

Gene Therapy for Genetic Diseases of Blood Cells

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Genetic Diseases of Blood Cells

Primary immune deficiencies:

SCID, WAS, CGD, HLH, LAD, X-HIM, IPEX

Hemoglobinopathies:

Sickle Cell Disease, Thalassemia

Lysosomal storage and metabolic disorders:

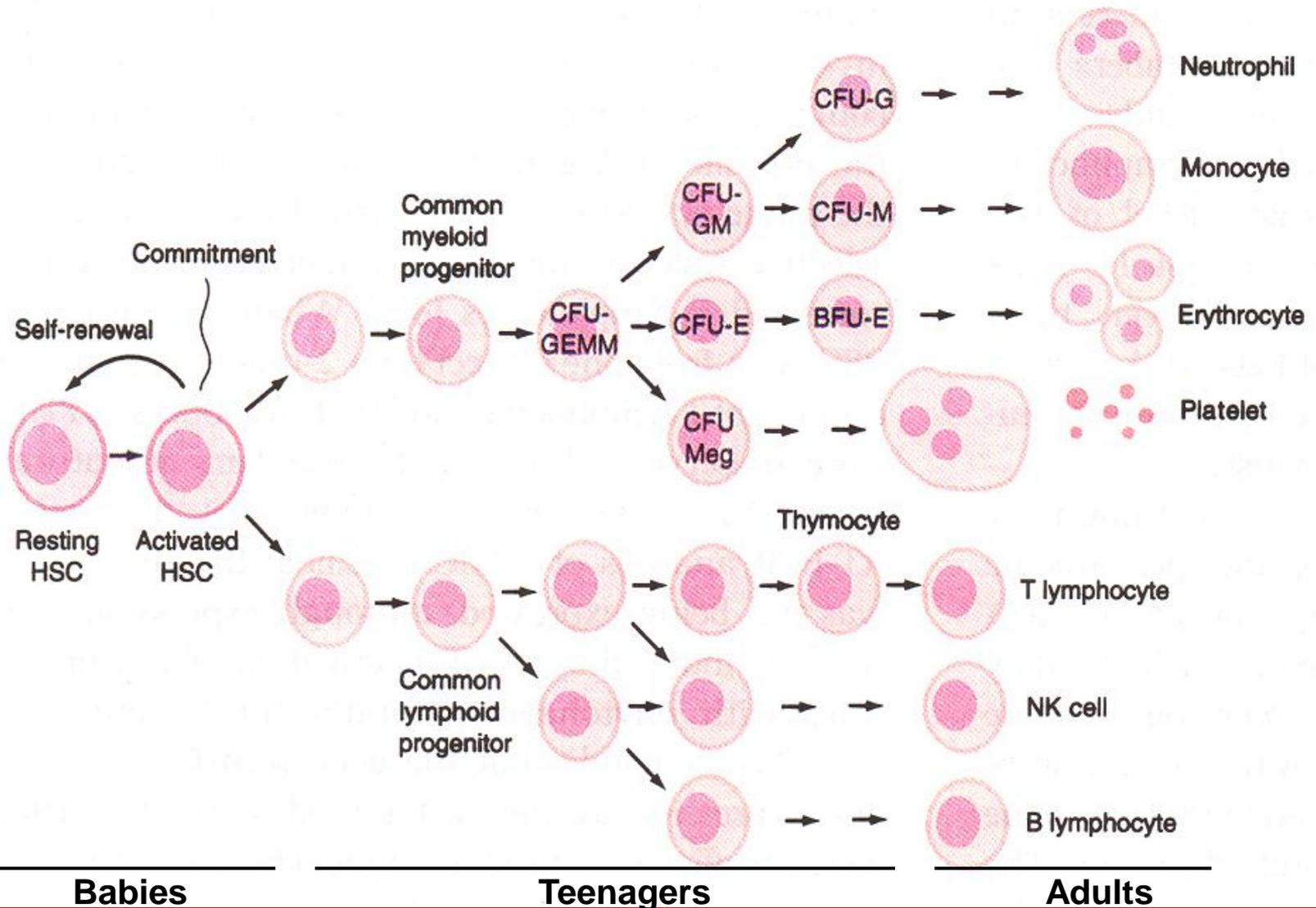
Gaucher, MPS, X-ALD, MLD, osteopetrosis

Congenital cytopenias:

Fanconi's, Blackfan-Diamond, Kostmann's, Glanzmann's

Ex HSC omni

Hematopoietic Stem Cells (HSC) Produce All of the Blood Cells



HSC Biology

Make all blood cells

Highly generative through progenitors-> millions of progeny

Transplantable. Durable.

Rare, often quiescent. Fragile *ex vivo*.

Better fresh than frozen, especially when alone.

We can't make more (yet), only less (one day: iPSC→HSC).

Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

~3% of all HSCT done for genetic diseases of blood cells

Outcomes vary by HSC source and disease co-morbidities:

--Matched siblings > unrelated adult (BM/PBSC), cord blood

Marrow and immune ablation are usually done with high dose combination chemotherapy (e.g. Bu/Flu/ATG)

--full, partial (reduced), none

Immune issues (Graft versus host disease {GVHD} and rejection) may limit availability and successful outcomes.

Bone Marrow Transplant -an example of Stem Cell Therapy



Harvesting bone marrow from the Donor

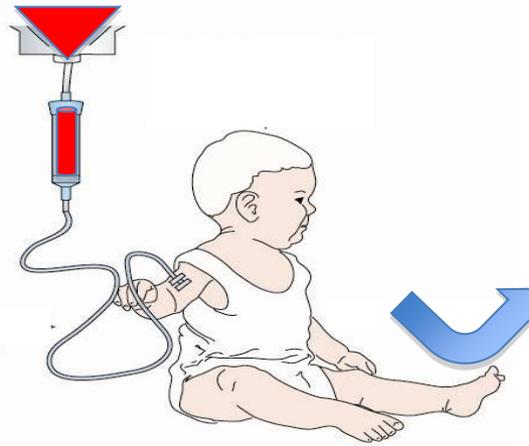


Processing Stem Cells

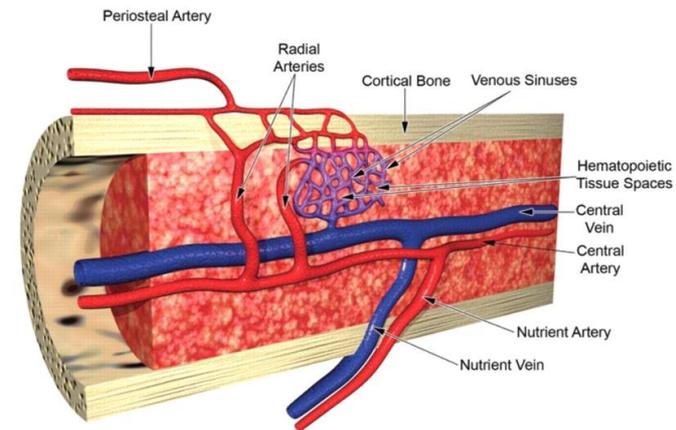


Patient is "conditioned" with high dose chemotherapy

Stem cells infused

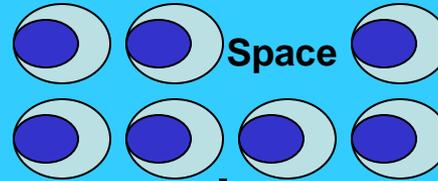


Intensive medical support until stem cells grow



Outcome after HSCT with Full, Partial or No Marrow Conditioning

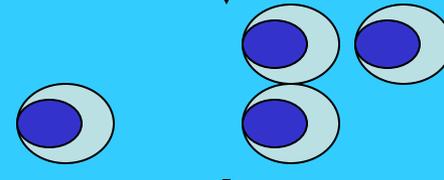
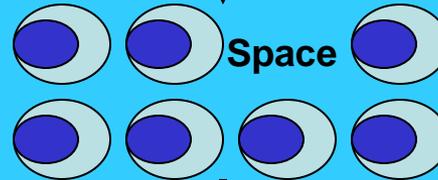
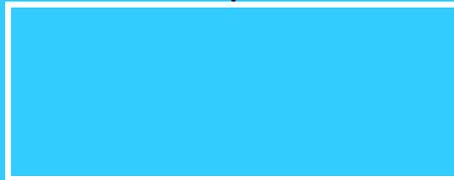
Patient's Bone Marrow HSC



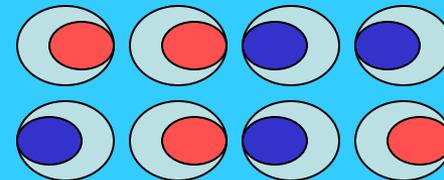
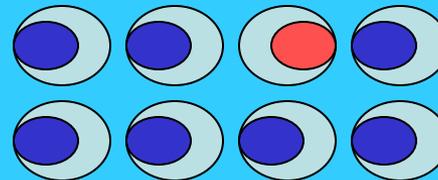
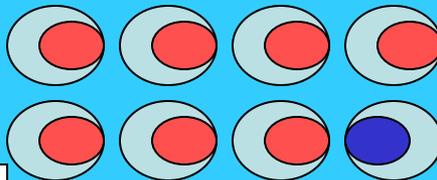
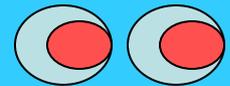
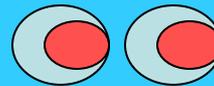
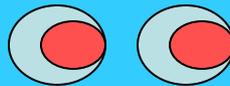
Full Marrow Conditioning

None

Partial Marrow Conditioning



Donor or Auto/Gtx HSC



Donor Chimerism

Full

Minimal

Mixed

Severe Combined Immune Deficiency - SCID

Fatal in infancy due to infections. >20 genetic causes.

First successful human BMT (1968).

First use of matched unrelated donor (MUD) BM(1974).

First transplanted with haplo-identical (parent) BM (1976).

First approached by gene therapy (1990).

First “cure” by gene therapy (1999).

BMT with MSD (no chemo) x 40 years – >90% successful

HSCT with haplo-identical (parent) or unrelated donors have poorer outcomes (55-75%) due to GVHD, infections,

Sickle Cell Disease - SCID

Causes significant morbidity, progressive organ damage

--Of ~80,000 affected in US, ~250-300 had BMT (0.4%).

--First done in 1976 for patient with SCD who also developed leukemia and needed BMT for leukemia.

→ SCD eliminated.

--Mostly done for children with SCD who had a matched sibling donor. ~90% success, 5% mortality, 5% rejection.

--More recently, transplants from unrelated donors (UD), either adults or cord blood have been done.

Sickle Cell Disease, Bone Marrow Transplant and Gene Therapy

Mark Walters (CHRCO) –
BMT for SCD with matched sibling donors.

59 transplanted. Bu/Cy/ATG. 10 year follow-up

93% Survival: 4 of 59 patients died.

85% (50/59) alive without sickle cell disease

5/59 patients rejected the donor's BM and
their SCD BM recovered, with return of SCD

(Walters MC et al. Biol Blood Marrow Transplant 16: 263-272, 2010)

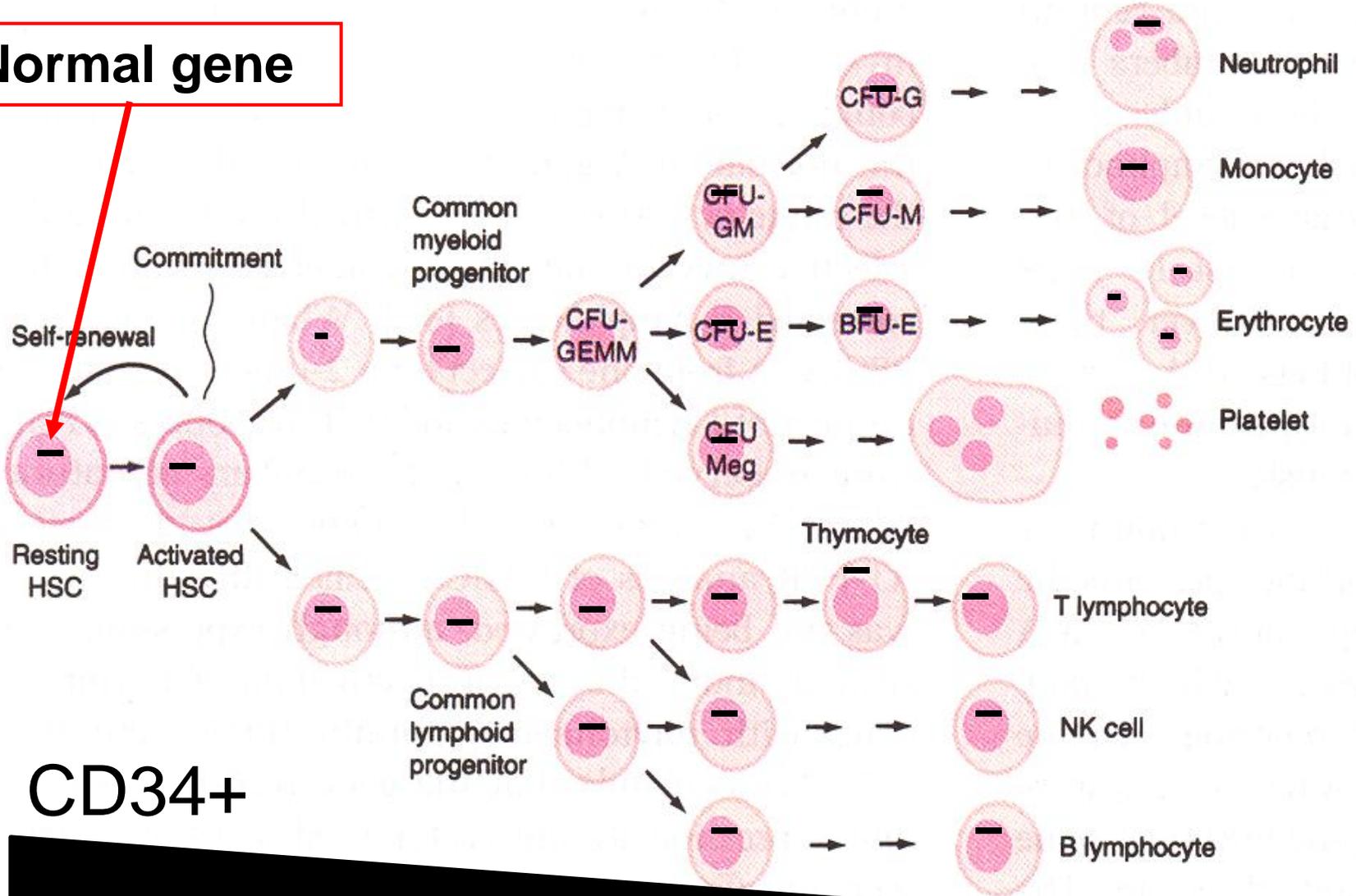
HSCT for Genetic Diseases of Blood Cells

Results with allogeneic HSCT continue to improve with better conditioning regimens, donor matching, immune manipulations, and medical management, but.....

Hypothesis: *HSCT for genetic diseases using gene-modified autologous HSC (gene therapy) may have similar benefits as allogeneic HSCT with less immunologic complications. Availability and outcomes may be improved.*

Hematopoietic Stem Cell Differentiation

Normal gene



CD34+

Gene Modification of HSC

Must be permanent (or able to self-replicate very fast – episomes, artificial chromosomes) due to massive HSC proliferation.

Stably add replacement gene (cDNA, mini-gene, genomic) or modify endogenous locus.

Transfection, electroporation, adenovirus, AAV
-- low efficiency, transient, +/- cytotoxic

Integrating retroviridae (RV, LV, FV), transposons
--- Incremental improvements in vectors design and production, HSC culture/stimulation methods

Gene Modification of HSC

HSC procurement: BM harvest, PBSC mobilization/pheresis

Isolate CD34+ cell fraction

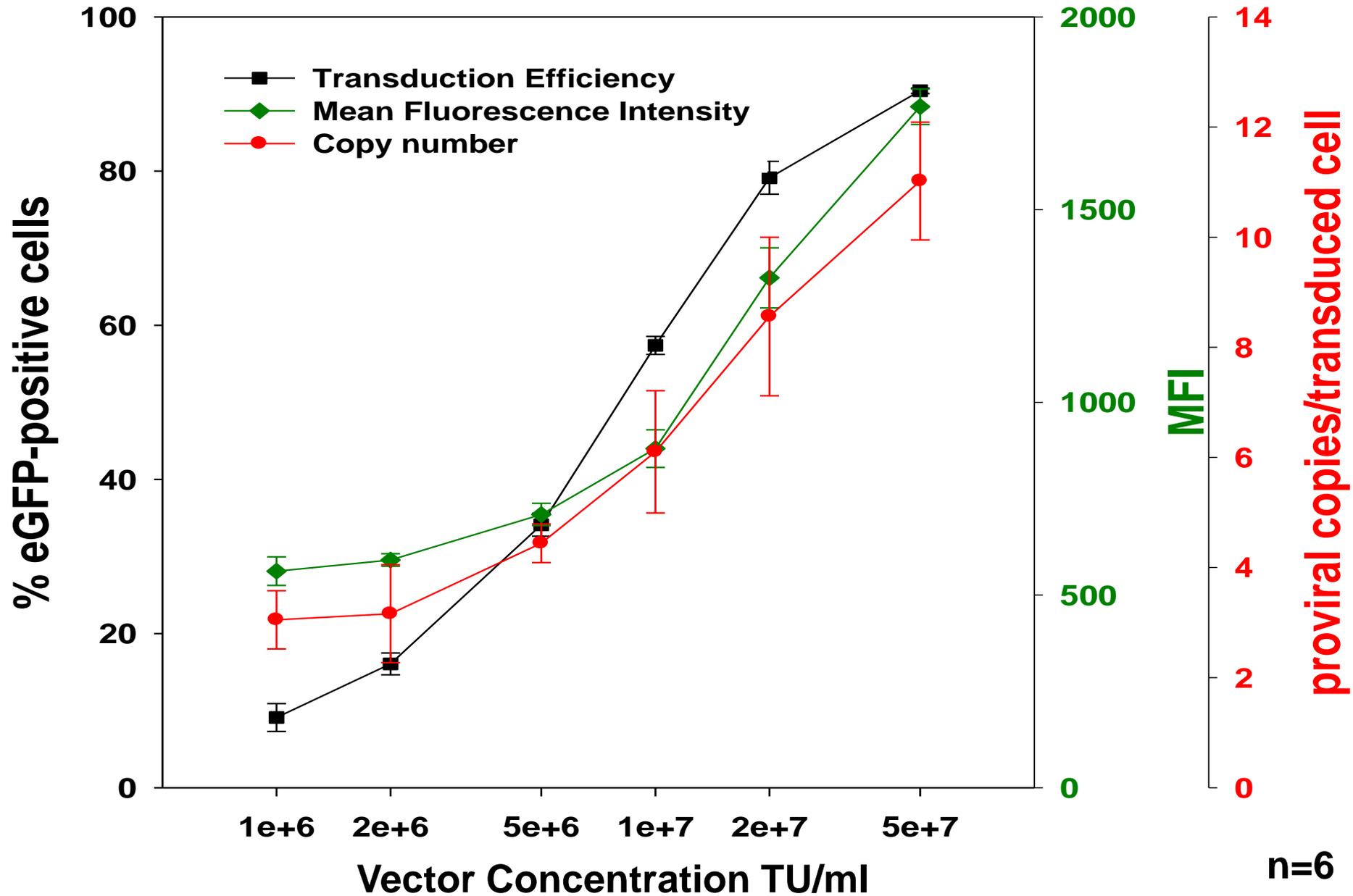
Culture with cytokine combination, ECM, vector 1-5 days

Wash, formulate, certify, release, infuse IV.

Challenge: genetically correct highest percentage of HSC
with least genomic perturbation.

---Poisson gets us every time....

Lentiviral Vector - Dose Response of Human CD34+ BM Cells: Transduction Efficiency, Mean Fluorescence Intensity and Copy Number



Retroviral Vector Era (1990 - ~2005)

ADA- SCID, XSCID, CGD, LAD, Gaucher, FA

ADA- SCID –

First disease approached. NIH - T cells 1990

Initial HSC trials in Europe and US – no benefit

Milan (HSR) – success with partial myeloreduction (Bu-4)

Later – UK and US similar clinical benefits

Works best in infants, some older with incomplete effect.

No SAE related to vector or procedure

Summary of ADA-Deficient SCID Patients

Retroviral Vectors, Myeloreductive Conditioning

Milan / London / Los Angeles-Bethesda

Center	# Pts	F/U (yrs)¹	Off Enzyme	Survival	DFS²
Milan	18	0.8 – 11.5	15/18	100%	83.3%
London	8	0.5 – 7.5	4/8	100%	50%
CHLA-NHGRI	6	3–7	3/6	100%	50%
UCLA-NHGRI	8	0.5-3	7/8	100%	87.5%
TOTAL	40	0.1 – 11.5	29/40	100%	72.5%

¹ As of May 2012

²DFS ≡ Alive without BMT or PEG-ADA re-start

Data: Courtesy HR Gaspar (London) and Alessandro Aiuti (Milan)

Retroviral Vector Era (1990 - ~2005)

XSCID –

Robust immune reconstitution without conditioning in trials in France and UK. Event-free survival with immune reconstitution = 83% (\geq haplo/MUD results).

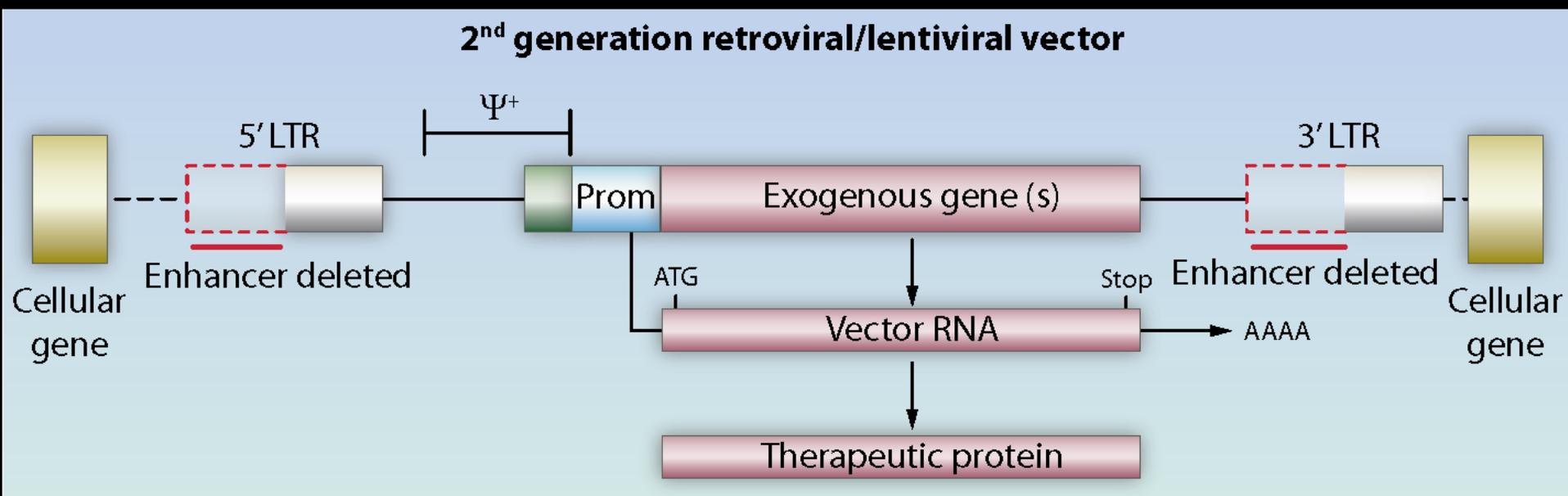
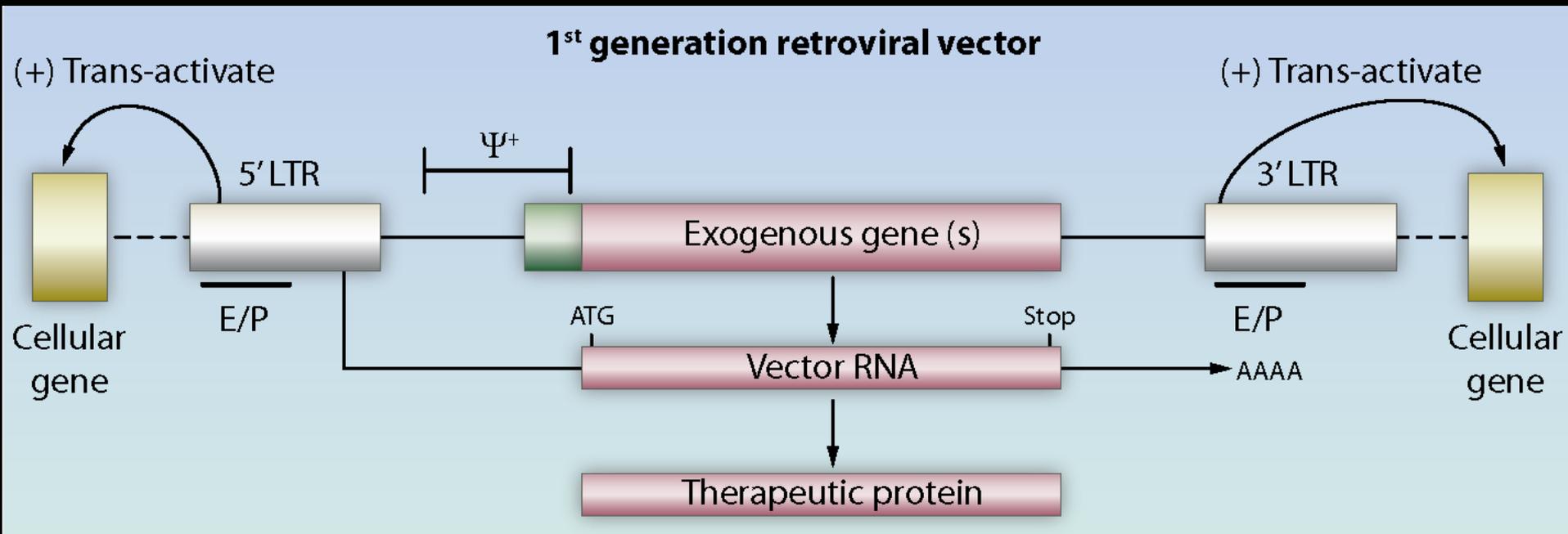
But, 5/20 subjects developed T leukoproliferation, due to vector-mediated insertional oncogenesis (IO).

Similarly, in trials for CGD and WAS, immune restoration achieved, but leukoproliferation also occurred in some (2/3 and 4/10, respectively).

Next Generation Vectors

Newer lentiviral (and γ -retroviral) vectors have been developed that show significantly reduced risks for causing IO in pre-clinical murine models (in vitro and BMT)

First and Second Generation Retroviral and Lentiviral Vectors



Lentiviral Vector Era (~2005-present)

X-ALD - full ablation and LV transduction led to 10-20% enduring gene-modified cells and neurologic stabilization

β -thalassemia – one subject became transfusion-independent, but in part due to expansion of dominant clone

New trials with next gen vectors open/coming for:

--WAS, MPS1, MLD, CGD, ADA-SCID, β -thal, X-ALD

Expect efficacy with less genotoxicity, but (semi-)randomly integrating vectors still may cause genome perturbations

--gene disruption, *trans*-activation, truncation, *trans*-splicing

In Situ Gene Modification

Two-step “hit and run” process (vs. persisting vectors):

1. Introduce targeted double-strand DNA break in genome using engineered site-specific endonuclease

- Zinc Finger Nucleases, TALENs, Homing Endonuclease

2. Induce homologous recombination (HR) with “donor”

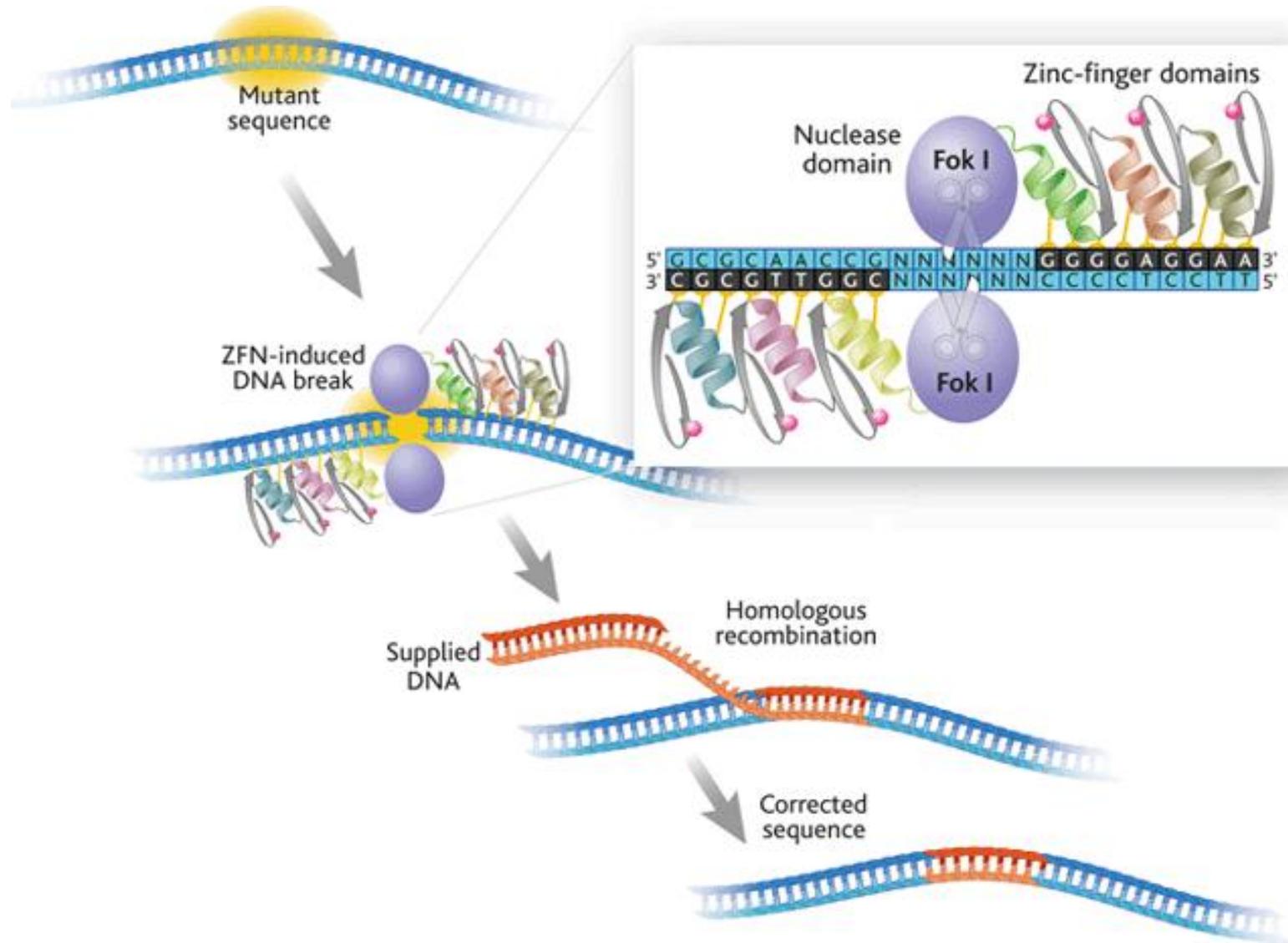
→ Disruption (endonuclease without donor – NHEJ)

→ Correction (homologous donor with corrective sequence)

→ Insertion (homology “arms” flanked insert

- marker, cDNA, mini-gene, siRNA.....)

Gene Correction By Zinc-Finger-Guided Endonuclease Cleavage and Homologous Recombination During DNA Repair



In Situ Gene Modification

Challenges-

---Delivery of multiple components to high percentage of HSC for transient expression yielding efficient HR:
Electroporation, IDLV, Ad/AAV....

Genotoxicities of *in situ* gene modification unknown.

Trials using ZFN-mediated gene disruption in HSC under development for HIV-1 co-receptor CCR5 and Bcl11a to induce γ -globin for SCD/thal.

Efficiency of targeted gene correction by HR in HSC currently too low for clinical applications.

Challenges of HSCT Gene Therapy – Technical

Efficiency of gene transfer/correction in HSC remains sub-optimal with some vectors.

Less toxic ways to facilitate engraftment are needed:

Other chemotherapy agents, antibodies, niche modifiers...

Production of transplantable HSC from iPSC

Challenges of HSCT Gene Therapy – Clinical

HSCT has high clinical acuity with significant inherent risks:

- Conditioning needed for engraftment (toxicity, infections)
- graft rejection / GVHD

Gene therapy should eliminate risks of rejection/GVHD, allow reduced conditioning and immune suppression, but has unique risks:

- sub-optimal frequency of gene correction of HSC
- poor transgene expression
- genome perturbation

Challenges of HSCT Gene Therapy- Translation

Long developmental time-line, with fragmented funding
--need to retain lab and regulatory staff with high expertise
in academic setting on intermittent, project-based funding

Toxicology studies costly, prolonged, uncertain predictive

Hard to fund pre-clinical studies with traditional awards for
toxicology, GMP vector and reagents,

NCATS: CTSI (GCRC), NGVB (NGVL), primate centers

NHLBI GTRP (PEGT), others: BRIDGs (RAID), TRND

EGPAF, DDCF, CIRM Disease Team

Need clinical trial grant “just-in-time”

The Slow Road to Lentiviral Vector Trial

	2009				2010				2011				2012				2013		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	
Pre-clinical studies (under P01 HL073104)	█	█	█	█	█	█	█	█	█	█	█	█	█	█					
NIH RAC Review: (December 2009)				█															
Initiate NHBLI GTRP Application (GMP vector and GLP toxicology)					█														
FDA Pre-IND (September 2010)							█												
NHLBI GTRP Approval (March 2011)									█										
Tox batch vector made										█									
Begin <i>in vitro</i> and <i>in vivo</i> tox studies											█								
UCLA IRB (Approved pending modifications)											█								
UCLA IBC (Approved pending modifications)											█								
Submit NIAID UO1 for UCLA clinical costs											█								
NIH IRB															█				
NIH IBC															█				
Perform <i>in vitro</i> insertional mutagenesis assay											█	█	█						
In vivo tox study													█	█	█	█	█		
Complete CRFs, SOPs, training															█	█			
Submit IND																	█		
Open trial																		█	

What Is Needed

Continued basic and applied research in HSC biology, gene transfer and regulation, DNA repair, transplantation.

Multi-phase, milestone-driven translational project funding to support clinical trial development, performance.