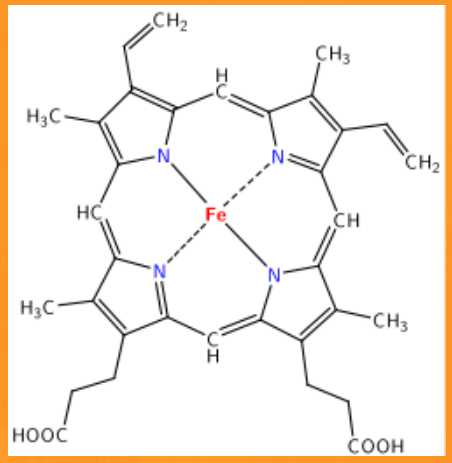


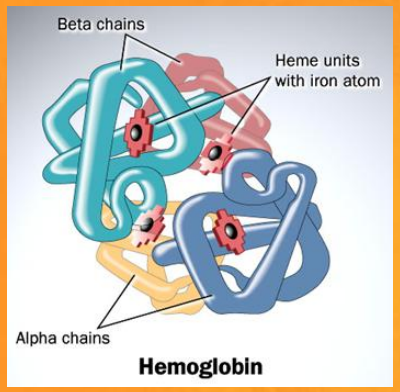


O_2 carrier

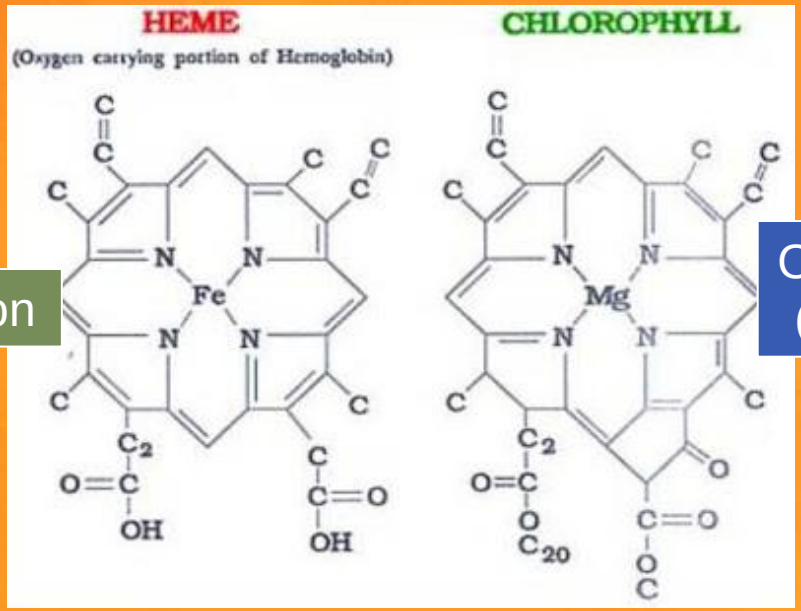
Hemoprotein; an ancient protein

O_2 trap





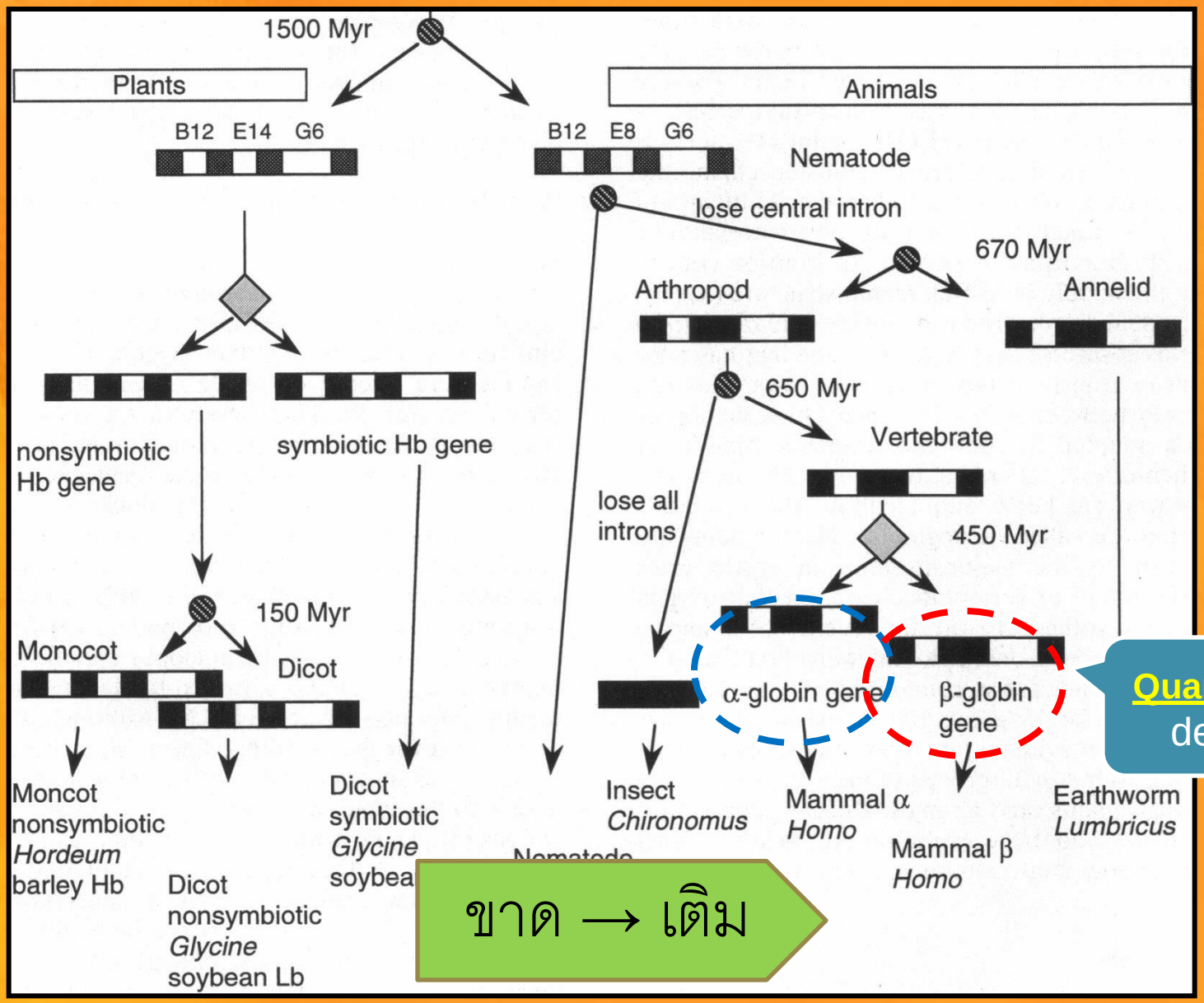
O_2 carrier



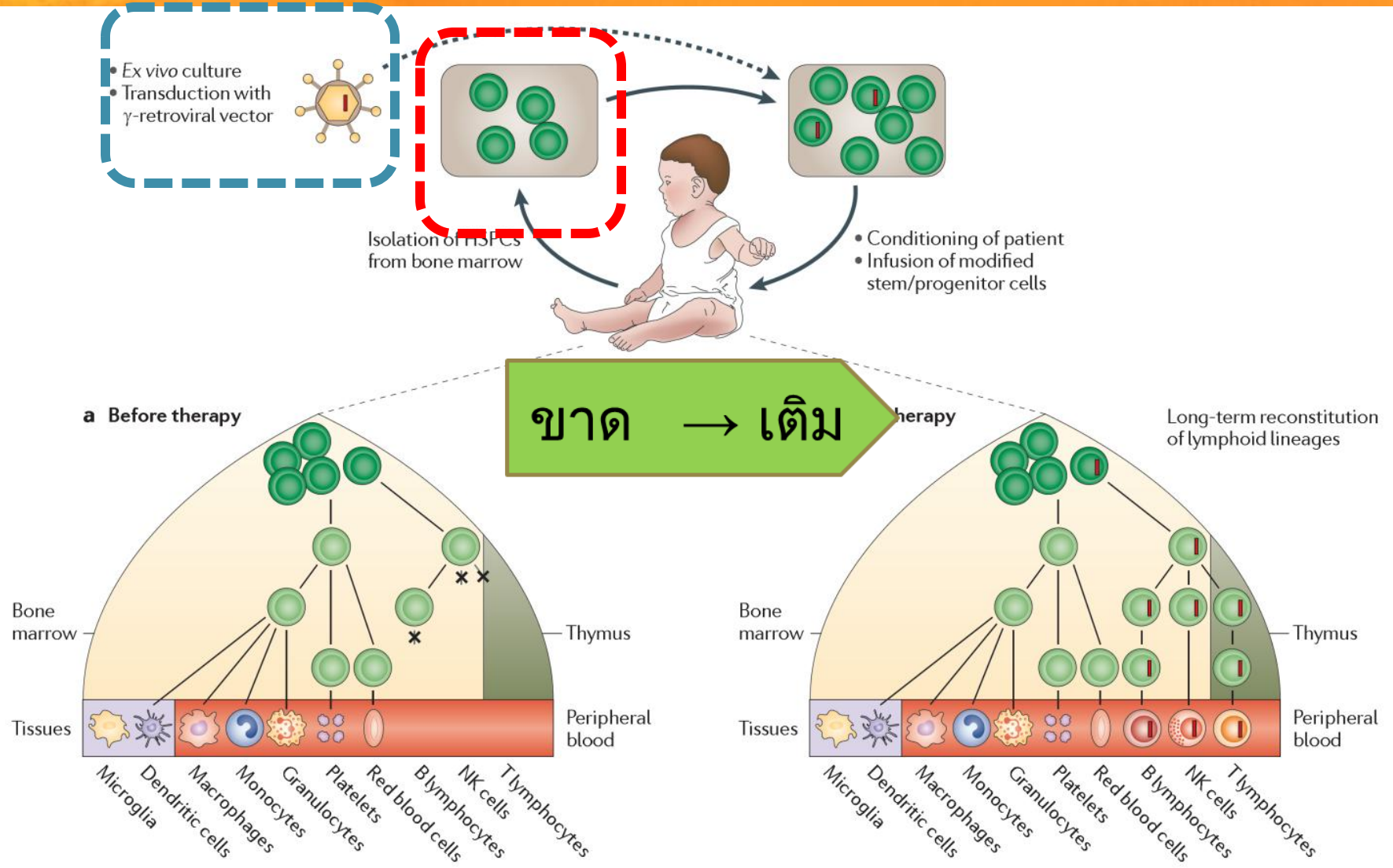
Oxidative phosphorylation

Oxygenic photosynthesis
(Energy splitting water)

Hemoprotein; an ancient protein



Gene therapy in SCID



Gene manipulation

- Replace
  - vector design
  - insertion site
- Repair
  - ZFN, TALEN, CRISPR
- Reconstruct

Cell manipulation

- HSCs
  - ex vivo expansion
- Reprogramming
  - somatic →iPSC→ HSCs
- Direct conversion
  - somatic→HSCs

Gene manipulation

1980

- Replace
  - vector design
  - insertion site
- Repair
  - ZFN, TALEN, CRISPR
- Reconstruct

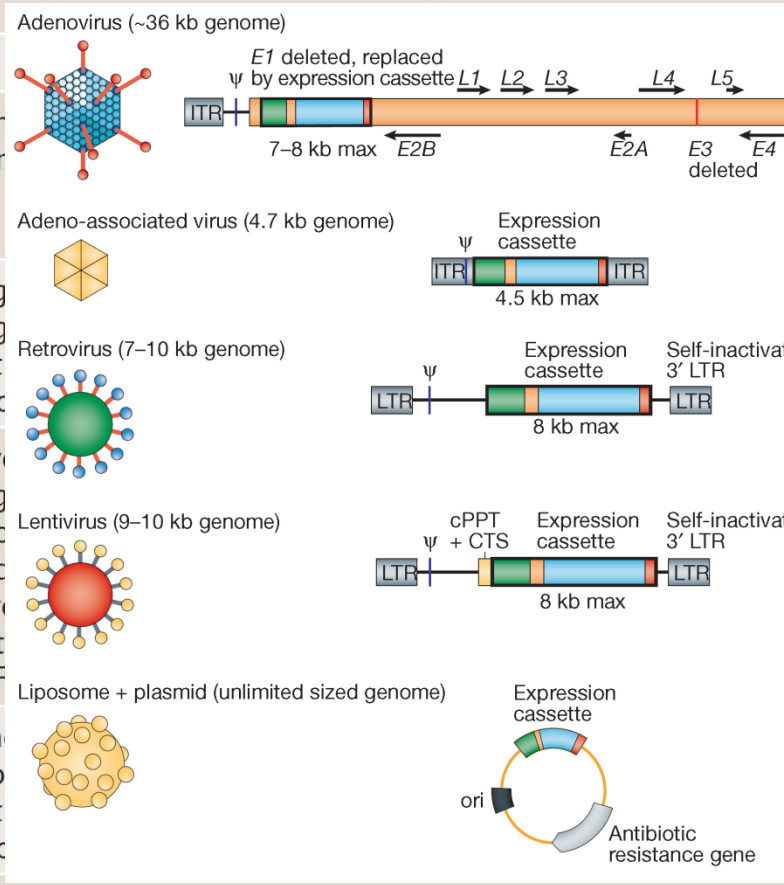
Cell manipulati

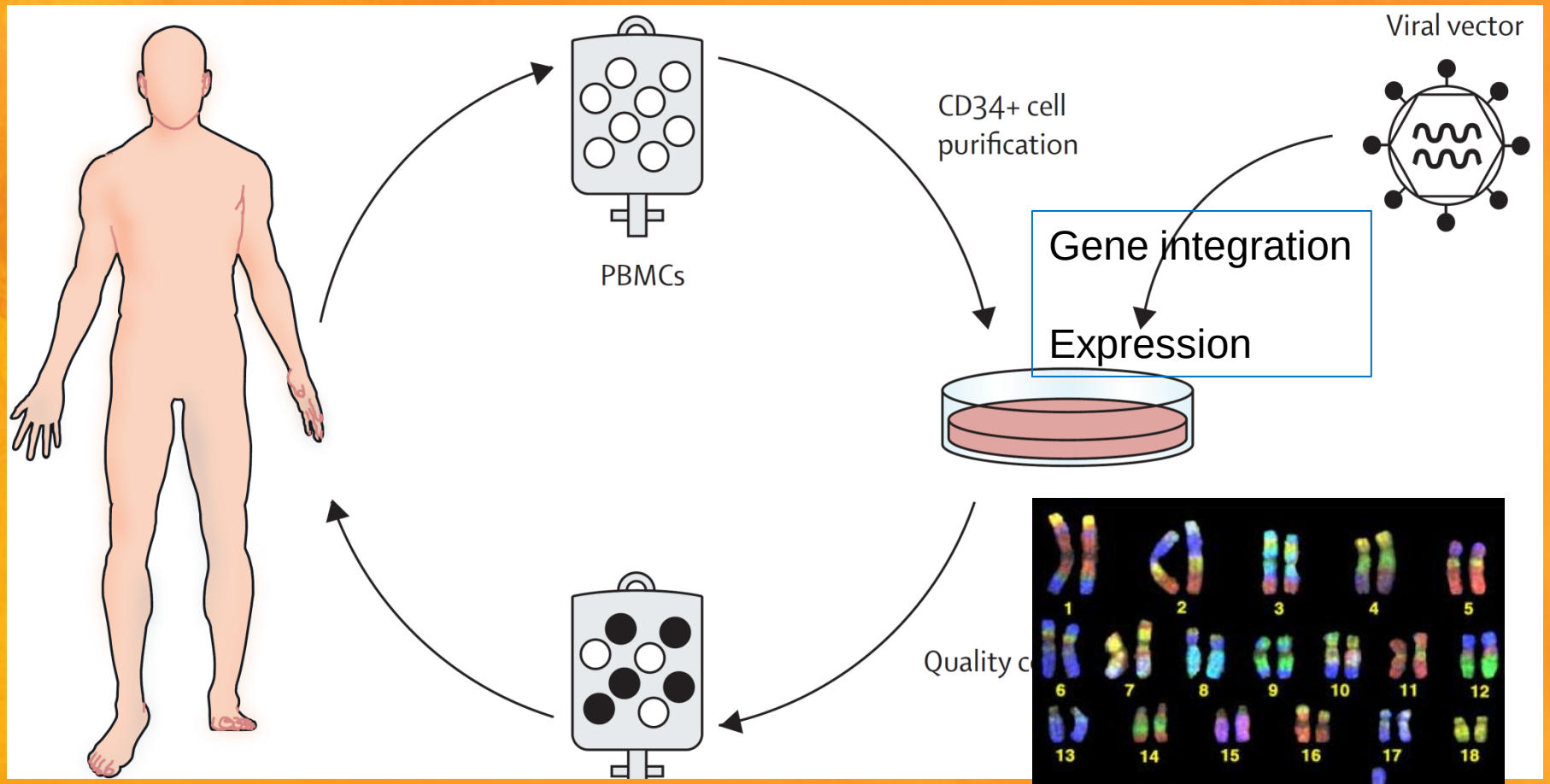
2011

- HSCs
  - ex vivo expansion
- Reprogramming
  - somatic →iPSC→ HSCs
- Direct conversion
  - somatic→HSCs

+

Disease type	Rationale and target cells	Gene vector	Stage of development	Safety	Efficacy	Comments
Haematological						
ADA-SCID	Lymphoid reconstitution	γ-RV (MLV or SFFV LTR) expressing ADA	Phase I/II trials completed or ongoing	Lor der		
X-linked SCID	Lymphoid reconstitution	γ-RV expressing IL-2R common gamma chain	Phase I/II trials completed	Hig trig act onc		
X-linked CGD	Myeloid reconstitution	γ-RV (SFFV or MFG LTR) expressing gp91$^{(phox)}$	Phase I/II trials recruitment closed, follow-up ongoing	My trig at c and my pat SFF		
WAS	Multi-lineage reconstitution	γ-RV (MPSV LTR) expressing WAS protein	Phase I/II trial recruitment closed, follow-up ongoing	On rep act onc		
WAS	Multi-lineage reconstitution	SIN LV expressing WAS from *WAS* gene promoter	Phase I/II trials recently started	-	-	-
β-thalassaemia	Erythroid reconstitution	SIN LV; large LCR expressing β-globin and cHS4 insulator	Phase I/II trial ongoing (one patient treated)	Clonal dominance possibly triggered by vector insertion in *HMGA2* gene	Transfusion independence	-
Fanconi's anaemia	Stem cell reconstitution	SIN LV expressing FANC-A protein from *PGK* promoter	Phase I/II trial approved	-	-	HSC gene transfer without stimulation to protect cells from DNA damage





Mixed population
QC?
Individual cell QC?

Uncontrolled + imprecise
Risk associated

nature Vol 467|16 September 2010|doi:10.1038/nature09328

LETTERS

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Marina Cavazzana-Calvo[1,2]*, Emmanuel Payen[3,4,5]*, Olivier Negre[3,4,5,6], Gary Wang[7], Kathleen Hehir[8], Floriane Fusil[3,4,5], Julian Down[8], Maria Denaro[8], Troy Brady[7], Karen Westerman[8,9], Resy Cavallesco[9], Beatrix Gillet-Legrand[6], Laure Caccavelli[1,2], Riccardo Sgarra[10], Leila Maouche-Chrétien[3,4], Françoise Bernaudin[11], Robert Girot[12], Ronald Dorazio[8], Geert-Jan Mulder[8], Axel Polack[8], Arthur Bank[13], Jean Soulier[5], Jérôme Larghero[5], Nabil Kabbara[5], Bruno Dalle[5], Bernard Gourmel[5], Gérard Socie[5], Stany Chrétien[3,4,9], Nathalie Cartier[14], Patrick Aubourg[14], Alain Fischer[1,2], Kenneth Cornetta[15], Frédéric Galacteros[16], Yves Beuzard[3,4,5], Eliane Gluckman[5], Frederick Bushman[7], Salima Hacein-Bey-Abina[1,2]* & Philippe Leboulch[3,4,9]*

The β-haemoglobinopathies are the most prevalent inherited disorders worldwide. Gene therapy of β-thalassaemia is particularly challenging given the requirement for massive haemoglobin production in a lineage-specific manner and the lack of selective advantage for corrected haematopoietic stem cells. Compound β^E/β^0-thalassaemia is the most common form of severe thalassaemia in southeast Asian countries and their diasporas[1,2]. The β^E-globin allele bears a point mutation that causes alternative splicing. The abnormally spliced form is non-coding, whereas the correctly spliced messenger RNA expresses a mutated β^E-globin with partial instability[1,2]. When this is compounded with a non-functional β^0 allele, a profound decrease in β-globin synthesis results, and approximately half of β^E/β^0-thalassaemia patients are transfusion-dependent[1,2]. The only available curative therapy is allogeneic haematopoietic stem cell transplantation, although most patients do not have a human-leukocyte-antigen-matched, geno-identical donor, and those who do still risk rejection or graft-versus-host disease. Here we show that, 33 months after lentiviral β-globin gene transfer, an adult patient with severe β^E/β^0-thalassaemia dependent on monthly transfusions since early childhood has become transfusion independent for the past 21 months. Blood haemoglobin is maintained between 9 and 10 g dl^{-1}, of which one-third contains vector-encoded β-globin. Most of the therapeutic benefit results from a dominant, myeloid-biased cell clone, in which the integrated vector causes transcriptional activation of *HMGA2* in erythroid cells with further increased expression of a truncated *HMGA2* mRNA insensitive to degradation by let-7 microRNAs. The clonal dominance that accompanies therapeutic efficacy may be coincidental and stochastic or result from a hitherto benign cell expansion caused by dysregulation of the *HMGA2* gene in stem/progenitor cells.

The design of integrative vectors for human β-globin gene transfer has been difficult. The genetic elements required for high and erythroid-specific expression are complex: the β-globin gene with its introns, promoter and β-locus control region (β-LCR)[3,4]. Lentiviral vectors have proven capable of transferring these elaborate structures with fidelity and high titres[5,6]. Hence, several mouse models of the β-haemoglobinopathies have been corrected, long-term, by *ex vivo* transduction of haematopoietic stem cells (HSCs) with β-globin lentiviral vectors[5–10]. These advances have prompted the prudent initiation of a human clinical trial (Supplementary Note 1).

The general structure of the β-globin-expressing lentiviral vector has been previously described[6,8] (Supplementary Fig. 1). It is a self-inactivating vector with two copies of the 250-base-pair (bp) core of the cHS4 chromatin insulator[11] implanted in the U3 region. It encodes a mutated adult β-globin ($\beta^{A(T87Q)}$) with anti-sickling properties[6] that can be distinguished from normal adult β-globin (β^A) by high-performance liquid chromatography (HPLC) analysis in individuals receiving red blood cell transfusions and/or β^+-thalassaemia patients.

This report focuses on the first treated patient (P2) who did not receive back-up cells: a male, aged 18 years at the time of treatment, with severe β^E/β^0-thalassaemia. A previous patient (P1) failed to engraft because the HSCs had been compromised by the technical handling of the cells without relation to the gene therapy vector. P1 failed to engraft after 5 weeks and was thus given back-up cells (Supplementary Note 2). P2 was first transfused at age three because of poorly tolerated anaemia (6.7 g dl^{-1} despite residual fetal haemoglobin (HbF)) and major hepatosplenomegaly. Transfusion requirements rapidly increased to once a month (2–3 red blood cell packs each time; 157 ml of red blood cells per kg the year before transplant). He was splenectomized at age 6. In spite of this, Hb levels decreased several times to as low as 4 g dl^{-1}, and hydroxurea therapy was ineffective. Iron chelation was initiated at age 8 by parenteral deferoxamine overnight, 5 times a week. The patient did not have a related human-leukocyte-antigen-matched donor and was thus enrolled in this trial after informed consent.

The *ex vivo* transduction efficiency of bulk bone marrow $CD34^+$ cells was 0.6 vector per cell after 1 week in culture after gene transfer. The patient was conditioned by intravenous Busulfex (3.2 mg kg^{-1} day^{-1} for 4 days) without the addition of cyclophosphamide, before transplantation with autologous gene-modified and cryopreserved cells

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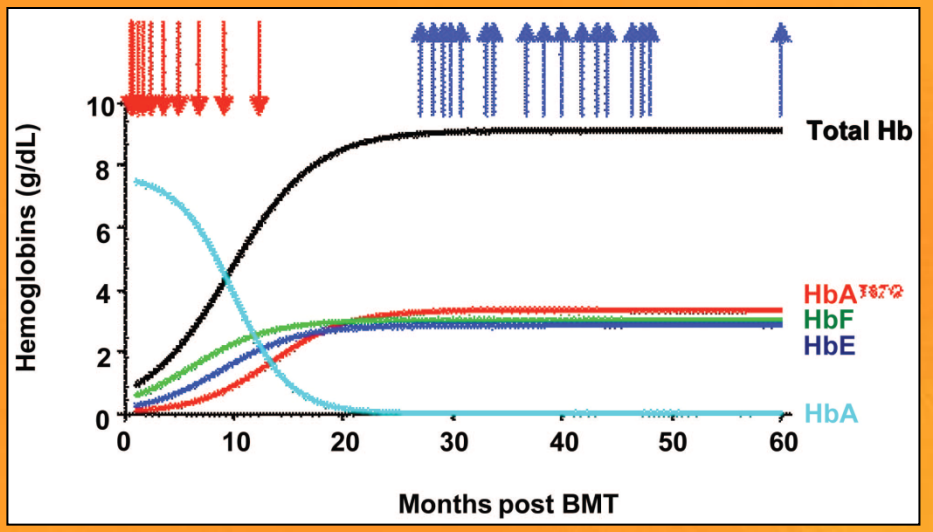
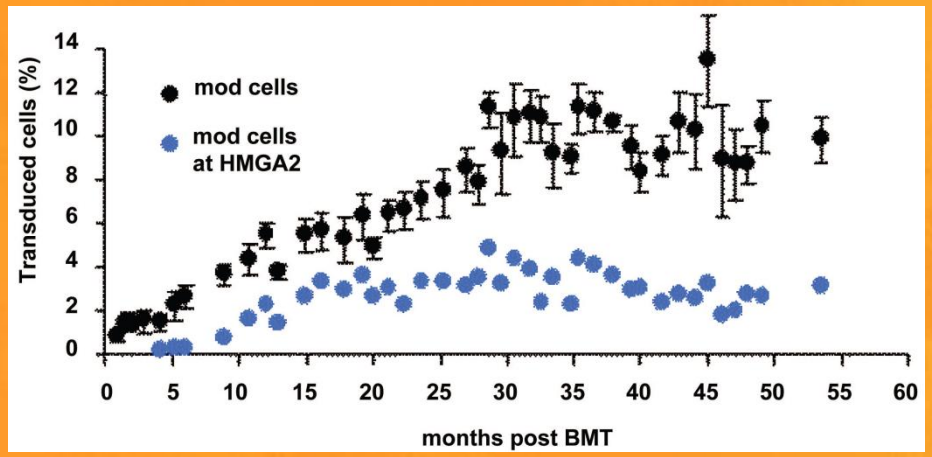
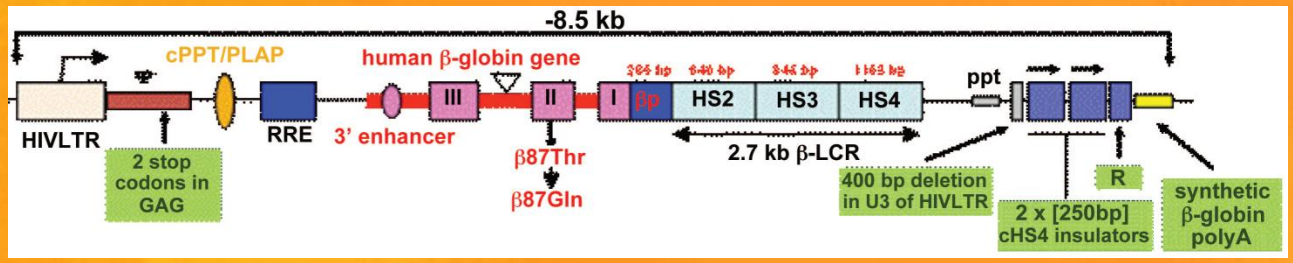
*These authors contributed equally to this work.



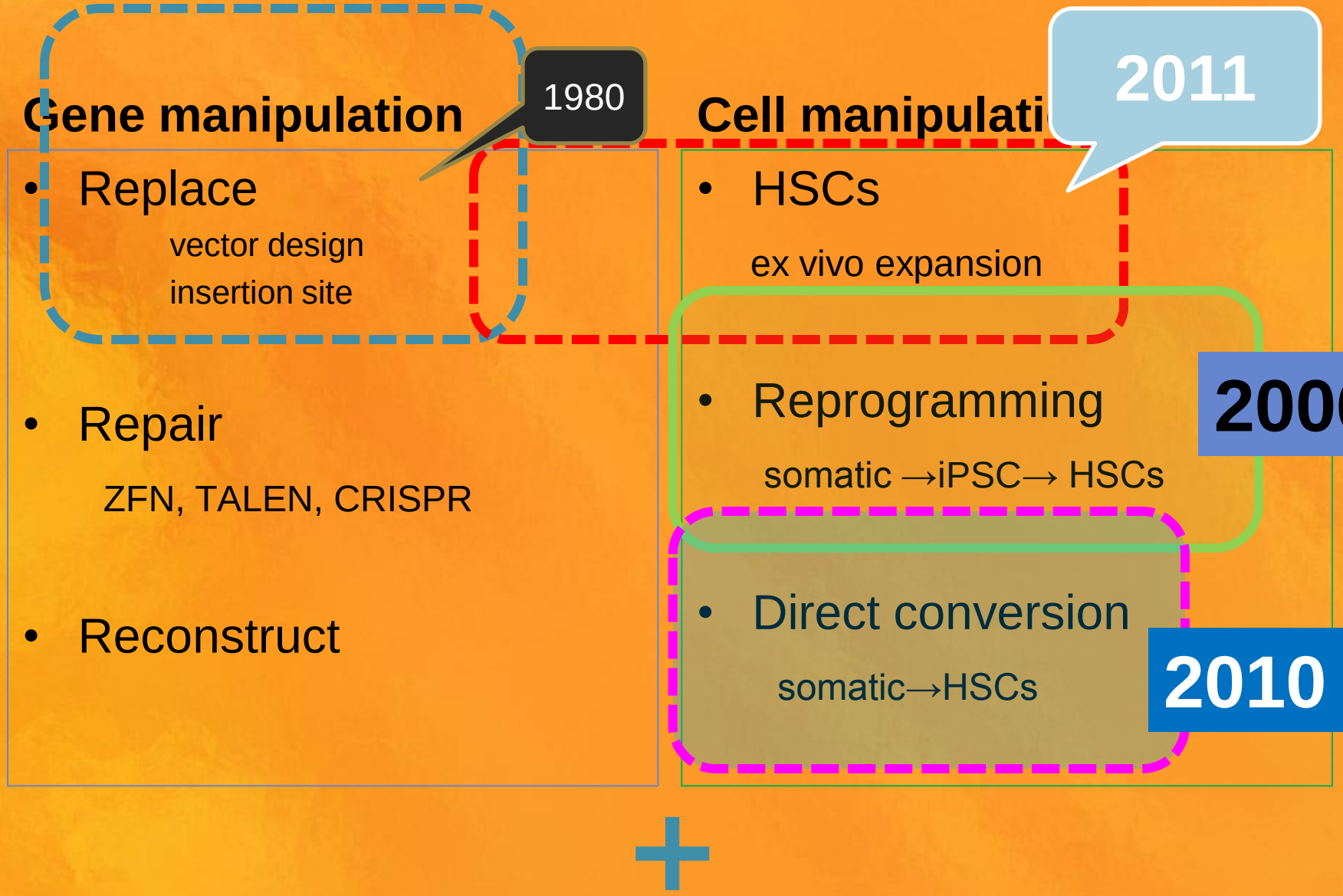




For subjects lacking a suitable donor, and even for those who do have a compatible donor but nevertheless face the risk of GVHD after allo-HSCT, much of the drawbacks may be avoided by gene therapy of HSCs. Direct homologous recombination/repair of the defective β-globin gene would be ideal, but is not yet feasible in HSCs. Gene addition by vector-based transfer and chromosomal integration of a therapeutic globin gene remains the approach of choice. However, efficient modification of HSCs and high expression of globin genes in erythroid cells have presented major

Emmanuel Payen[1,2] and Philippe Leboulch[1-4]

2012


Gene manipulation
1980
Cell manipulati
2011
• Replace
vector design
insertion site
• Repair
ZFN, TALEN, CRISPR
• Reconstruct
• HSCs
ex vivo expansion
• Reprogramming
somatic →iPSC→ HSCs
2006
• Direct conversion
somatic→HSCs
2010

Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

INTRODUCTION

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Evans and Kaufman, 1981; Martin, 1981). Human ES cells might be used to treat a host of diseases, such as Parkinson's disease, spinal cord injury, and diabetes (Thomson et al., 1998). However, there are ethical difficulties regarding the use of human embryos, as well as the problem of tissue rejection following transplantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from the patients' own cells.

Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997) or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

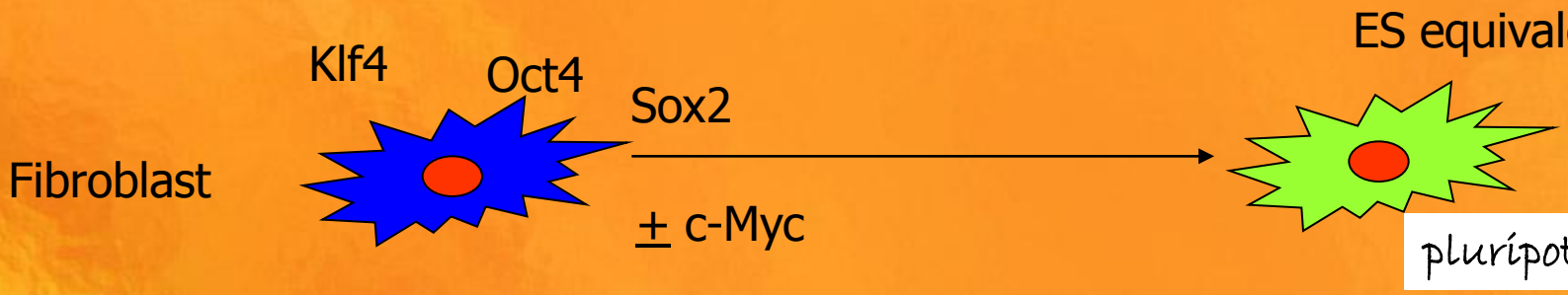
Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), c-*myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and β-*catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

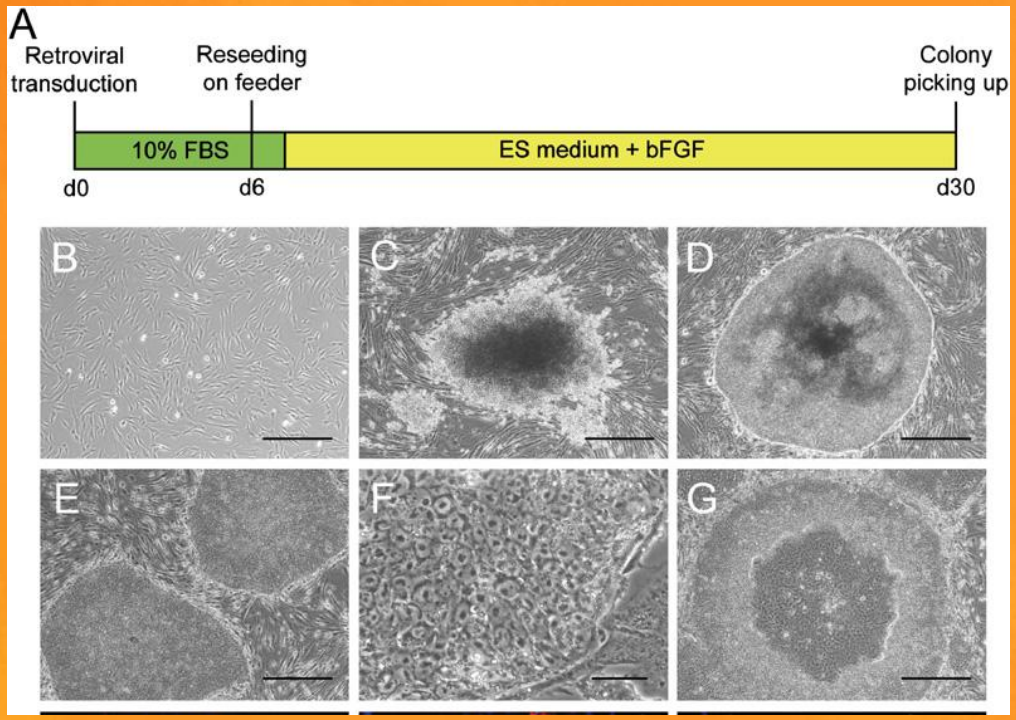
In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

RESULTS

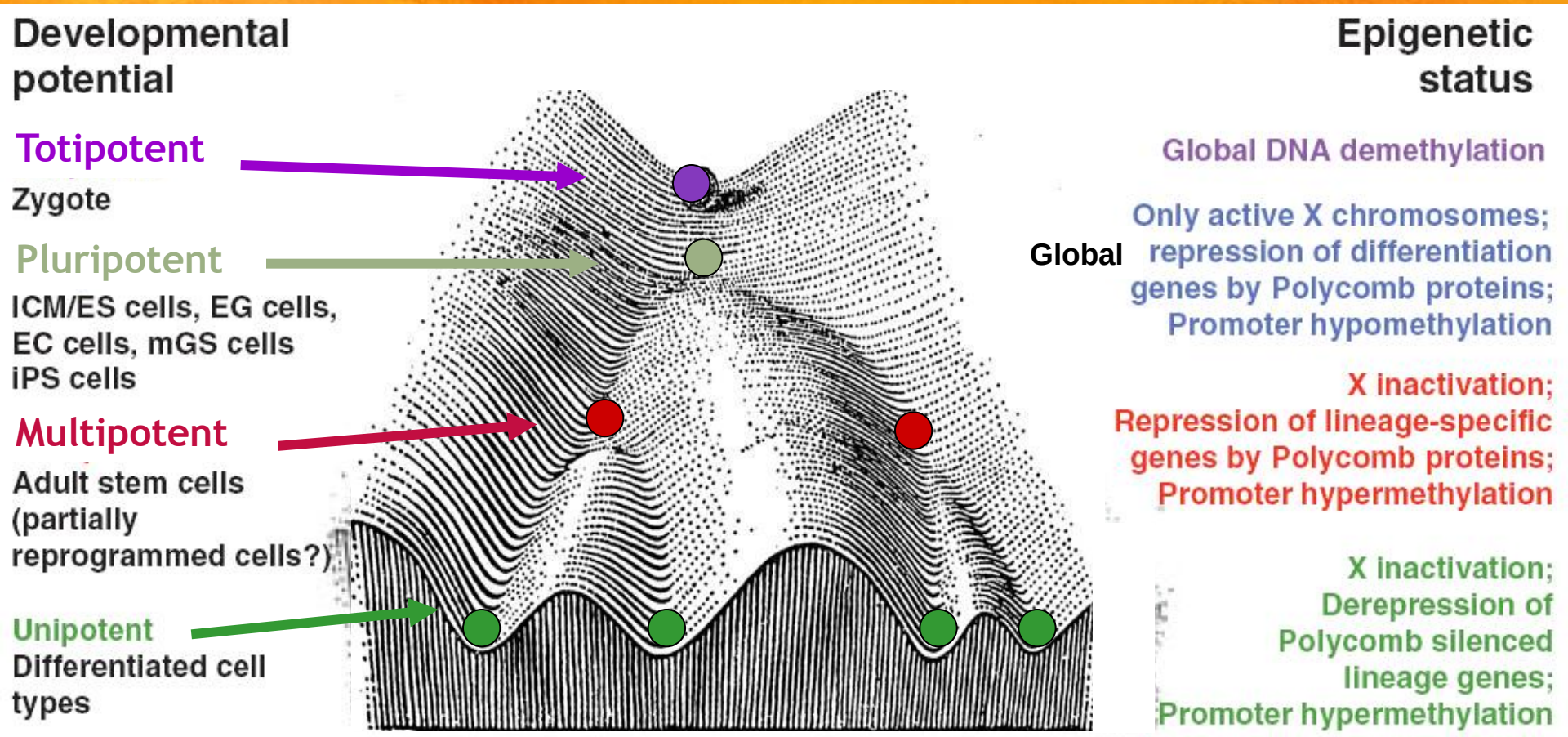
We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β-catenin, c-Myc, and Stat3, we used active forms, S33Y-β-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2ΔSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

Induced pluripotent stem cells





Waddington's landscape



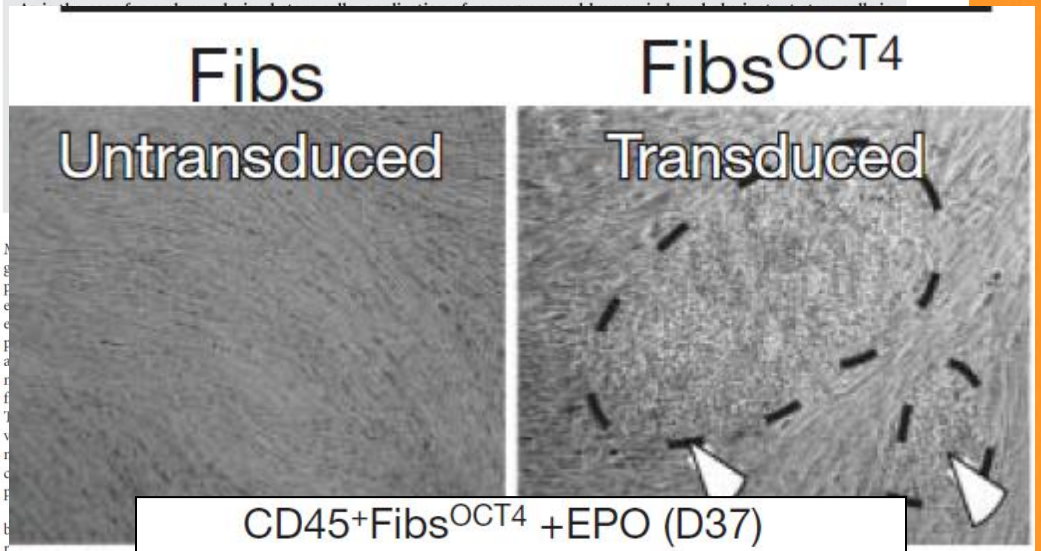
Hochedlinger K & Plath K. 2009

ARTICLE

doi:10.1038/nature09591

Direct conversion of human fibroblasts to multilineage blood progenitors

Eva Szabo[1], Shravanti Rampalli[1], Ruth M. Risueño[1], Angelique Schnerch[1,2], Ryan Mitchell[1,2], Aline Fiebig-Comyn[1], Marilyne Levadoux-Martin[1] & Mickie Bhatia[1,2]

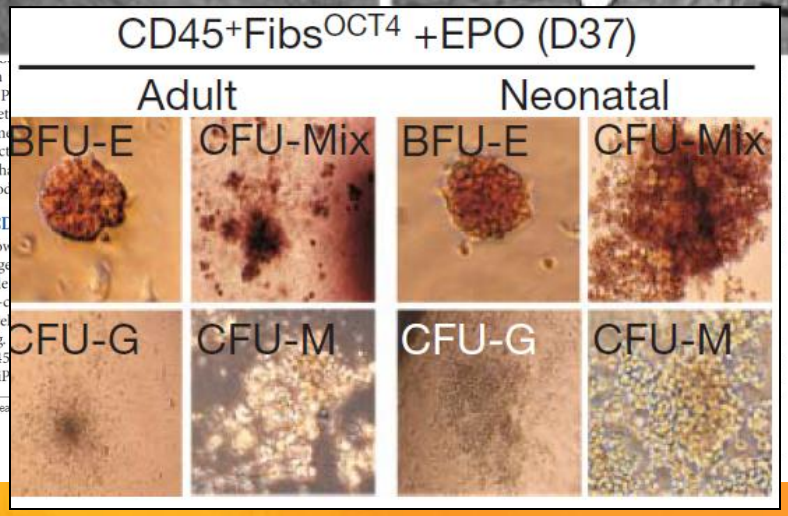


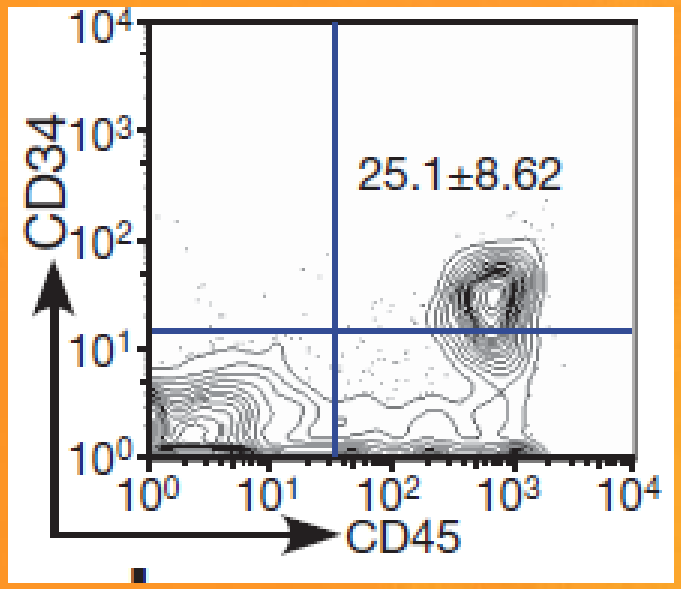
include the human
OCT2 (also called P
similar DNA target
lymphoid developme
poiesis. Here, by ect
demonstrate and cha
to multipotent blooc

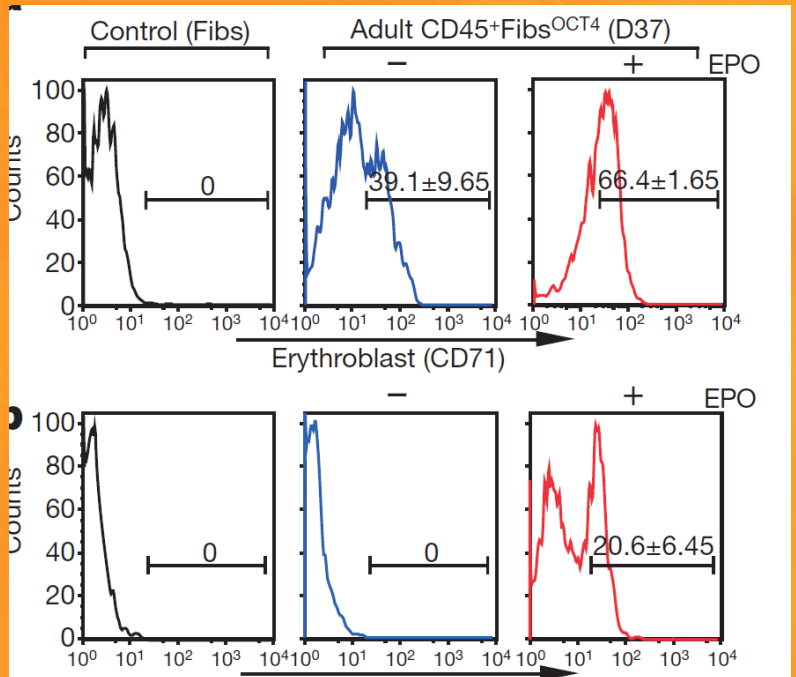
Emergence of CI

Reprogramming tow
that encompasses ge
rare subset of stable
plementary Fig. 1a–c
that possess round cel
(Supplementary Fig.
marker CD45 (CD45
that is indicative of iP

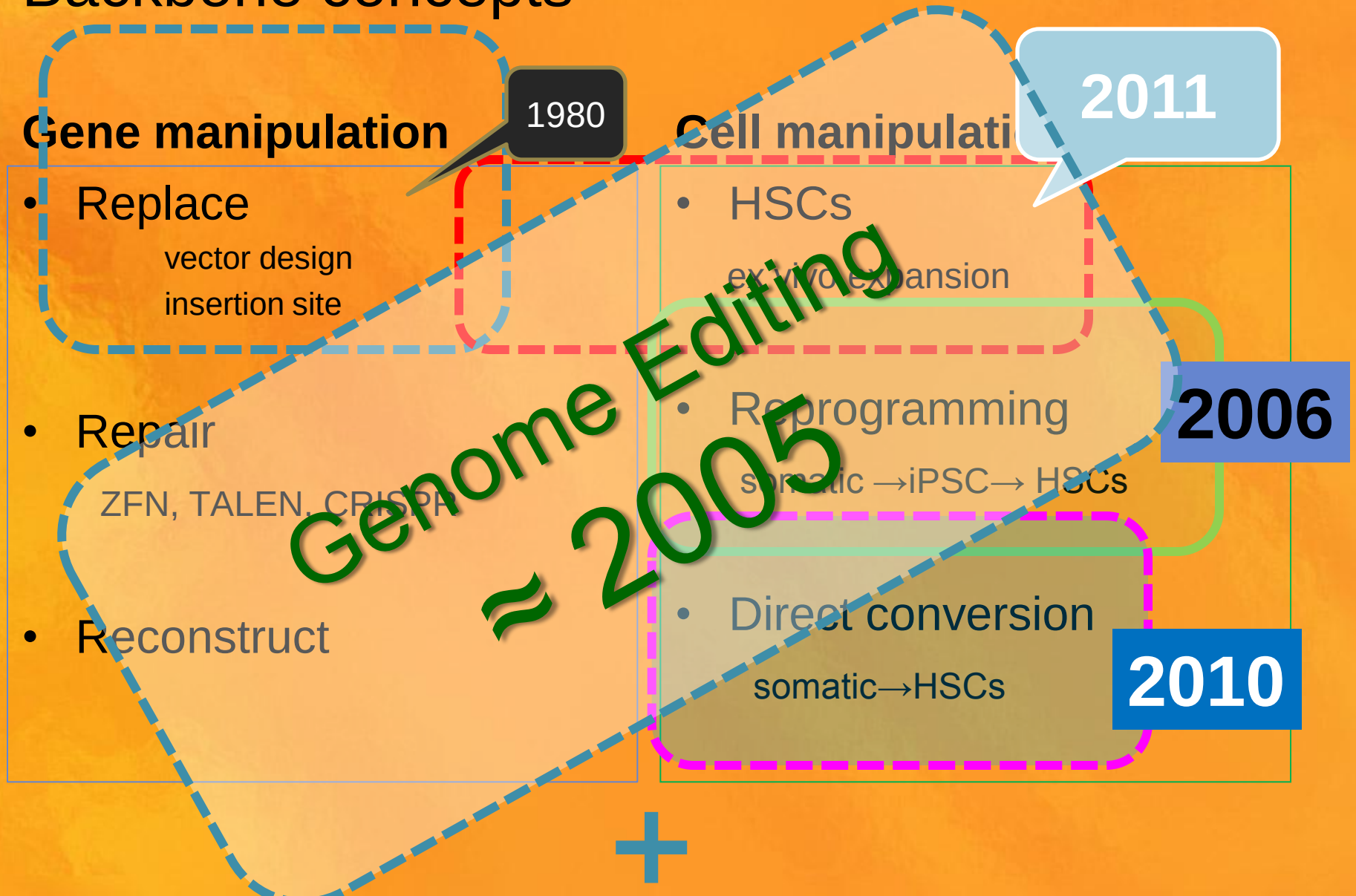
[1]Stem Cell and Cancer Resea
Canada L8N 3Z5.





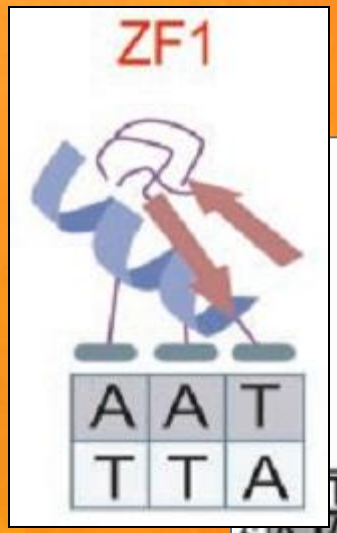


Backbone concepts

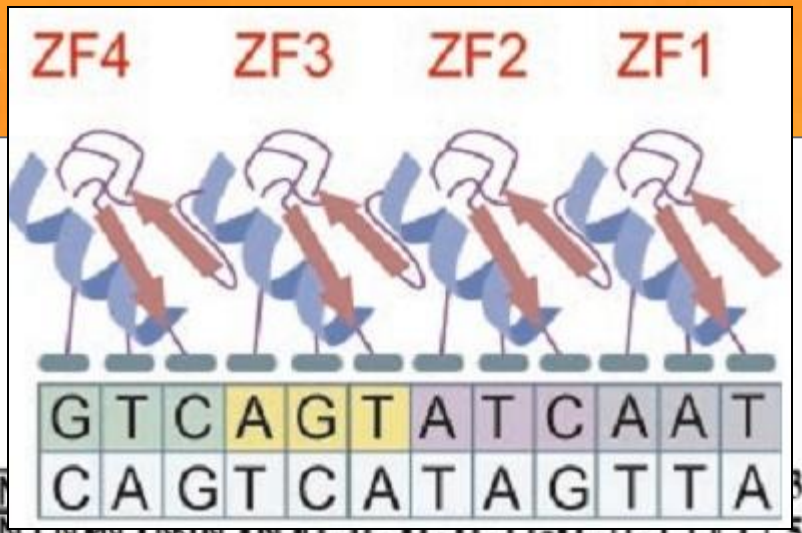


ZFN; Targeted Gene Editing

Zing finger
DNA binding



Modular assembly



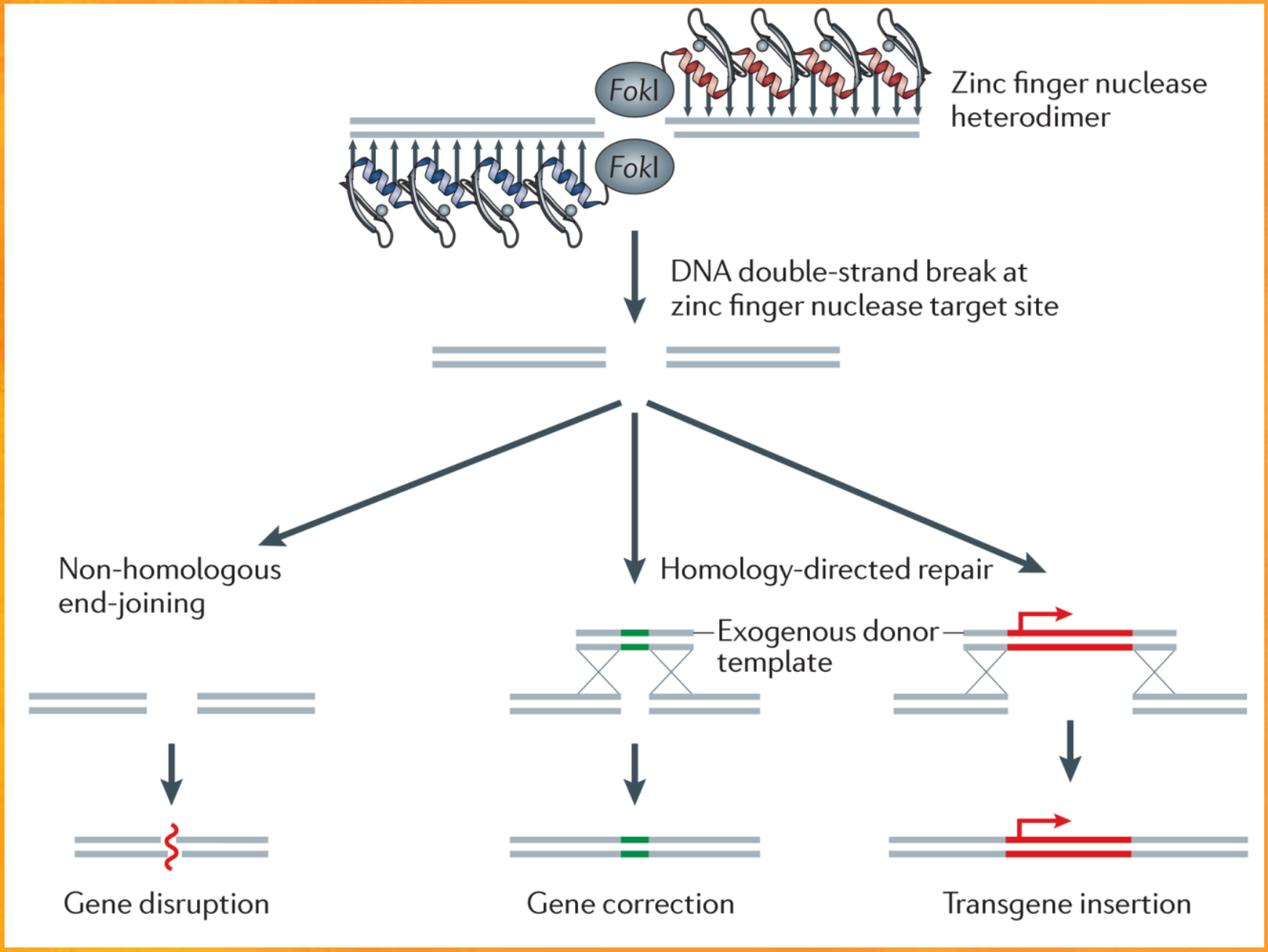
Double Strand Break(DSB)

Repairing

-NHEJ (error prone)

-Homology direct repair (HDR)

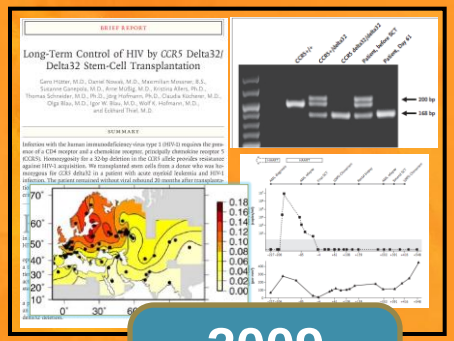
2005

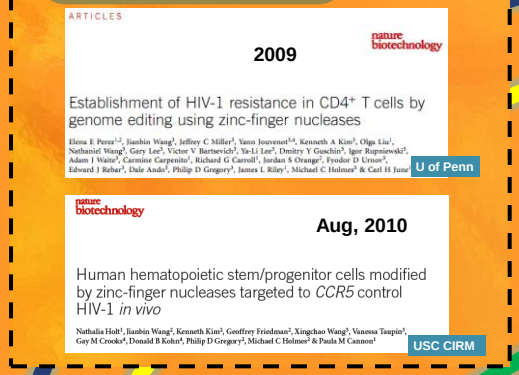

FokI
FokI
Zinc finger nuclease heterodimer
DNA double-strand break at zinc finger nuclease target site
Non-homologous end-joining
Homology-directed repair
Exogenous donor template
Gene disruption
Gene correction
Transgene insertion

The BERLIN Patient

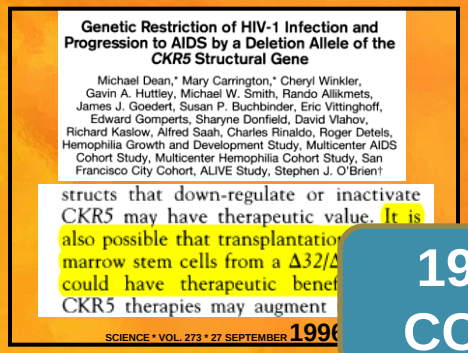
Bridging across disciplines

2010 ZFN CCR5


Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation



2009 BMT

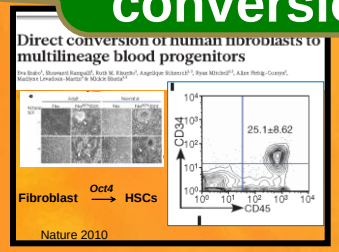

Genetic Restriction of HIV-1 Infection and Progression to AIDS by a Deletion Allele of the *CKR5* Structural Gene

structs that down-regulate or inactivate CKR5 may have therapeutic value. It is also possible that transplantation marrow stem cells from a Δ32/Δ could have therapeutic benef CKR5 therapies may augment

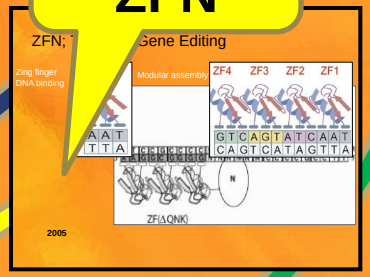
SCIENCE * VOL. 273 * 27 SEPTEMBER 1996

1996 CCR5

2010 Direct conversion

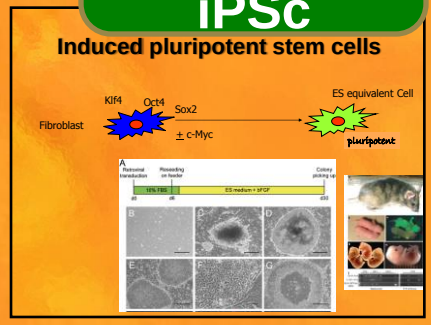


2005 ZFN



1983 discovery

2006 Yamanaka iPSc

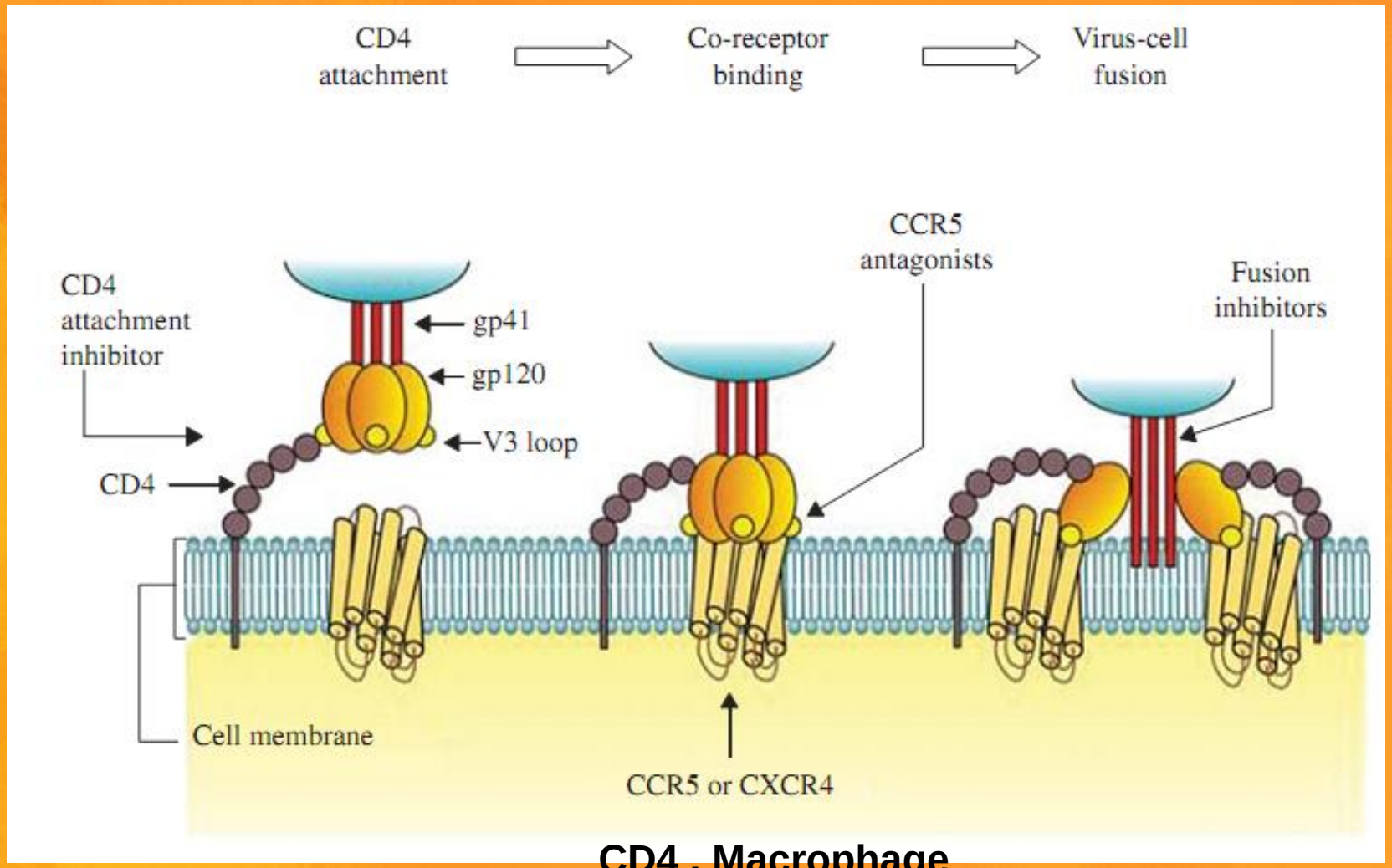


HIV

GT

Stem cell

HIV cellular entry



CD4 , Macrophage

ARTICLES

nature biotechnology

2009

Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases

Elena E Perez[1,2], Jianbin Wang[3], Jeffrey C Miller[3], Yann Jouvenot[3,4], Kenneth A Kim[3], Olga Liu[1], Nathaniel Wang[3], Gary Lee[3], Victor V Bartsevich[3], Ya-Li Lee[3], Dmitry Y Guschin[3], Igor Rupniewski[3], Adam J Waite[3], Carmine Carpenito[1], Richard G Carroll[1], Jordan S Orange[2], Fyodor D Urnov[3], Edward J Rebar[3], Dale Ando[3], Philip D Gregory[3], James L Riley[1], Michael C Holmes[3] & Carl H June[1]

U of Penn

nature biotechnology

Aug, 2010

Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to *CCR5* control HIV-1 *in vivo*

Nathalia Holt[1], Jianbin Wang[2], Kenneth Kim[2], Geoffrey Friedman[2], Xingchao Wang[3], Vanessa Taupin[3], Gay M Crooks[4], Donald B Kohn[4], Philip D Gregory[2], Michael C Holmes[2] & Paula M Cannon[1]

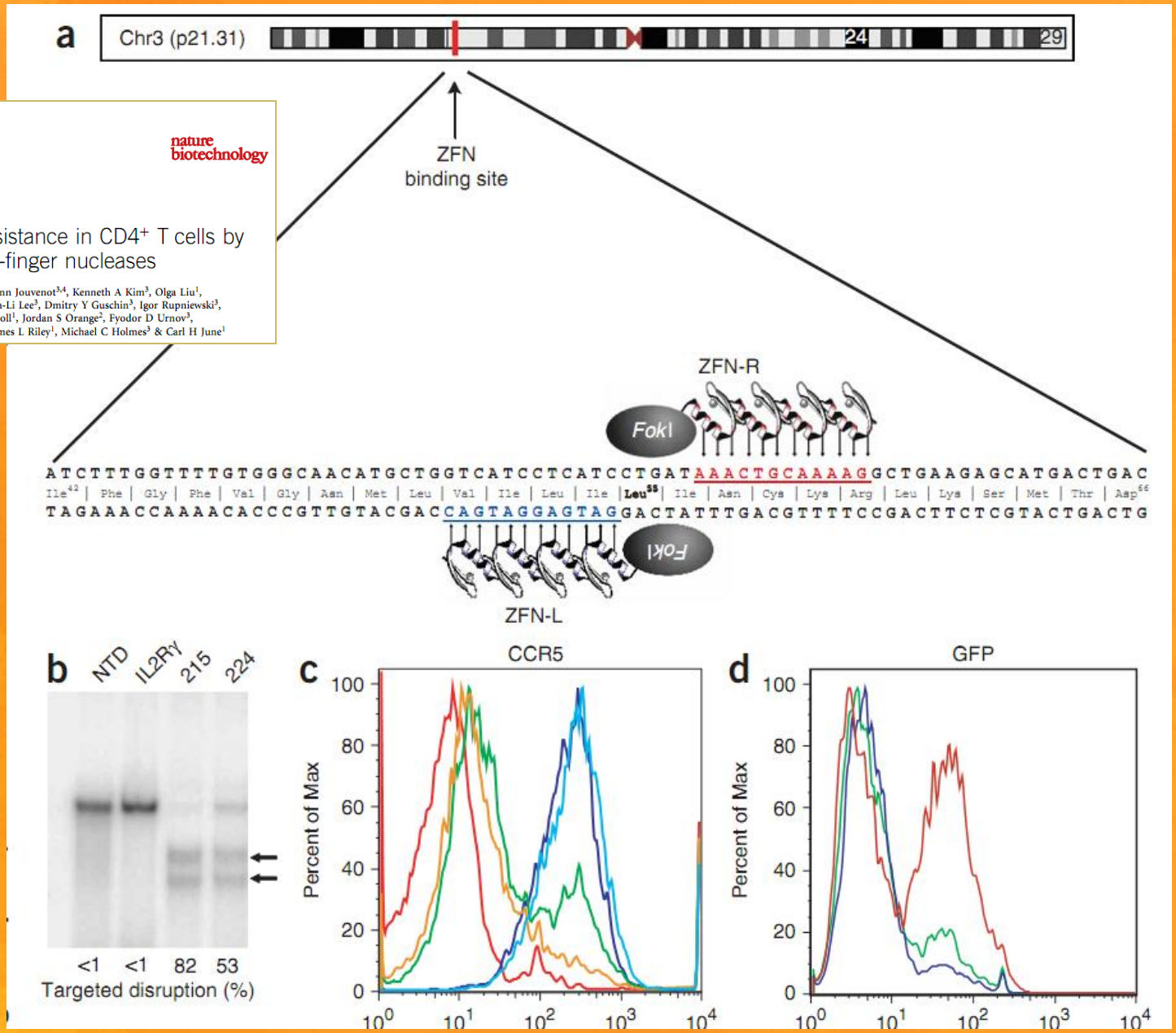
USC CIRM

ARTICLES

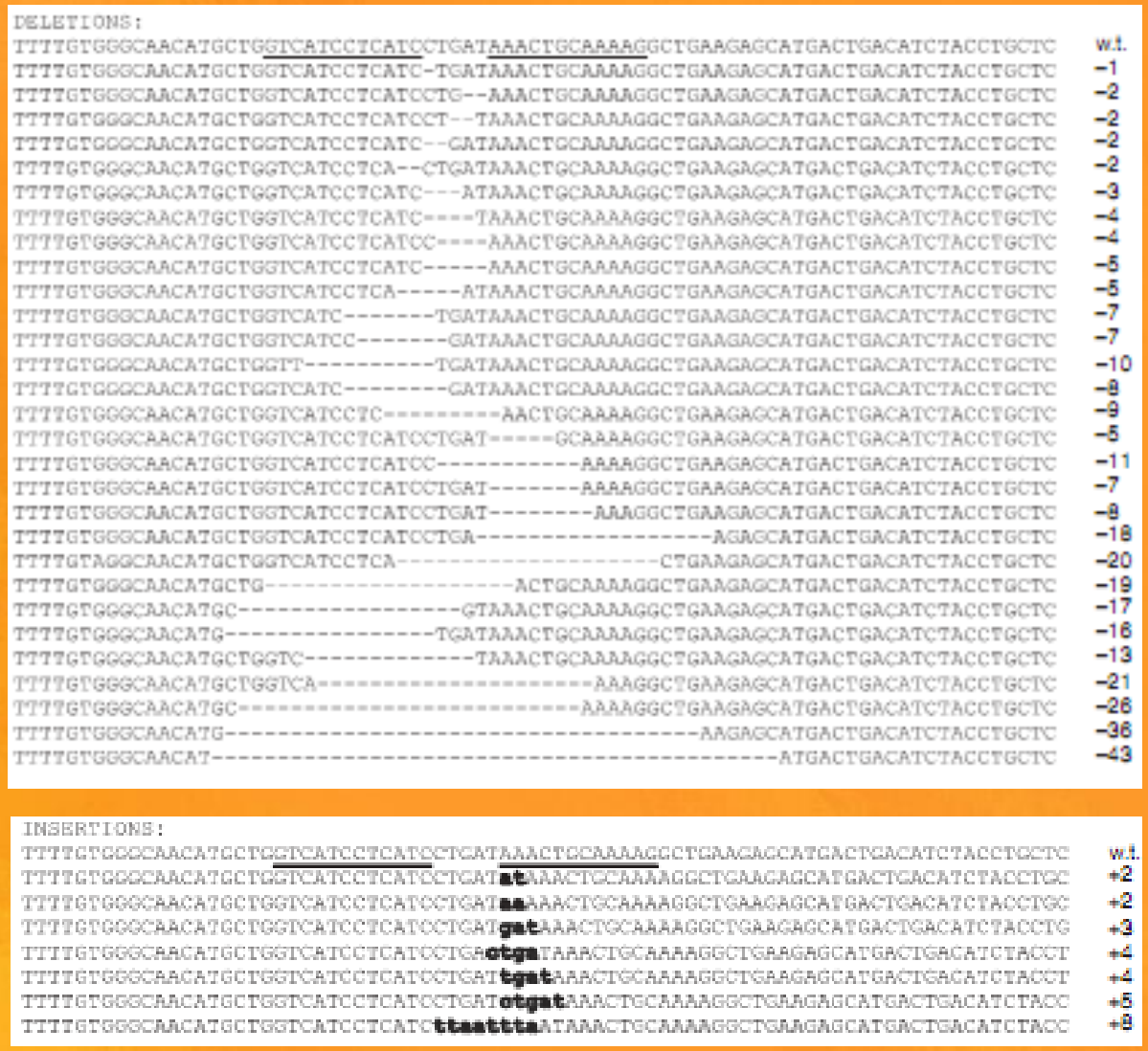
nature biotechnology

Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases

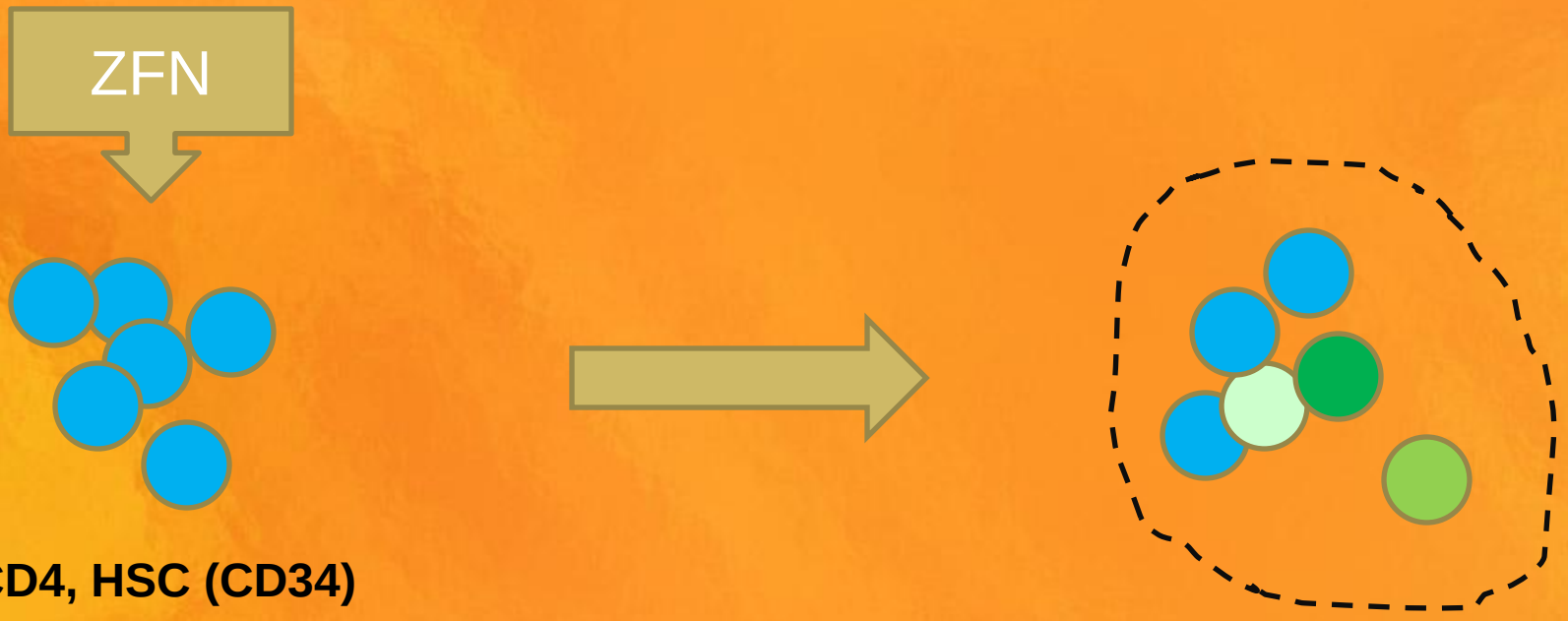
Elena E Perez[1,2], Jianbin Wang[3], Jeffrey C Miller[3], Yann Jouvenot[3,4], Kenneth A Kim[3], Olga Liu[1], Nathaniel Wang[3], Gary Lee[3], Victor V Bartsevich[3], Ya-Li Lee[3], Dmitry Y Guschin[3], Igor Rupniewski[3], Adam J Waite[3], Carmine Carpenito[1], Richard G Carroll[1], Jordan S Orange[2], Fyodor D Urnov[3], Edward J Rebar[3], Dale Ando[3], Philip D Gregory[3], James L Riley[1], Michael C Holmes[3] & Carl H June[1]



CCR5 genome editing



ZFN targeted to CCR5



CD4, HSC (CD34)

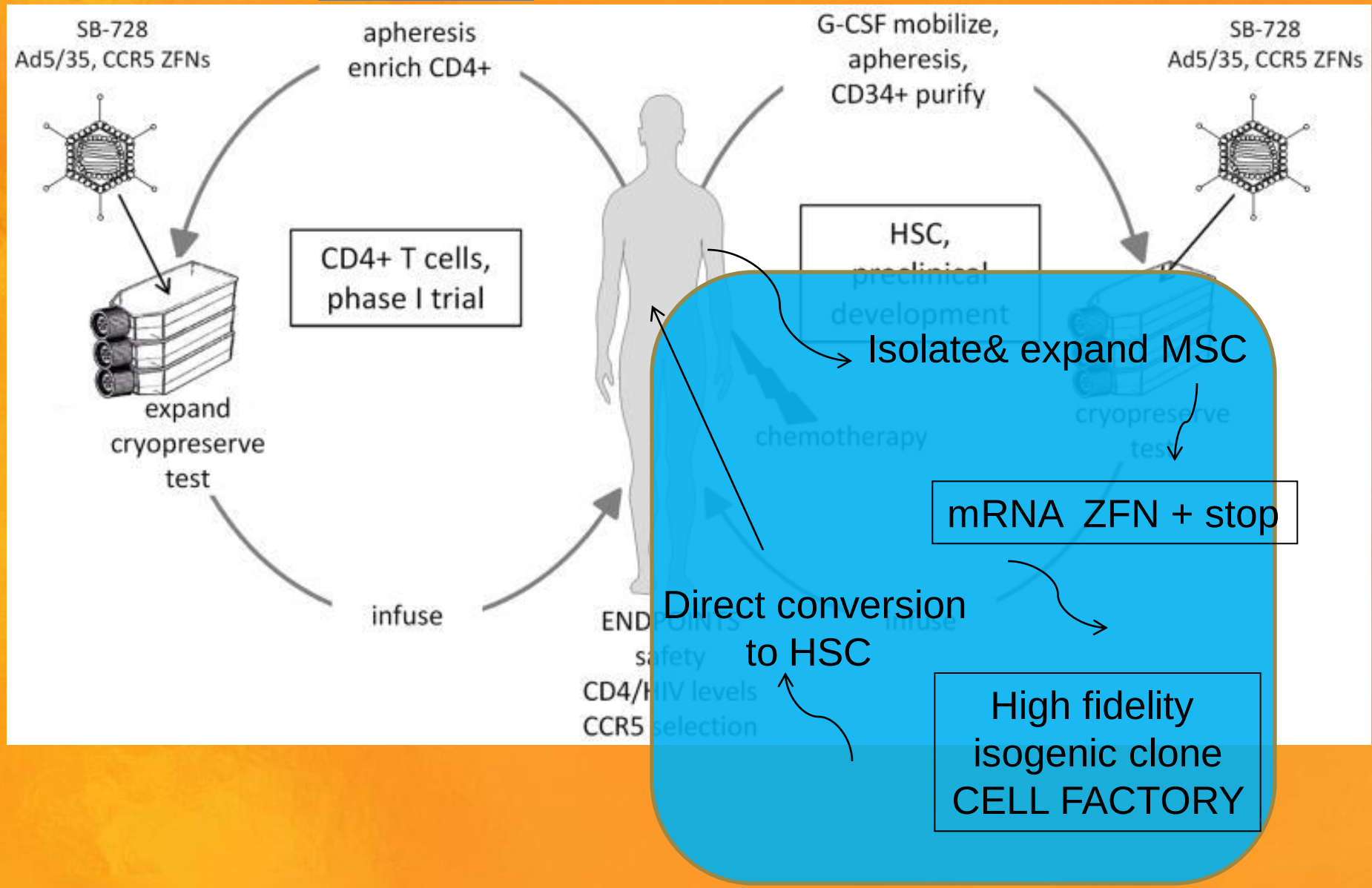
NHEJ is imprecise.
InDel mutations are unpredictable
Indel ; not equal with loss function

CD4, HSC in vitro expansion

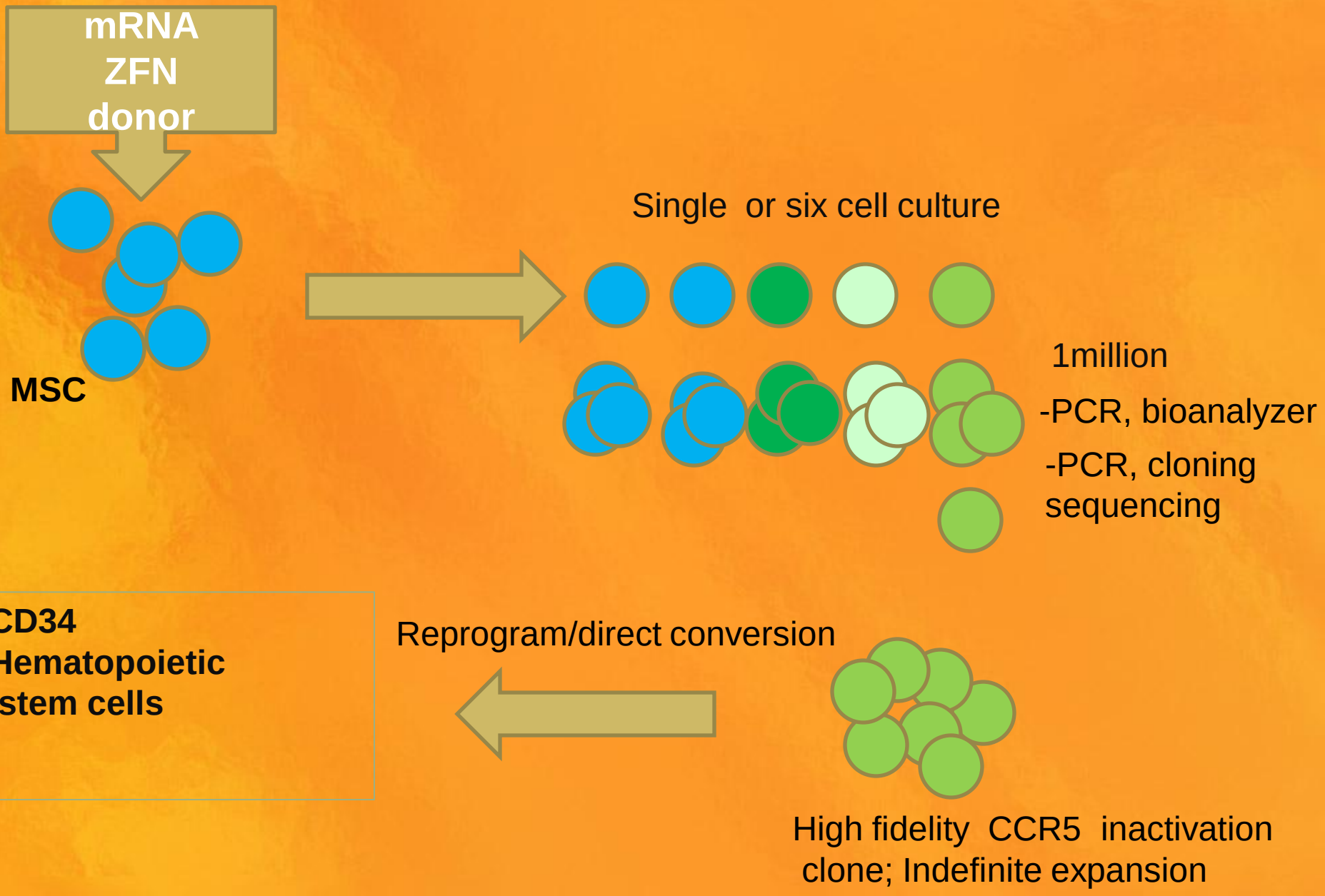
HDR

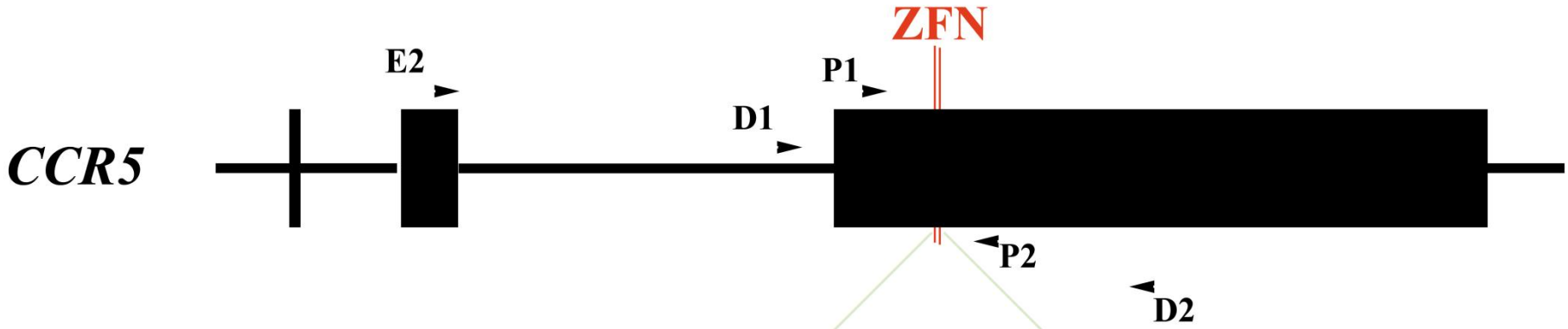
Other cells

High Fidelity Cell Clones


U of Penn
USC CIRM
SB-728
Ad5/35, CCR5 ZFNs
apheresis
enrich CD4+
CD4+ T cells,
phase I trial
expand
cryopreserve
test
infuse
G-CSF mobilize,
apheresis,
CD34+ purify
HSC,
SB-728
Ad5/35, CCR5 ZFNs
Isolate& expand MSC
mRNA ZFN + stop
Direct conversion
to HSC
High fidelity
isogenic clone
CELL FACTORY

ZFN targeted to CCR5

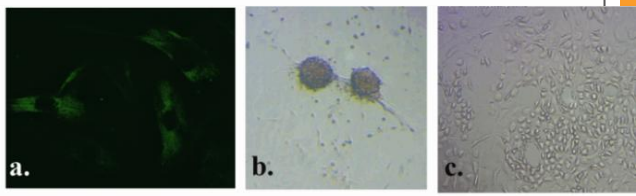


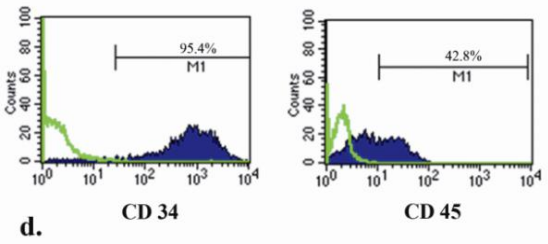


Donor ACACCTGCAGCTCTCAT*A*tt*TAGATAGTTAG*cc**ATACAGTCAGTATC**

CCR5 (NM_000579)	ggaatcatctttaccagatctcaaaaagaaggtcttcattacacctgcagctctcatttt----------ccatacagtcagtatcaattctggaagaatttccag
single-cell clones	ggaatcatctttaccagatctcaaaaagaaggtcttcattacacctgcagctctcatatttagatagttagccaaacagtcagtatcaattctggaagaatttccag ggaatcatctttaccagatctcaaaaagaaggtcttcattacacctgcagctctcatatttagatagttagccatacagtcagtatcaattctggaagaatttccag
six-cells clones	ggaatcatctttaccagatctcaaaaagaaggtcttcattacacctgcagctctcatatttagatagttagccatacagtcagtatcaattctggaagaatttccag ggaatcatctttaccagatctcaaaaagaaggtcttcattacacctgcagctctcatatttagatagttagccatacagtcagtatcaattctggaagaatttccag

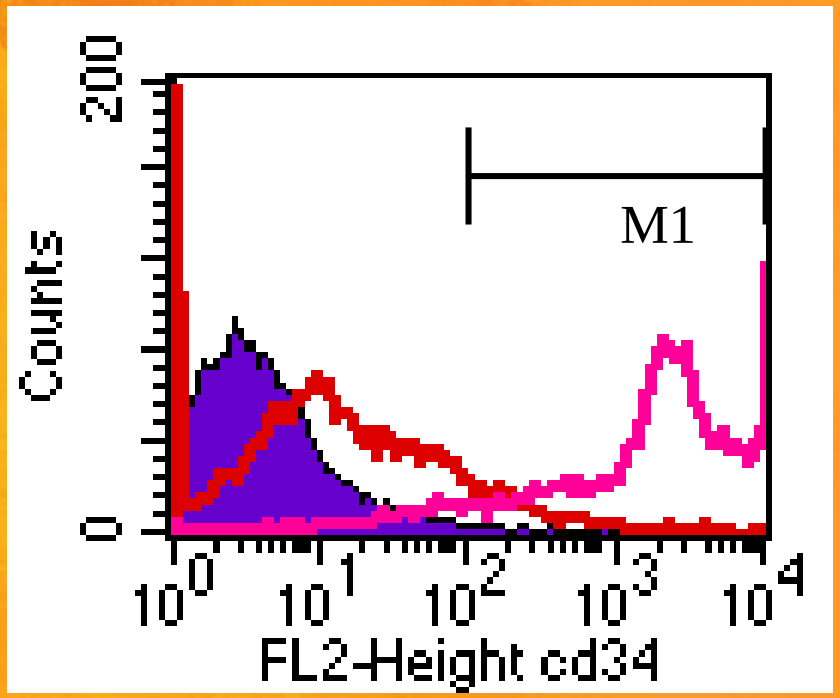
Single cell	4320	88	2
6 cells	986 (5916)	42	4

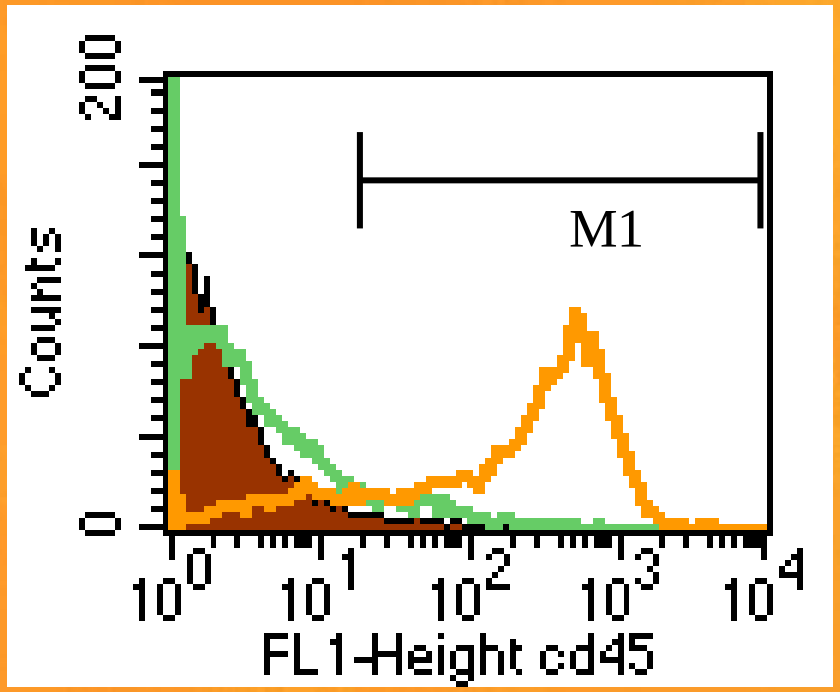


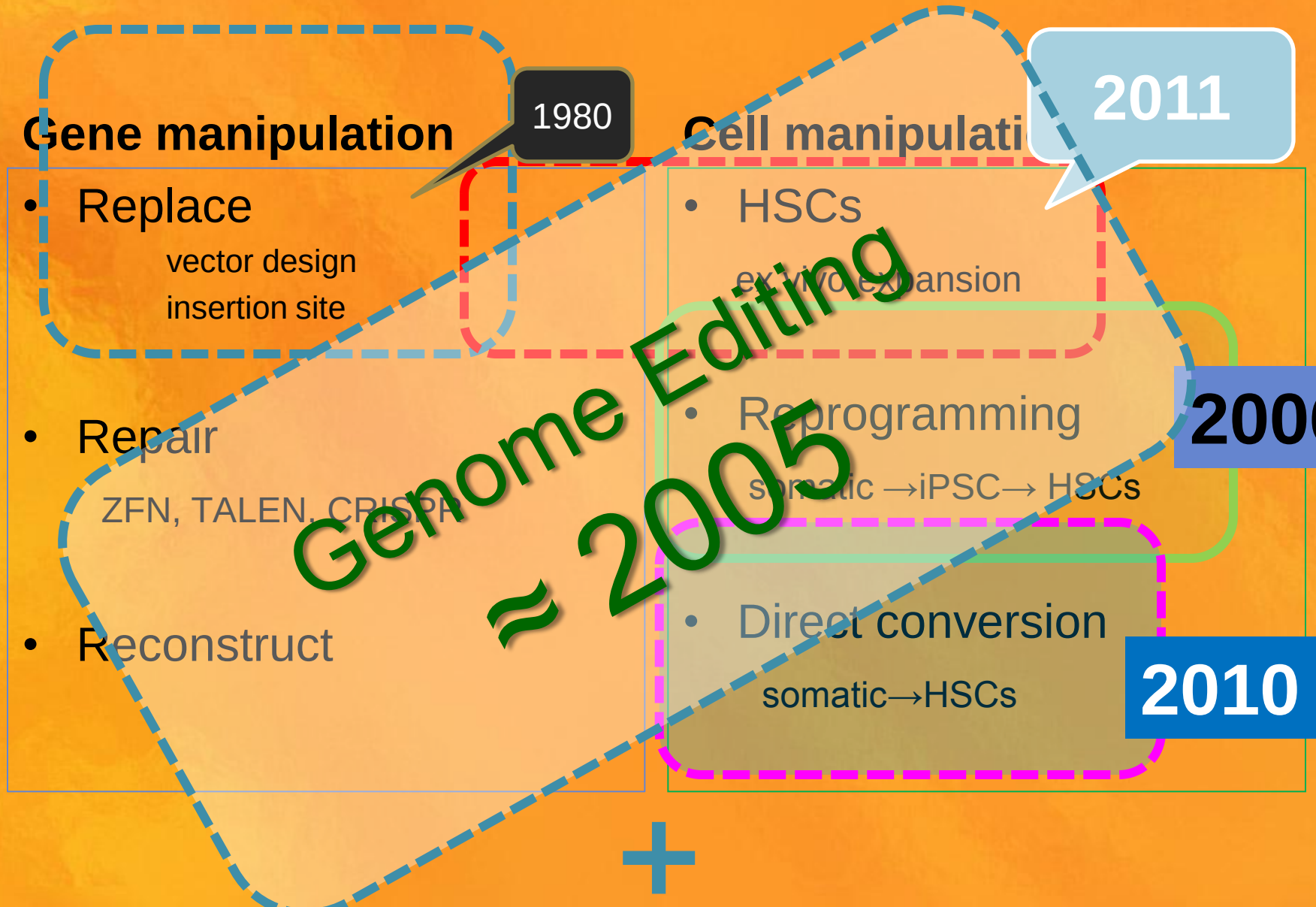


MSC

HSC


Counts
200
0
M1
10^0 10^1 10^2 10^3 10^4
FL2-Height cd34


Counts
200
0
M1
10^0 10^1 10^2 10^3 10^4
FL1-Height cd45

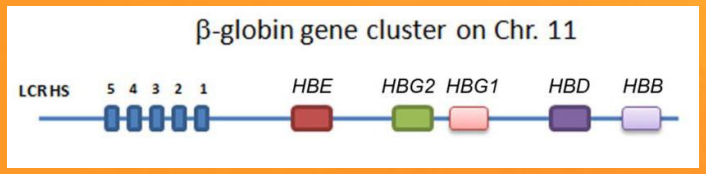

Gene manipulation
1980
Replace
vector design
insertion site
Repair
ZFN, TALEN, CRISPR
Reconstruct
Cell manipulati
2011
HSCs
ex vivo expansion
Reprogramming
somatic →iPSC→ HSCs
2006
Direct conversion
somatic→HSCs
2010
Genome Editing
≈ 2005
+

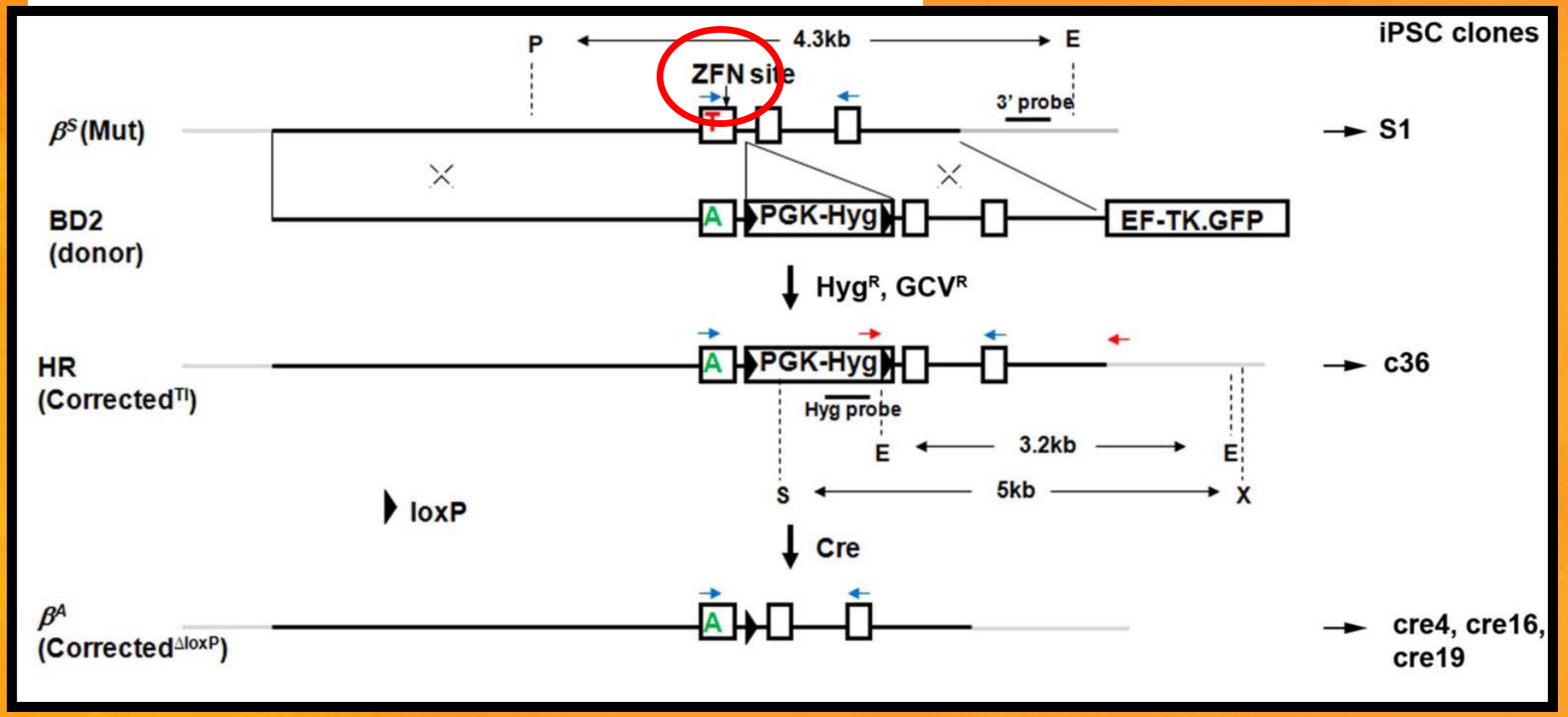
blood

2011 118: 4599-4608
doi:10.1182/blood-2011-02-335554

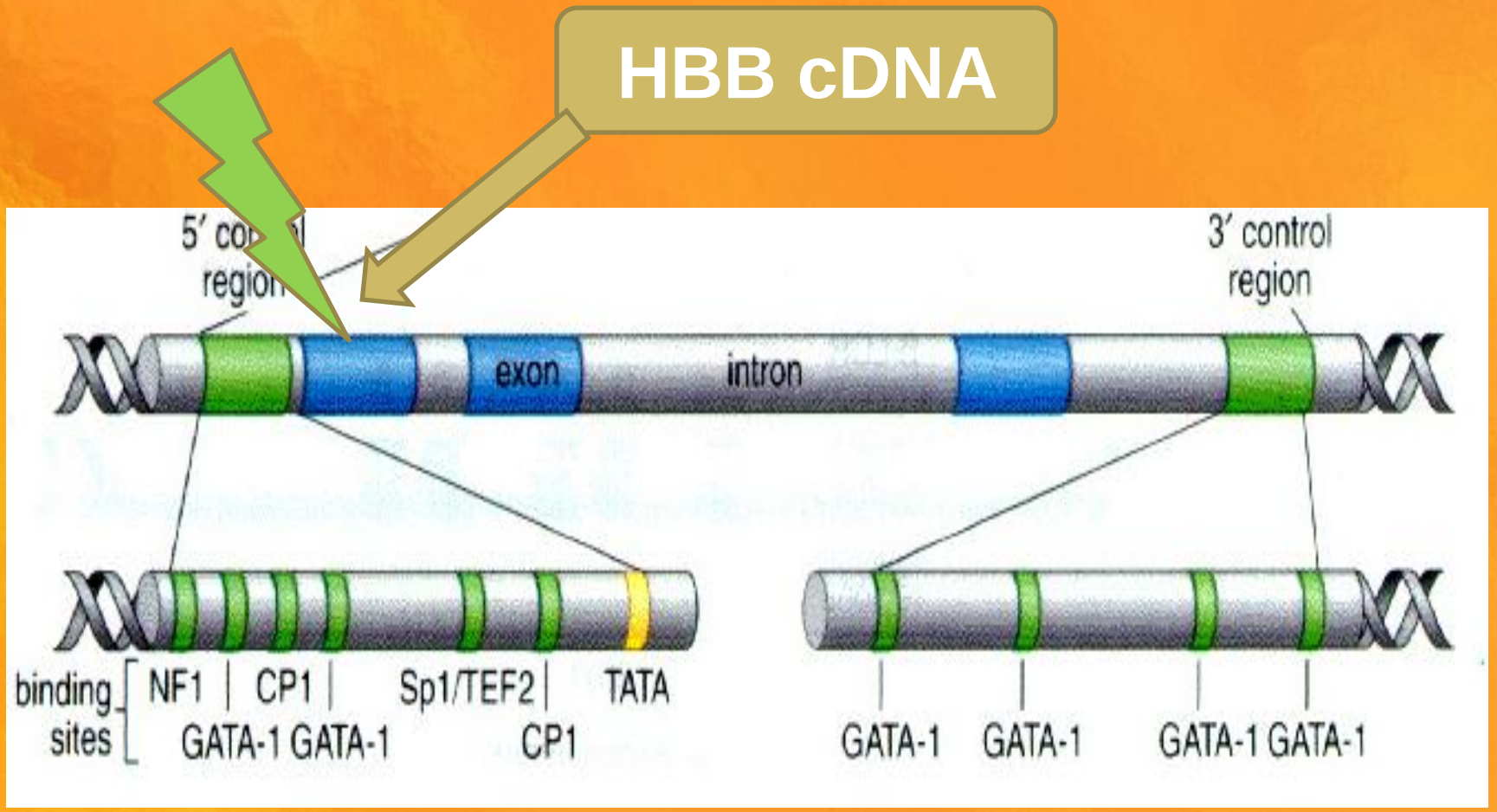
Site-specific gene correction of a point mutation in human iPS cells derived from an adult patient with sickle cell disease

Jizhong Zou, Prashant Mali, Xiaosong Huang, Sarah N. Dowey and Linzhao Cheng

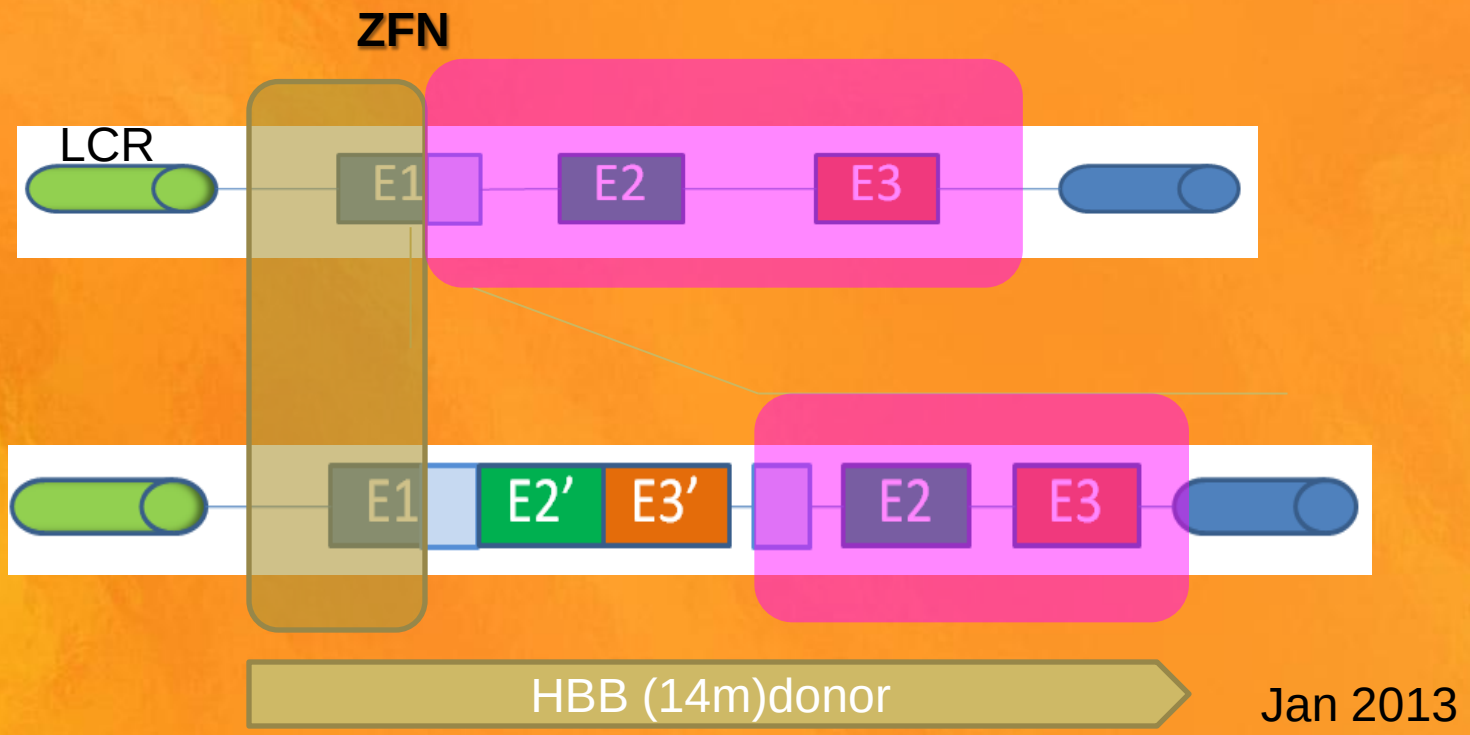




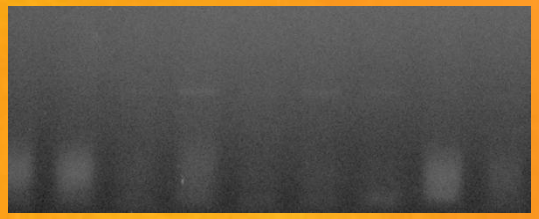
Reconstruction



Dec 2012



Jan 2013



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doi:10.1093/nar/gkt947

Nuclease-mediated gene editing by homologous recombination of the human globin locus

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[1]Department of Pediatrics, Stanford University, 1291 Welch Rd. Stanford, CA 94305, USA and [2]Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75390, USA

Received August 6, 2013; Revised September 24, 2013; Accepted September 25, 2013

ABSTRACT

Tal-effector nucleases (TALENs) are engineered proteins that can stimulate precise genome editing through specific DNA double-strand breaks. Sickle cell disease and β-thalassemia are common genetic disorders caused by mutations in β-globin, and we engineered a pair of highly active TALENs that induce modification of 54% of human β-globin alleles near the site of the sickle mutation. These TALENS stimulate targeted integration of therapeutic, full-length beta-globin cDNA to the endogenous β-globin locus in 19% of cells prior to selection as quantified by single molecule real-time sequencing. We also developed highly active TALENs to human γ-globin, a pharmacologic target in sickle cell disease therapy. Using the β-globin and γ-globin TALENs, we generated cell lines that express GFP under the control of the endogenous β-globin promoter and tdTomato under the control of the endogenous γ-globin promoter. With these fluorescent reporter cell lines, we screened a library of small molecule compounds for their differential effect on the transcriptional activity of the endogenous β- and γ-globin genes and identified several that preferentially upregulate γ-globin expression.

INTRODUCTION

Sickle cell disease is the most common monogenic disease worldwide and is caused by a single point mutation in the β-globin gene. Painful clinical symptoms begin shortly after birth as mutated β-globin subunits replace non-defective γ-globin chains in the predominant form of hemoglobin. Current pharmacological treatment with hydroxyurea partially reverses this globin switching by increasing the production of γ-globin (1,2). This has led to broad interest in developing other compounds and discovering new mechanisms that preferentially upregulate γ-globin (2–5), and also in developing methods to study globin regulation (6,7). Analyses of differential expression of β- and γ-globin generally have been limited to hemoglobin electrophoresis or qRT-PCR, but recent reports have described a method of using the expression of fluorescent molecules driven by the β- and γ-globin promoters as a readout of differential globin regulation. In those studies, the authors integrated into the genome a bacterial artificial chromosome containing the entire 200 kb β-globin locus (which includes both β-globin and γ-globin among other genes), modified such that the β- and γ-globin promoters drive expression of fluorescent proteins (6,7). The integration of the complete genomic locus presumably maintains much of the physiologically relevant regulation of expression, but it does not allow for the direct analysis of the endogenous locus and is confounded by the fact that integration is in a random genomic location and that some cells gain multiple copies of the BAC. In addition, a BAC-based strategy creates a system in which the globin locus is triploid rather than diploid and this change may also affect the regulatory dynamics. Alternatively, direct modification of the endogenous β- and γ-globin loci eliminates those confounding variables.

Endogenous genomic loci can be precisely altered using engineered zinc finger nucleases (ZFNs) (8–11) and Tal-effector nucleases (TALENs) (12–14). ZFNs and TALENs are comprised of a specifically engineered DNA binding domain fused to the FokI endonuclease domain. Binding of a pair of ZFNs or TALENs to contiguous sites leads to the dimerization of the FokI domain, resulting in a targeted DNA double-strand break. Repair of the break can proceed by mutagenic non-homologous end joining or by high-fidelity homologous recombination with a homologous DNA donor template. Compared to ZFNs, TALENs seem to cause lower levels of cytotoxicity (15). Their recognition domain is characterized by repeated arrays of 34 conserved amino acids, except in positions 12 and 13. These two amino acids comprise the repeat variable domain (RVD), which contacts the DNA and provides the nucleotide recognition specificity

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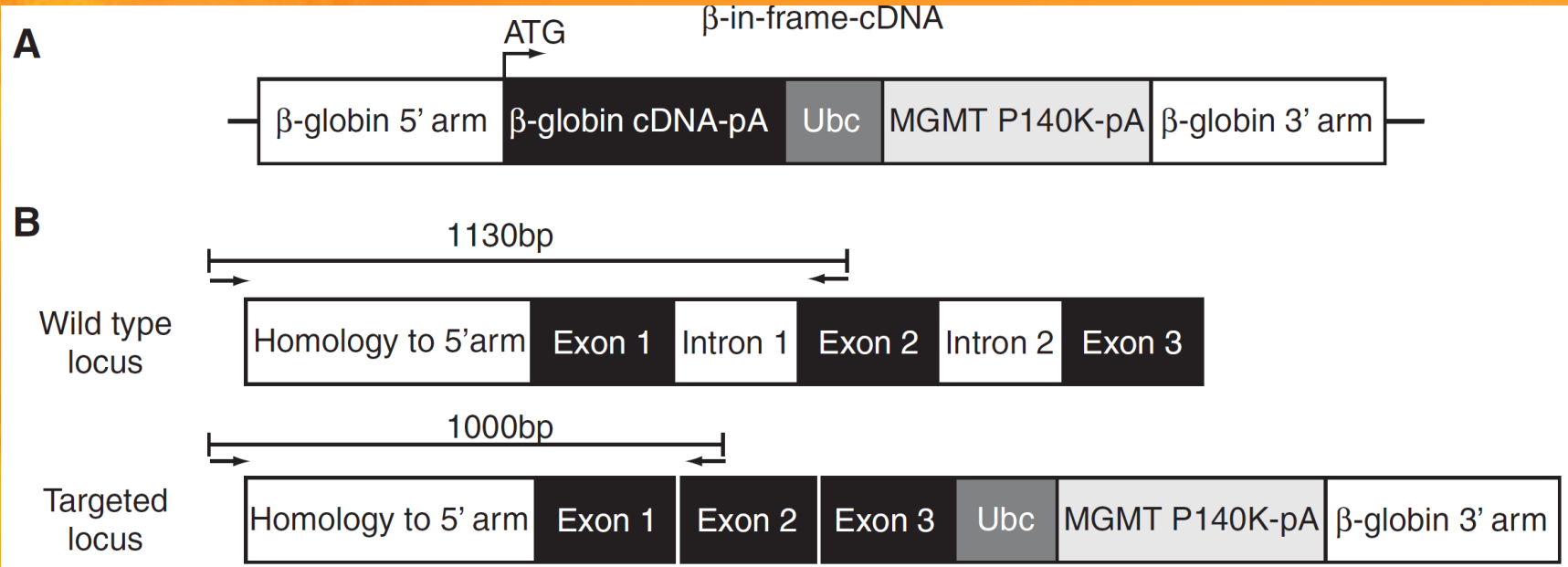
Matthew H. Porteus, Department of Pediatrics, 1291 Welch Rd. G3040, Stanford University, Stanford, CA 94305, USA.



C

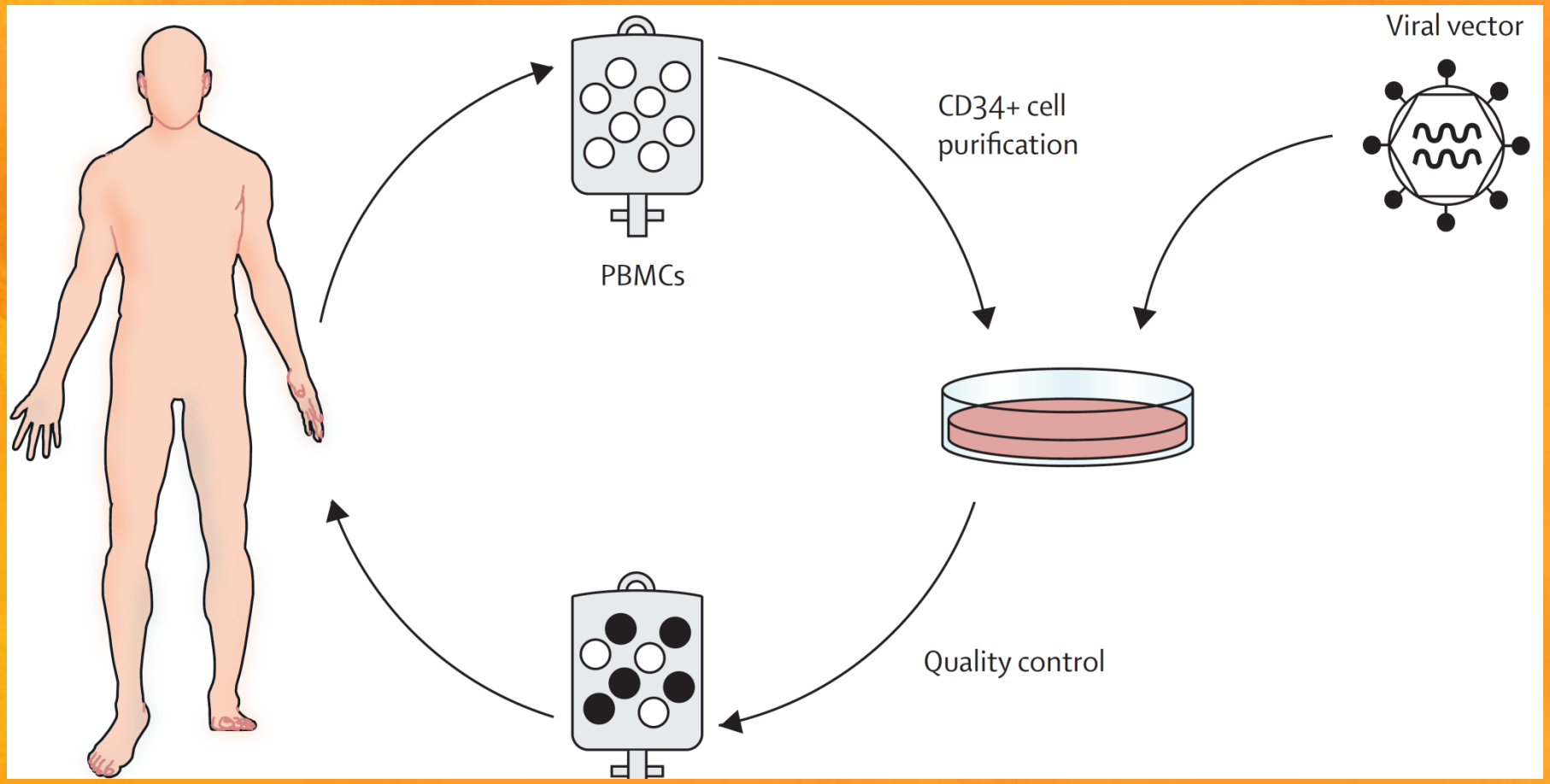
Drug pulses	-TALENs		+TALENs	
	# Sequences	% Targeting	# Sequences	% Targeting
0	941	0%	1100	8%
1	1101	0%	1319	69%
2	1119	0%	1100	61%
3	1100	0%	1493	61%

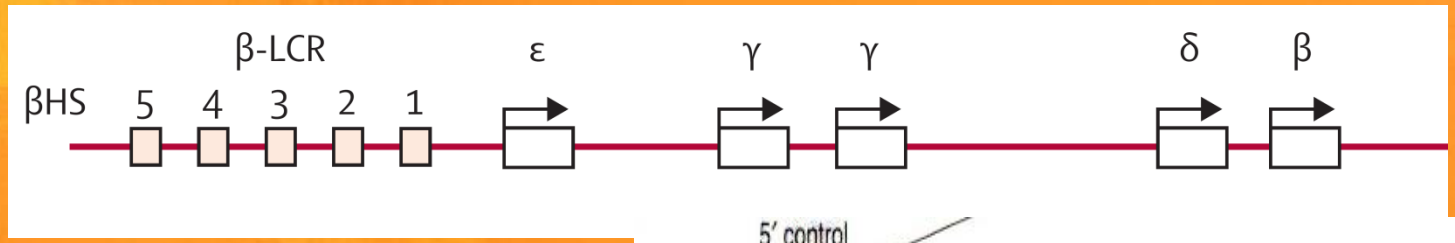
Gene manipulation

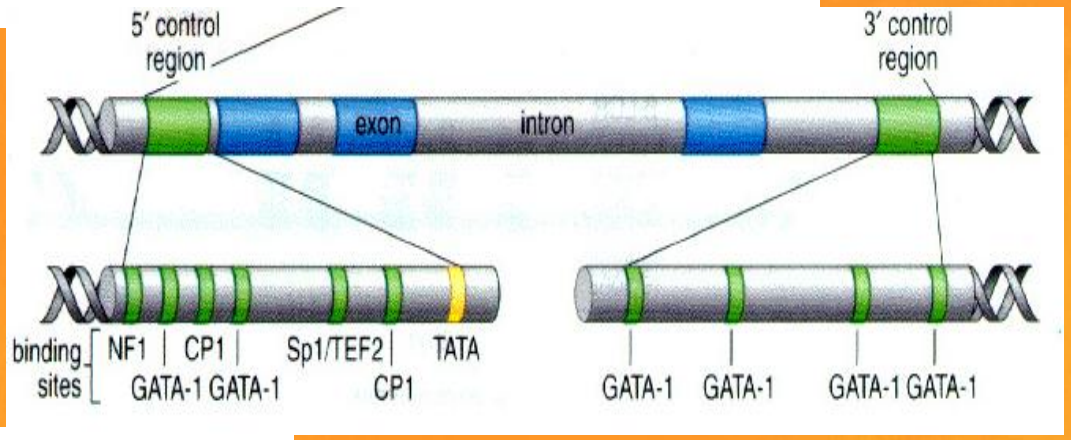
- Replace
  - vector design
  - insertion site
- Repair
  - ZFN, TALEN, CRISPR
- Reconstruct

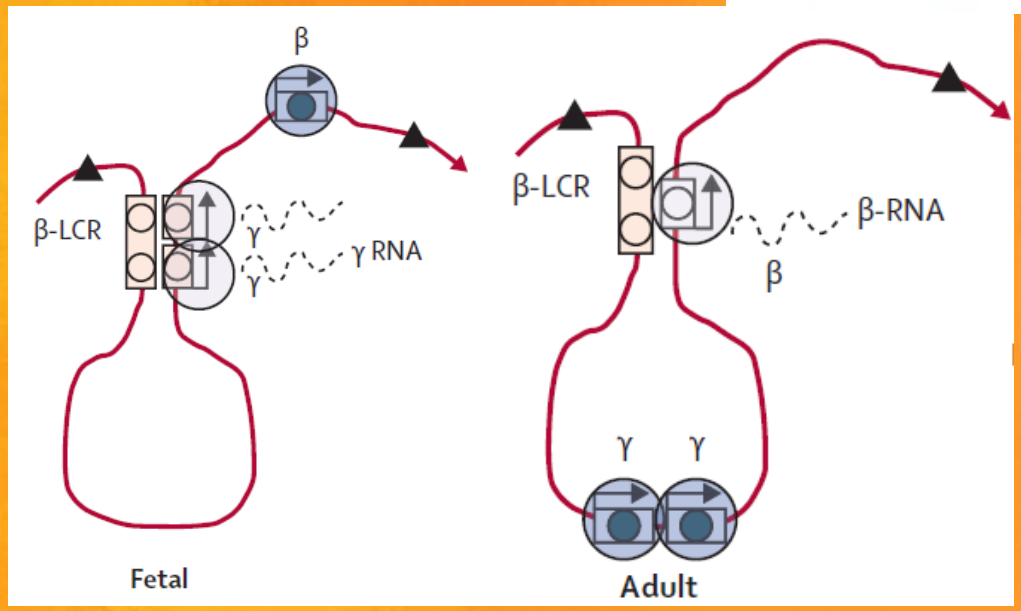
Cell manipulation

- HSCs
  - ex vivo expansion
- Reprogramming
  - somatic →iPSC→ HSCs
- Direct conversion
  - somatic→HSCs


Viral vector
CD34+ cell
purification
PBMCs
Quality control


β-LCR
ε
γ
γ
δ
β
βHS
5
4
3
2
1


5′ control region
3′ control region
exon
intron
binding sites
NF1
CP1
Sp1/TEF2
TATA
GATA-1 GATA-1
CP1
GATA-1
GATA-1
GATA-1 GATA-1


β
β-LCR
γ
γ
γ RNA
Fetal
β-LCR
β-RNA
β
γ
γ
Adult