Gene Therapy of FA: Premises and Promises

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Treat marrow failure
Prevent leukemia
Reduce endocrine problems
Lower risk of head/neck cancer
Improve quality of life
A unified trial theory
Success in Bone Marrow Failure: Blood and Marrow Transplant

Life-saving Measure

The Slings and Arrows
1. Graft-versus-host disease
2. Infectious complications
3. Long-term effects

Years
0 1 2 3 4 5

1982-1999 23%
1999-2003 67%
2003-2006 75%
2006 89%
Lessons of Nature: Mosaicism → Gene Therapy

Natural Gene Therapy

Gene Addition

Selection Neutral

Selection Neutral
Biological Hurdles for Gene Therapy

- **Uptake, transport and uncoating**
  - Receptor
  - Endosome

- **Vector genome persistence**
  - Nuclear matrix

- **Transcriptional activity**
  - RNA
  - Protein
  - MHC
  - Nucleus

- **Immune response**
  - TCR
  - CTL
Custom-designed Cells for Gene Therapy

- **Gene addition**
  - Pasting in a whole new gene
  - Printing up a new addendum or errata page

- **Genome engineering**
  - Editing the original text
  - Correcting gene typos
How It Works | The procedure the SCID-X1 trial will use

Stem cells are isolated from bone marrow harvested from a baby’s hip. The normal gene is inserted into the stem cells in the lab. The corrected cells are then transfused back into the baby and populate over time, repairing the baby’s faulty immune system.

Proof-of-concept
1. Naldini, Antoniou, Hanenberg
2. Kiem, Verhoeyen, Bueren, Rio
3. Thrasher, Cavazzana-Calvo, Renella
4. Bartholomae, Galy

- Prospects
  1. Autologous BMT
  2. Renewable source
  3. Cooperation

- Challenges
  1. Off-target effects
  2. Cell purity
  3. Cooperation

1st International Fanconi Anemia Working Group
Genotoxicity
Transduction
Production

Self-inactivating
Viral envelope
Int Working Grp

Gene Editing
New envelopes
Collaboration
Fragile $\rightarrow$ Few

Collection

Minimal, short manipulation

Collect early

Better survival

Hypoxia, antioxidants

CD34+, Lin-, CXCR4 antagonist, mesenchymal stromal/stem cells
Inclusion criteria

- FANCA diagnosis
- BM with normal karyotype

Exclusion criteria

- Uncontrolled viral, bacterial, or fungal infection
- Patients with an HLA-identical sibling donor
Future
Problem
Strategy
Future

Rare
Tight criteria
Other FA groups

Efficacy
Interpretatable data
Matched sibs

Vector
FA cells
Trials

Conditioning before and after infusion
Vector → FA cells → Trials → Safety

Problem → Strategy → Future

Safety

Quantitative models of cancer risk

- Better vectors
- Safe harbor integration
- Gene editing
New Data

- Prospects
  1. First trials
  2. Open platform
  3. Cooperation

New Tools

- Challenges
  1. Off-target effects
  2. Funding
  3. Coordination

New Horizons

2nd International Fanconi Anemia Working Group
NEW DATA

• Thrasher
• Renella/Williams
• Becker/Kiem
• Navaro/Bueren

• Immunodeficiencies: SCID, CGD, ADA, WAS
• Metabolic disease: ALD, MLD, MPS I
NEW TOOLS

• Verhoeven: New viral envelopes
• Hanenberg: Foamy viruses-LV
• Schmidt: Off-target effects
• Sevilla: HSC mobilization
• Kiem: HSC expansion
• Rio: Gene transfer
New Horizons

- Clapp: Design for studies
- Bueren: Conditioning
- Qasba: NHLBI resources
Pasting in a new gene → correcting gene typos

Gene Addition

Viruses
Transposons

Genome Engineering

Gene editing

5'-ACTGCTACGGATTAAAGTGATGATGT-3'
3'-TGACGGATGCCGATTTCACTTACACACA-5'

Nucleosome domain
DNA binding
Zinc-finger domains

N
F1
F2
F3
Genomic Surgery

Structure

Function


Using Physiologic DNA Repair for Gene Repair of FA Mutations

**FA-A: Gene Addition**

- Endogenous
- FA-A Promoter
- Zinc Finger Nuclease
- Corrected
- FA-A Promoter
- Wild type Exons 1-43
- Exons 1-43

- Most common
- Scattered mutations

**FA-C: Gene Editing**

- Endogenous
- FA-C Exon 1
- 2
- 3
- 4
- 5-13
- IVS-4
- Zinc Finger Nuclease
- Corrected
- FA-C Exon 1
- 2
- 3
- 4
- 5-13

- Ashkenazi Jewish
- Single site

Together:
Same reagents useful to > 75% of FA patients
Cells for FA Gene Therapy

Hematopoietic Stem Cells

1. Difficult to transf ect
2. Easy to transplant

Hematopoietic Progeny of Induced Pluripotent Stem Cells

1. Easy to transf ect
2. Difficult to transplant

GFP

DsRED
Build and Correct Your Own Cells

- S Agarwal
- M Barragan
- S Navarro
- T Graf
- A Raya
- T Cathomen
- J Bueren

- Induced pluripotency
- Targeted correction of C295T
- iPSC from FA-D1 mice
- Lineage conversion
- FA iPSC
- Zinc Finger Nucleases
- Safe Harbor for FANCA

Barcelona 2011
• First successes in cancer were in children
• Genotoxicity of chemo versus gene tx
• Collect as many efficacy markers as possible, continuous assessment
• National Institutes of Health, NHLBI
• FA families, FARF, Fanconi Hope
• Access + endpoints + cooperation
• Excitement + good will
• Will the gene-corrected cell population fully replace the mutant blood and marrow cells?
• Will late cancers due to cumulative acquisition of secondary mutations occur?
• Will the loss of natural FA gene regulation be clinically relevant?
• Will host immune response target the gene-modified cells?
• How will the genome integration data prospectively impact the clinical care of the patients?
Challenges Remain

- Objective readouts
- When to go to BMT
- Mixed chimerism with myelodysplasia
- Regulatory redundance
- Funding fragmentation
- Short-term justification
- Roadmap with one change at a time
• Risk stratification
• Patient-specific therapy
• When is experimental therapy no longer experimental?
• Gene therapy for extramedullar tissues
• Lessons learned from cellular reprogramming
• “The system has (not) failed us”
• FA gene therapy: concept → reality
Cross-functional Team

- Research
- Clinical Transplant
- Vector Production and Testing
- Regulatory and Quality Assurance
- FA families FARF

GT