Emerging Gene Therapy Strategies - Challenges, Risks and Potential for Cure

Sung-Yun Pai, MD - Associate Professor in Pediatrics - Boston Children’s Hospital/Dana-Farber Cancer Institute

Mark Walters, MD - Director, Blood and Marrow Transplant Program - UCSF Benioff Children’s Hospital Oakland

November 9, 2018
Disclosures

The following faculty and planning committee staff have the following financial disclosures:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Disclosure</th>
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<tbody>
<tr>
<td>Sung-Yun Pai, MD</td>
<td>Boston Children’s Hospital/Dana-Farber Cancer Institute</td>
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</table>
| Mark Walters, MD      | UCSF Benioff Children’s Hospital, Oakland              | Bluebird bio, inc, Stipend, Consultant
Sangamo Biotherapeutics, inc., Stipend, Consultant
Bioverativ, Stipend, Consultant
AllCells, inc., Stipend, Medical Director
ViaCord Processing Lab, Stipend, Medical Director |
| Stephen Spellman      | CIBMTR                                                | None                                                                       |
| Alexandra Erickson    | CIBMTR                                                | None                                                                       |
| Misty Evans           | Vanderbilt                                             | Jazz Pharmaceuticals, Monetary, Speakers Bureau                           |
Learning objectives

• At the conclusion of this session, attendees will be able to:

  • Define diseases that have the potential to be treated using gene therapy strategies

  • Analyze current strategies and methodologies such as CRISPR and viral vectors being used in gene therapy

  • Evaluate how these gene therapy strategies are being applied in current clinical trials
Gene therapy for primary immunodeficiency: Important firsts and iterative progress

Sung-Yun Pai MD
Associate Professor in Pediatrics
Co-Director, Gene Therapy Program
Division of Hematology-Oncology BCH, Department of Ped Onc DFCI
Minneapolis, MN
NMDP Council Meeting 2018
Ex vivo genetically modified human cells

1) Isolation of the target cells (autologous or allogeneic)
2) Gene transfer
3) Re-Infusion of the genetically modified cells

In vivo delivered vectors, nucleic acids, replicating micro-organism (not including live vaccines)

Direct application:
- viral vector
- non-viral vector
- naked DNA
- replicating rec. micro-organism (adenovirus, Salmonella)
Limitations of allogeneic transplant for genetic blood disease

Healthy matched donor

Problems:
- Most patients lack a matched sibling donor
- GVHD

Sick → prepare the patient → Transplant → Well
Gene therapy is an alternative to allogeneic transplant using the patient’s own cells.

Advantages:
- Patient is own donor
- No GVHD
- Vector integrates into the DNA of the cell, and passes the gene to all progeny
Retroviruses are used for ex vivo gene transfer into HSC.

**Family**
- Family: Retroviridae
  - Subfamily: Orthoretrovirinae
    - Genus: Alpharetrovirus
    - Species: Ex: mouse mammary tumor virus (MMTV)
  - Subfamily: Betaretrovirus
    - Genus: Gammaretrovirus
      - Species: Ex: murine leukemia virus (MuLV)
    - Subfamily: Betaretrovirus
      - Species: Ex: human T lymphotropic virus (HTLV)
    - Subfamily: Lentiretrovirus
      - Species: Ex: human immunodeficiency virus (HIV)
  - Subfamily: Deltaretrovirus
  - Subfamily: Epsilonretrovirus
    - Species: Ex: human immunodeficiency virus (HIV)
  - Subfamily: Betaretrovirus
    - Genus: Gammaretrovirus
    - Species: Ex: murine leukemia virus (MuLV)
  - Subfamily: Gammaretrovirus
    - Species: Ex: human immunodeficiency virus (HIV)

**Subfamily**
- Subfamily: Spumaretrovirinae
  - Genus: Spumaretrovirus (foamy)
  - Species: Ex: Parvovirus B19

**Family**
- Family: Parvoviridae
  - Subfamily: Parvovirinae
    - Genus: Amdovirus
    - Species: Ex: Parvovirus B19
    - Species: Ex: adeno-associated virus
  - Subfamily: Bocavirus
  - Subfamily: Dependovirus
    - Species: Ex: adeno-associated virus
  - Subfamily: Erythrovirus
  - Subfamily: Partetavirus
  - Subfamily: Parovirus

**Subfamily**
- Subfamily: Densovirinae
  - Genus: Brevidensovirus, Contravirus, Densovirus, Iteravirus, Pefudensovirus

**Integrate into DNA**
- Transcriptional start sites
- Within transcriptional units
Simplified retroviral life cycle

Viral genome (RNA)

ψ

\[RU5\]

\[RU5\]

\[gag\]

\[pol\]

\[env\]

reverse transcription
integration
expression
uncoating

translation

Viral genome

budding

assembly
Gene transfer vectors are made to avoid replication competent retrovirus (RCR) by split packaging.

Gene of interest

Gene transfer vectors are made to avoid replication competent retrovirus (RCR) by split packaging.

Packaging cell line e.g. Human embryonic kidney 293T adherent cell line
Vector transduces stem cells without RCR

Gene transfer (addition) —

- Genetic material is added to the cell
- Location is not controlled and is different for each cell
- Insertion pattern varies depending on the type of virus used

Gene of interest

CD34+ hematopoietic stem cell

uncoating

reverse transcription integration expression
What is severe combined immunodeficiency (SCID)?

A congenital disease in which babies are born without T lymphocytes

The first disease to be successfully treated with long-term engraftment of donor cells (Gatti 1968)

Multiple genetic causes

IL2RG (X-linked)

ADA (adenosine deaminase)

Without treatment, death in the first year of life of infection

ADA SCID can be partially treated with enzyme replacement therapy which is expensive, non-curative and requires lifelong treatment

Standard treatment is allogeneic HSCT, can be performed without conditioning
The groundwork for successful gene therapy arose from HSCT for immunodeficiency

- Sib BMT for ADA def (Parkman)
- URD BMT for CGD (Westminster)
- γRV transfer into mouse BM cells (Joyner)
- Retronectin improves efficiency of murine retroviral transfer (Williams)
- Early trials of GT for ADA deficiency in PBL (Blaese, Bordignon, Kohn, Onodera)
- IL2RG cloned, cause of SCID-X1 (Noguchi, Puck)
- WAS gene discovered (Derry)

- ADA def as cause of SCID (Giblett)
- ADA gene cloned (Orkin, Wiginton)
- Gene for XCGD cloned (Royer-Pokora)
- CYBB identified as component of NADPH oxidase (Dinauer)

- 66-70
- 71-75
- 76-80
- 81-85
- 86-90
- 91-95
- 96-00
- 01-05
- 05-10
- 11-15
Gene therapy for primary immunodeficiency has led the way

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Vector</th>
<th>Year</th>
<th>Groups</th>
<th>Efficacy?</th>
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<td>Adenosine deaminase deficient SCID</td>
<td>ADA</td>
<td>γ-RV</td>
<td>2002-2009</td>
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<td>X-linked SCID</td>
<td>IL2RG</td>
<td>γ-RV</td>
<td>2002-2004</td>
<td>Paris, London</td>
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Introduction of low dose conditioning propeled the success of gene therapy for ADA SCID

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<tr>
<th>Year</th>
<th>Reference</th>
<th>Vector</th>
<th>Stop ADA?</th>
<th>Bu dose</th>
<th>N</th>
<th>Significant Gene Marking?</th>
<th>Off ADA?</th>
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<tr>
<td>1995</td>
<td>Bordignon et al</td>
<td>γ-RV</td>
<td>No</td>
<td>0</td>
<td>2</td>
<td>No</td>
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<td>1995</td>
<td>Kohn et al</td>
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<td>Hoogerbrugge et al</td>
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<td>2012</td>
<td>Candotti et al</td>
<td>γ-RV</td>
<td>No</td>
<td>0</td>
<td>4</td>
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Rivat et al Hum Gene Therapy 2012, includes personal communications
Introduction of low dose conditioning propelled the success of gene therapy for ADA SCID

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<tr>
<td>2002</td>
<td>Aiuti et al</td>
<td>γ-RV</td>
<td>Yes</td>
<td>4 mg/kg</td>
<td>2</td>
<td>Yes</td>
<td>1/2</td>
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<tr>
<td>2009</td>
<td>Aiuti et al</td>
<td>γ-RV</td>
<td>Yes</td>
<td>4 mg/kg</td>
<td>18</td>
<td>Yes</td>
<td>15/18</td>
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<td>2011b</td>
<td>Gaspar et al</td>
<td>γ-RV</td>
<td>Yes</td>
<td>4 mg/kg (or Mel)</td>
<td>8</td>
<td>Yes</td>
<td>4/8</td>
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<tr>
<td>2012</td>
<td>Candotti et al</td>
<td>γ-RV</td>
<td>Yes</td>
<td>4 mg/kg</td>
<td>14</td>
<td>Yes</td>
<td>10/14</td>
</tr>
</tbody>
</table>

Rivat et al Hum Gene Therapy 2012, includes personal communications
Gammaretroviral gene therapy for ADA SCID is safe and effective

- 10 pts received with autologous CD34+ BM cells transduced with MND-ADA-γRV after 4 mg/kg busulfan

- 100% survival
- Excellent T cell reconstitution
- 9 of 10 off enzyme replacement
- 3 of 10 off of IVIG
- No gene therapy related SAE

Shaw et al JCI 2017
First generation gene therapy for X-linked SCID (SCID-X1) was efficacious but caused insertional oncogenesis

Gammaparetroviral vector
(with viral enhancers)
(Paris, London)

- Viral promoter (Moloney leukemia virus) with strong expression of the IL2RG transgene
- Transduced autologous CD34+ bone marrow cells, infused without conditioning
- Excellent T cell reconstitution, 17/18 long-term survivors, no opportunistic infections

Safety concerns

Insertional oncogenesis:
5/20 developed T cell leukemia at 2-5.6 years post-GT
1/20 developed T cell lymphoma at 15 years post-GT
1 patient died of leukemia
5 patients treated and in remission with normal T cells

Occurred due to insertion near and activation of oncogene (LMO2 in 5 of 6 cases)

Gammaretroviral vectors were associated with insertional oncogenesis in multiple diseases

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<tr>
<th>Disease</th>
<th>Gene</th>
<th>Vector</th>
<th>Year</th>
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<th>Efficacy?</th>
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<td>Milan</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2009</td>
<td>UCLA/NIH</td>
<td></td>
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<tr>
<td>X-linked SCID</td>
<td>IL2RG</td>
<td>γ-RV</td>
<td>2002</td>
<td>Paris, London</td>
<td>Yes</td>
<td>No 6/20 ALL 1/20 T lymphoma</td>
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<td>X-linked chronic granulomatous disease</td>
<td>CYBB</td>
<td>γ-RV</td>
<td>2006</td>
<td>Frankfurt</td>
<td>Transient (silencing)</td>
<td>No 2/2 MDS</td>
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<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>WAS</td>
<td>γ-RV</td>
<td>2010</td>
<td>Hannover</td>
<td>Yes</td>
<td>No 7/9 ALL/AML</td>
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</table>
Possible strategies to avoid insertional oncogenesis

**Strategies**

- **Modify vector**
  - delete strong enhancers
  - use weak cellular
  - insulators

- Change vector class
  - lentiviral vector
Can modification of the SCID-X1 vector retain efficacy and improve safety?

Previous γRV

- No sibling donor
- No matched unrelated donor OR active treatment-resistant infection at diagnosis

autologous BM harvest
CD34+ selection

SCF
IL3
TPO
Flt3L

3 rounds of transduction in retronecctin coated bags

24h 24h 6h

Infuse fresh

NO CONDITIONING

SIN-γRV (self-inactivating)
Viral enhancers deleted

(David Williams, Adrian Thrasher, Christopher Baum)

Vector produced by Cincinnati Children’s Hospital Medical Center

IND #14067, Sponsor David A. Williams, NCT01129544
Funding: NIAID U01-AI087628 (Williams/Pai)

Boston (Pai, Notarangelo), Los Angeles (Kohn, DeOliveira), Cincinnati (Marsh, Malik), Paris (Hacein-Bey-Abina, Cavazzana, Fischer), London (Thrasher, Gaspar)
Robust reconstitution equivalent to previous vector

CD3 count

Vector copies in CD3

Copies/cell in CD3+

10/13 patients successful

Kinetics of T cell reconstitution similar between vectors

Hacein-Bey-Abina, Pai et al NEJM 2014 and unpublished
SIN-γRV appears to be safer than γRV

Insertion pattern of SIN-γRV still typical for γRV

Proportion of insertions near cancer-causing genes decreased in SIN-γRV pts

No leukemias to date, median 6.5y (1.3 to 7.9y)

Frederic Bushman, University of Pennsylvania (studies of all sites including European patients)

Hacein-Bey-Abina, Pai et al, NEJM 2014 and updated
Possible strategies to avoid insertional oncogenesis

**Strategies**

- Modify vector
  - self-inactivating format
  - use cellular promoter
  - insulators
- Change vector class
  - lentiviral vector
Next generation trials all use lentiviral vectors (US only)

<table>
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<tr>
<th>Promoter</th>
<th>ADA SCID</th>
<th>X-linked SCID</th>
<th>Wiskott-Aldrich</th>
<th>X-linked CGD</th>
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<tbody>
<tr>
<td></td>
<td>EFS</td>
<td>EFS</td>
<td>Human WAS 1.6kB</td>
<td>Chimeric myeloid specific</td>
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| Codon optimized? | Yes | Yes | No | No |

| Frozen cells?    | No  | Yes | Yes | Yes | Yes | No |
| Centers          | UCLA, NIH | NIH | St. Jude, UCSF, Seattle | Boston, UCLA | Boston | UCLA, NIH, Boston |
| N treated        | 20  | 10  | 8  | 9  | 2   | 5   | 6   |
| Longest follow-up| ~5y | ~2y | >5y| ~2y| 0.5y | 5y  | 2.7y|

| Efficacy?        | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Oncogenesis?     | No  | No  | No  | No  | -   | No  | No  | No  |

Unpublished data, Kohn, UCLA; Sorrentino, St. Jude/Cowan, UCSF; Pai/Williams, Boston; Malech, NIH
Gene editing methods seek to modify or repair endogenous genes, rather than adding a new copy of the relevant gene.

Advantages of editing over addition:

1. Regulation in native context
2. Avoid insertional oncogenesis

Strategy:
Target a double stranded break (DSB) to gene of interest
Repair the break
  ➢ with or without a donor template
  ➢ using nonhomologous or homologous recombination
Two double stranded break repair pathways

- **Non-Homologous End Joining (NHEJ)**
  - Inaccurate repair
  - No donor template required

- **Homologous Recombination (HR)**
  - Accurate repair
  - Homologous donor template required

NHEJ:HR ratio depends on:

- Cell type
- Cell cycle phase
- Presence of donor template

Donald B. Kohn
Different outcomes of gene repair

**Site-Specific Endonuclease:**
- Zinc finger nuclease
- Homing endonuclease
- TALEN
- CRISPR/Cas9

**Repair Template:**
- No Donor
- Homologous Donor

**Patient’s Gene**

**Gene Disruption**

**Gene Correction**

**Gene Insertion**

**Homology Arms**

**Gene Cassette**

**M** = mutant bp  
**N** = normal bp

Donald B. Kohn
ZFN, TALENs and CRISPRs– Oh My!

Artificial protein with ZnF array, each recognizing 3-4 nt, connected to FokI nuclease

Artificial protein with TAL effector protein array, each recognizing 1 nt, connected to FokI nuclease

Bacterial system in which the Cas9 protein nuclease is guided to target by bacterial trcrRNA fused to guide RNA (gRNA) with specificity

Donald B. Kohn
Site-specific Gene Editing of Autologous Hematopoietic Stem Cells for Gene Therapy

Autologous HSC (BM, PBSC or CB)

CD34 select

Pre-stimulate S/F/T (24-48 hr.)

Electroporation

Site-Specific Endonuclease (as mRNA or RNP)

Homologous Donor (as Oligo or by Viral Vector)

Formulate Graft. Infuse. (Fresh or Cryopreserve)
Practical considerations in gene editing

Similar to gene addition

- Efficiency, efficiency, efficiency
- Delivery into appropriate cell type
- Toxicity of the process to HSC, maintaining pluripotency
- Ethical issues of somatic versus germline manipulation

Distinct from gene addition

- Off target cutting
- Delivery that is transient yet effective (no integration)
- Need to deliver both the nuclease and for HR, donor template
- If strategy is mutation specific, need personalized materials for each patient
On the road to gene therapy as standard care

1990-2000
- Successful ex vivo gene addition

2005-2015
- Changes in vector format to overcome oncogenesis
- Centralized manufacturing
- Cryopreservation
- Gene editing
- Dissemination
- Pivotal trials
- Licensure

YOU ARE HERE
Principals of gene therapy for transfusion-dependent β-thalassemia and severe sickle cell disease

Mark Walters, MD
Financial Disclosure

In accordance with the ACCME® standards for Commercial Support Number 6, my relevant financial relationships are disclosed:

Medical Director:
ViaCord Processing Lab
AllCells, Inc

Consultant:
bluebird bio, Inc
Sangamo Biosciences/Bioverativ
Global Blood Therapeutics
Trucode
BMT for SCD at UCSF BCHO

Oct 2000 – June 2018

N = 26

URD – 2
HaploID - 1
Unrelated UCB - 1
MMRD – 1
Adults >20y - 4

Alive – 26/26

DFS – 25/26 (96%)
How is a curative outcome depicted – HLA-Id sibling HCT?

Complication incidence – Graft-versus-host disease

Successful outcome – overall survival

E Gluckman et al Blood 2017 129:1548-1556
Barriers to Transplant for SCD

• Only 18% of families have HLA-ID sibling donor
• Only 19% have well-matched unrelated donor
• Clinicians do not refer patients because of GVHD and risks of dying/long-term toxicity
• The problem of graft rejection/recurrent SCD has not been eliminated, especially in mismatched donor HCT
‘Genomic’ therapies for hgb disorders

- Gene addition therapy (anti-sickling β-globin or γ-globin) in autologous HCT
- Gene editing for γ-globin expression in autologous HCT
- Gene editing for sickle allele correction in autologous HCT
- In vivo gene editing
Curative therapies – fraction of ‘corrected’ HSCs

• After allogeneic HCT, stable mixed hematopoietic chimerism is sufficient to establish a curative effect
• Benchmark of >20-25% donor myeloid chimerism has been suggested, but there is inter-individual variability
• Ideally, fraction of corrected HSPCs might be even higher

Curative therapies – stable mixed chimerism after allo-HCT


How a curative outcome is depicted – Gene therapy

Avg vector copy number (VCN) is surrogate for fraction HSCs transduced

Dilution of HbS by 50%

HbA$^{T87Q}$ ~50%

Rebeil et al. NEJM 2017; 376:848-55
Modulators of HbF expression

GWAS observations

1. β-globin locus (chromo 11)
2. HBS1L-MYB intergenic region (chromo 6)
3. BCL11a (chromo 2)
BCL11A is an epistatic suppressor fetal Hb

How is a curative outcome depicted – Hb F induction after gene editing?

What is the HbF and F-cell induction target?

Unpublished, Daniel Bauer
Curative therapies – VCN

- After vector transduction, VCN and % transduced HPSCs directly proportional
- VCN of 0.5 – 1 corresponds to 20-30% HSPC transduction
- Stochastic nature of HSPC contribution to erythropoiesis challenges a direct prediction
- However, VCN and %transduction are important endpoints that should be tracked in the short- and long-term

Thompson AA et al NEJM 378:1479, 2018
LentiGlobin gene therapy for transfusion-dependent β-thalassemia

HGB-204: 8/10 patients with non-β⁰/β⁰ genotypes achieved and maintain transfusion independence

**Median duration of transfusion independence** to date of **33 months**
(min – max: 16 – 38) in 8/10 patients

*Indicates male patients. Transfusion independence is defined as weighted average Hb ≥9 g/dL without any RBC transfusions for ≥12 months. Hb, hemoglobin

HGB-204: 3/8 patients with $\beta^0/\beta^0$ genotypes are free from chronic transfusions

Transfusion independence is defined as weighted average Hb $\geq 9$ g/dL without any RBC transfusions for $\geq 12$ months. Hb, hemoglobin.

**Patients 1103 and 1123 achieved transfusion independence with a duration to date of 14 and 16 months, respectively**

* Indicates male patient
‡ Patient had a single transfusion for an acute event of cat scratch disease
Transfusion independence is defined as weighted average Hb $\geq 9$ g/dL without any RBC transfusions for $\geq 12$ months. Hb, hemoglobin

Data as of 7 March 2018
Curative therapies – VCN

Correlation between Blood HbA^{T87Q} Level and VCN in PBMCs at 6 Mo

Blue dots: US/Austr/Thai
Red dots: France

\[ r^2 = 0.75; \ P < 0.001 \]

Thompson AA et al NEJM 378:1479, 2018
Peripheral blood VCN is higher in patients in Group B and C

For Group A patients, medians (Q1, Q3) depicted; Group A patients with month 30 study visit (N=3)

VCN, vector copy number (vector copies/diploid genome)


Data as of May 15, 2018
Patients in Group B and C demonstrate higher HbA$^{T87Q}$ production

For Group A patients, medians (Q1, Q3) depicted; Group A patients with month 30 study visit (N=2)

HbA$^{T87Q}$, vector derived hemoglobin

Vector-derived hemoglobin in treated patients

Data as of May 15, 2018


Hb, hemoglobin; HbA, adult hemoglobin; HbA^{T87Q}, vector derived hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin

Cas9 for programmable gene correction

- targeted
- fast
- efficient
- simple
- inexpensive
How is a curative outcome depicted – Gene editing?

Fraction of Sickle allele corrected

Frequency of off-target modification

Dilution of HbS by 50%

Vakulskas CA et al, Nat Med 24:1216, 2018
In vivo and in vitro experiments

Do edited HSCs produce non-sickle hgb?

Are human edited cells true HSCs?
In vitro erythroid expansion
In vivo experiments: xenografts

Are human edited cells true HSCs?

correct

engraftment?

edited blood lineages?

sickle donors
Optimized sickle correction in xenotransplant model with plerixafor-mobilized HbSS CD34+ cells

ssDNA donor directed editing had an average of 22.15% ± 7.66% correction in marrow
Curative therapies – % allele

Magis W et al.; bioRxiv 432716; doi: https://doi.org/10.1101/432716
• LentiGlobin BB305 gene therapy shows promising results in TDT
• LentiGlobin VCN strongly correlated with HbAT87Q level
• Clinical benefit in SCD has been appears to follow HbAT87Q levels – approach 50% non-HbS
• The future of curative therapies that will have broad availability might follow advances in gene therapy and genomic correction of the sickle mutation in HSCs – availability of the treatment will be a limiting factor
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IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy
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University College London Hospital, London, UK
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