Genome editing for the therapy of β-haemoglobinopathies

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Outline

- Gene therapy and its tools
- Adding, breaking & fixing things
- Trials (and tribulations)
- Gene therapy for the masses?!
- Current trends
Gene therapy and its tools
**Approaches**

1. **Gene Addition**
   - Universal for SCD and β-thalassaemia
   - With integrating lentiviral vectors
   - E.g. addition of β-like globins

2. **Gene Correction**
   - Mutation-specific
   - With designer nucleases or gene editors
   - E.g. correction of the IVS I-110 mutation

3. **Reactivation of γ-globin**
   - Universal for SCD and β-thalassaemia
   - With various tools and approaches
   - E.g. deactivation of γ repressor BCL11A

**Current Clinical Trials**
- Addition of “β”
- Curative.
- Repair of β
- Curative.
- Activation of γ
- Curative.
- Truncation of BCL11A-XL

**Focus of current development**

**Combination therapy with RNAi**
Procedure for blood disorders

1. corrected stem cells
2. isolation of haematopoietic stem and progenitor cells
3. vector production
4. nuclease transduction
5. preincubation
6. re-engraftment

Volunteers.
Informed Consent.

Safety? Efficacy?
Analysing all Patients

Blood sample

Count

Cryostorage and thawing procedure
  Alternative freezing media

Cell extraction procedure
  Alternative cell isolation protocols

Count / viability / recovery

Lentiviral transduction

CFU scoring

Stephanou et al. 2017 Cytotherapy PMID 28088294
Repairing and breaking: designer nucleases

CRISPR/Cas9 (RGEN)
RNA-DNA basepairing

ZFN
Approximate protein code

Left ZFN

Right ZFN

DSB

Left TALEN
Precise protein code

Right TALEN

Repair
5'
3'

3'
5'

HDR
required for precise (ORF) editing

Repair
5'
3'

3'
5'

NHEJ
ideal for disruption & tagging

Disruption
5'
3'

Repairing and stopping: base editor

Targeted C→T or A→G conversion, stimulated by nick, without DSB
Based on engineered single protein based on mutated Cas9
Fix missense mutation (repair) or introduce non-sense mutations (stop)

mediates recognition

gRNA

creates ssDNA loopout to allow base conversion
nicks & prompts mismatch repair of non-target strand

non-target DNA strand
target DNA strand

uracil DNA glycosylase inhibitor prevents removal of base edit
cytidine deaminase C→U conversion in ssDNA loopout within 2–5 bp

Rees et al. (2017) Nature Communications PMID 28585549
Kim et al. (2017) Nature Biotechnology PMID 28191901
Breaking things
Key components

α-globin locus Chr. 16

β-globin locus Chr. 11

HbF (<1%) αγγα

HbF (>20%) αγγαβ

HbA (>75%) MYB KLF1 GATA1 NuRD BCL11A NuRD LRF KLF1 MYB
**Tools & targets**

Highest efficiencies are based on research protocols involving iPS cells (or cell lines) and clonal selection using integrating lentiviral vectors.

<table>
<thead>
<tr>
<th>Target (HGVS name)</th>
<th>Nuclease</th>
<th>Model</th>
<th>Reported Efficiencies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^\gamma$-175 T&gt;C HPFH (HBG1:c.-228T&gt;C)</td>
<td>TALEN</td>
<td>K562</td>
<td>Ay promoter activity x2 (K562 &amp; MEL) &amp; $\beta$-globin promoter activity x0.5 (MEL) clonal</td>
<td>(Wienert et al. 2015)</td>
</tr>
<tr>
<td>$^\gamma$-117 G&gt;A HPFH (HBG1:c.-170G&gt;A)</td>
<td>TF-PNA</td>
<td>TG MEL, TG Mm BM</td>
<td>HDR 1.63% (CD34+) bulk</td>
<td>(Chin et al. 2013)</td>
</tr>
<tr>
<td>$^\gamma$ -114 to -102 deletion (HBG1:c.-157_-155delCAATAGCCTTGAC)</td>
<td>RGEN</td>
<td>Hs mPB CD34+</td>
<td>HDR 4.6% (16/344 clones), 5x HbF induction in normal CD34+, 100% F cells in clonal HUDEP-2</td>
<td>(Traxler et al. 2016)</td>
</tr>
<tr>
<td>$^\gamma$ -198 T&gt;C British HPFH (HBG2:c.-198T&gt;C)</td>
<td>RGEN</td>
<td>Hs PB CD34+</td>
<td>77% HbF clonal</td>
<td>(Wienert et al. 2017)</td>
</tr>
<tr>
<td>Sicilian HPFH (HPFH-5) 13-kb deletion</td>
<td>RGEN</td>
<td>Hs mPB CD34+</td>
<td>2x HbF induction by biallelic deletion, 1.5x HbF induction by heterozygous deletion, 10 of 32 clones with ≥1 deletion</td>
<td>(Ye et al. 2016)</td>
</tr>
<tr>
<td>$BCL11A$ h +55, +58, +62 (NC_000002.11: g.60718000-255000)</td>
<td>RGEN (x533)</td>
<td>HUDEP-2</td>
<td>40% HbF, F cells 3x bulk (CD34+, for +58 sgRNA-1621)</td>
<td>(Orkin et al. 2015; Canver et al. 2015)</td>
</tr>
<tr>
<td>$BCL11A$ exon 2, +58</td>
<td>ZFN (x2, x2)</td>
<td>Hs CD34+</td>
<td>40% HbF (biclonal), human bulk LTRCs in NSG mice @ 22% ko/ko and 18% wt/ko chimerism</td>
<td>(Chang et al. 2017)</td>
</tr>
<tr>
<td>$HBS1L-MYB$ 98 DHS sites</td>
<td>RGEN (x4690)</td>
<td>HUDEP-2</td>
<td>None</td>
<td>(Canver et al. 2017)</td>
</tr>
<tr>
<td>$Ehmt2$ knockout</td>
<td>RGEN</td>
<td>MEL</td>
<td>80x Hbb-ey and Hbb-Bh1 clonal</td>
<td>(Renneville et al. 2015)</td>
</tr>
<tr>
<td>$KLF1$ knockout</td>
<td>3x RGEN</td>
<td>K562</td>
<td>24% NHEJ, $\gamma$ mRNA x8.1 and 7.7 (exon 2), x1.8 (exon 3) bulk</td>
<td>(Shariati et al. 2016)</td>
</tr>
<tr>
<td>$ZBTB7A/LRF$ knockout</td>
<td>RGEN</td>
<td>HUDEP-2</td>
<td>3x &gt;60% HbF clonal</td>
<td>(Masuda et al. 2016)</td>
</tr>
</tbody>
</table>
Truncation of BCL11A-XL

“basic research+”
Reactivation of endogenous γ-globin

E. g. truncation of BCL11A XL isoform

BCL2 → MDM2 → p53 → apoptosis

Alternate cut sites

CFU-E

BCL11A mRNA

β-globin

CFU-TL/BL

BCL11A mRNA

NuRD

LRF

NuRD

BCL11A

KLF1

MYB

NuRD

FOG1

GATA1

BCL11A

Chr. 11

12345 LCR HBE HBG2 HBG1 HBD HBB

γ

γ

δ

β

High HbF

High HbF?

Toxic

Toxic?
Fixing things

HBV, E6V
<table>
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</thead>
<tbody>
<tr>
<td>θ^S (HBB:c.20A&gt;T)</td>
<td>ZFN</td>
<td>K562</td>
<td>NHEJ 2%, HDR 0.2% bulk; HDR 13% clonal</td>
<td>(Vannocci et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>TALEN</td>
<td>K562</td>
<td>HDR 63% clonal</td>
<td>(Voit et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>ZFN</td>
<td>IPSC</td>
<td>HDR 67% (2 out of 3) clonal</td>
<td>(Sun et al. 2012, 2014)</td>
</tr>
<tr>
<td></td>
<td>ZFN (x3)</td>
<td>HEK293T</td>
<td>HDR 12.9%, 2.5% &amp; 1.2% (HEK293T) clonal</td>
<td>(Zou et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>RGEN, TALEN</td>
<td>iPSC</td>
<td>HDR 33.3%, 37.9%, 26.3% (iPSK) clonal</td>
<td>(Sebastianio et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>ZFN</td>
<td>iPSC</td>
<td>HDR 33% (TALEN), 12.3% (RGEN) clonal</td>
<td>(Xu et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSC</td>
<td>HDR 67% (iPSK) bulk</td>
<td>(Huang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>HEK293T</td>
<td>HDR 45% (HEK293T), 3.2-3.6% (iPSK) bulk, RGEN</td>
<td>(Xie et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSK</td>
<td>NHEJ 12%, HDR 45%</td>
<td>(Wen et al. 2017)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSK</td>
<td>HDR 33% clonal</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>HEK293T</td>
<td>HDR 52% (zygote)</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>HEK293T</td>
<td>HDR 48.3% (HEK293T) &amp; 7.4% (zygote) bulk</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>HEK293T</td>
<td>NHEJ &gt;50% (HEK293T)</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSK</td>
<td>NHEJ &gt;65-85%, bulk 1.5x erythropoiesis &amp; 1.6-2.9x HBB</td>
<td>(our unpublished results)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>HEK293T</td>
<td>HDR undetectable (HEK293T)</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>TALEN</td>
<td>iPSK</td>
<td>HDR 40% clonal</td>
<td>(Ma et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSC</td>
<td>HDR 42% clonal</td>
<td>(Xie et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>RGEN</td>
<td>iPSC in NSI mice</td>
<td>10 weeks tumor-free survival</td>
<td>(Ou et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>TF-PNA</td>
<td>TG CHO, K562</td>
<td>HDR 0.4% (CHO), PCR^- (K562 &amp; CD34^) bulk</td>
<td>(Chin et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>TALEN</td>
<td>iPSC</td>
<td>HDR 68% clonal</td>
<td>(Ma et al. 2013)</td>
</tr>
<tr>
<td>Mutation of HBB exon 1 W16X</td>
<td>Base Editor</td>
<td>Hs zygotes</td>
<td>Base editing in 8 of 19 embryos</td>
<td>(Zhou et al. 2017)</td>
</tr>
</tbody>
</table>
Correction of $HBB_{\text{IVS I-110(G>A)}}$

“disruption of aberrant regulatory elements (DARE)”
### IVS I-110 β-thalassaemia

**Relative carrier frequency:** 75.9%

**Absolute carrier frequency:** 9.1%

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**Country** | **Relative Frequency (%)**
--- | ---
Cyprus | 75.87%
Greece | 42.1%
Turkey | 38.38%
Macedonia | 37.8%
Egypt | 36.9%
Lebanon | 34.2%
Saudi Arabia | 33.45%
Algeria | 26.4%
Jordan | 25%
Germany | 24.8%
Argentina | 23.2%
Italy | 23%
Bulgaria | 23%
Israel | 21.5%
Syria | 19.85%
Slovakia | 17%
Tunisia | 15.91%
Mexico | 13%
Portugal | 12.24%
Czech Republic | 11.15%
Cuba | 9.17%
Spain | 8.1%
United Kingdom | 4.9%
Iran | 4.85%
Kuwait | 4.75%
Morocco | 4.45%
Guadeloupe | 1.1%
Oman | 0.35%
India | 0.2%

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**Sources:**

Kountouris et al. (2014) PLoS1 PMID 25058394
Kountouris et al. (2016) Scientific Reports PMID 27199182

**Website:** [http://www.ithanet.eu/db/ithamaps](http://www.ithanet.eu/db/ithamaps)
Gene therapy for the masses?!
Accessibility of technology

- Lowered bar for translation
- Streamlining regulatory frameworks (e.g. GMP requirements)
- Incentivising companies by EU & US orphan drug regulation
- Falling cost through increasing competition around genetic medicines
- Democratisation of research
- Away from viral vectors and their costly production
- Straightforward design of RGENs
- Effective delivery of RGENs as RNPs by nucleofection
- GMP in a box
- Closed-system prototype as GMP facility replacement in Fanconi trial
- One-off cost of $150,000
- 1/5 staffing requirement
- 1/2 processing time
- No cleanroom requirements

Adair et al. 2016 Nature Communications PMID27762266
https://www.fredhutch.org/content/dam/public/communications/Photo/2016/10-October/Adair/JennAdairGraphic.pdf
“Catching them early”

- Infant gene therapy with incremental benefits
  - Higher relative HSC yield
  - Higher success rate
  - More substantial correction of disease parameters
  - Lowered vector requirements
    patient from 80 kg to 4 kg → vector cost from $100,000 to $5,000

- *In utero* gene therapy as quantum leap
  - Treatment also of disorders lethal *in utero* (hydrops fetalis)
  - Minimal cell and vector requirements (1/1000 of that in adults)
    - Postnatal: $5 \times 10^6$ cells/kg and $5 \times 10^8$ vector particles/kg
    - *In utero*: $3 \times 10^5$ cells and $1.5 \times 10^7$ vector particles total
  - Direct vector injection possible as outpatient treatment

- Pending
  - Biosafety issues (germline transmission)
  - Bioethics issues (justification of treatment)
  - Large-animal studies

Ramachandra et al. 2014 Frontiers in Pharmacology PMID25566071
Shaw et al. 2014 Stem Cells PMID25186828
GG2020 Fringe Meeting in Thessaloniki

Grand Hotel Palace, Thessaloniki
Sunday, 19 November 14:00 h – 16:30 h
Immediately after the TIF Meeting
Access Free

The Global Globin 2020 Challenge – Towards Comprehensive Global Epidemiology and Prevention

Human Variome Project – Global Globin 2020 Challenge Executive Committee

www.humanvariomeproject.org/gg2020/
www.ithanet.eu

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Cyprus Antianaemia Association
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All patients and sample volunteers!

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«ERASMUS+ BLC11A-»
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«ThalaMoSS»
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Current trends
General trends

- Involvement of companies
  - GSK with GLOBE, Bluebird Bio with LentiGlobin
  - Editas Medicine, CRISPR Therapeutics, Intellia Therapeutics

- Universal approaches
  - Induction of γ-globin
  - Gene addition of anti-sickling β-like globins

- Translational research focus
  - Away from cell lines and even iPS cells towards primary HSPCs
  - Efforts to boost *in vivo* long-term repopulation after treatment

- Editing & CRISPR/Cas9
  - New preclinical publications mostly on editing approaches
  - Editing studies towards CRISPR/Cas9 platform

- Safety
  - Towards genome-wide assessment of integration sites and off-targets
  - Safe-harbour approaches for gene addition
  - Many studies on innovations to reduce off-target editing
Trials (and tribulations)
**Key trials**

- Bluebird Bio (LentiGlobin vectors) based on $\beta^{T87Q}$-globin
- First authorised human trials with HPV569 vector

- Transfusion independence of 1 out of 3 patients reported
- Temporary clonal dominance for HMGA2 insertion event
- Shortcomings in vector production, transduction efficiency, stability

- Follow-up trials with modified BB305 vector

- CMV-driven production and no cHS4 insulator: higher titres & stability
- Many subjects with speedy transfusion independence
  - #NCT01745120 phase-1/2 trial for $\beta$-thalassaemia
  - #NCT02140554 phase-1 trial for severe SCD
  - #NCT02151526 phase-1/2 trial for SCD and $\beta$-thalassaemia
  - #NCT02633943 long-term (15-year) follow-up of extant patients
  - #NCT03207009 phase-3 trial for $\beta^0/\beta^0$

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https://clinicaltrials.gov/

Ribeil et al. (2017) NEJM PMID 28249145

Cavazzana et al. (2017) Molecular Therapy PMID 28377044
Key trials

- Memorial Sloan Kettering Cancer Center (TNS9.3.55 vector)
  - #NCT01639690 phase-1 trial
  - Mild conditioning (8 mg/kg busulfan)
  - No transfusion independence in 4 out of 4 subjects
  - Trial unofficially stopped (“ongoing but not recruiting”)
  - Possible future trials with insulated vector

- IRCCS San Raffaele (GLOBE vector)
  - #NCT02453477 phase-1/2 trial
  - Intraosseous injection, three cohorts of different ages (>18, 8-17, 3-7 years)
  - 7 out of 10 patients treated, greater benefit in younger patients

https://clinicaltrials.gov/
Cavazzana et al. (2017) Molecular Therapy PMID 28377044
Key trials

- Boston Children's Hospital (David Williams BCL11A vector)
  - #NCT03282656 phase-1 trial
  - Three cohorts of different ages (18-35, 12-18, 3-12)
  - Recruiting up to 7 SCD patients

- Children's Hospital Medical Center, Cincinnati (Punam Malik γ-globin vector)
  - #NCT02186418 phase-1 (SCD) / phase-2 (SCA) trial
  - Recruiting approximately 10 SCD patients (aged 18-35)

- Children's Hospital Medical Center, Cincinnati (Donald B. Kohn βAS3-FB vector)
  - #NCT02247843 phase-1 trial
  - Estimated enrolment 6 SCD patients (aged 18+)

https://clinicaltrials.gov/
Cavazzana et al. (2017) Molecular Therapy PMID 28377044
Adding things

\[
\begin{align*}
2 + 1 &= 2 \\
0.5 + 1 &= 1 \\
0 + 2 &= 1 \\
0 + (-1) &= 1
\end{align*}
\]
Adding: lentiviral vectors

- Transfer vehicles for efficient transduction also of HSPCs
- Disarmed and compartmentalised (e.g. 2nd-generation vector)
- <8.5 kb capacity
- Replication-defective
Major strategies

- Addition of expression cassettes for β-like globins
  - β-globin for β-thalassaemia
  - β^{T87Q}-globin, γ-globin, β^{G16D,E22A,T87Q}-globin for β-thalassaemia and sickle cell disease

- Addition of shRNA expression cassettes
  - RNAi of BCL11A to induce endogenous γ-globin
  - Co-expression of anti-β^S shRNA and γ-globin
  - RNAi of aberrant β-globin RNA to boost endo- or exogenous β-globin

- Addition of expression cassettes for γ-globin activators
  - LDB1/Zinc finger fusion to loop chromatin from LCR to γ-globin promoter and induce endogenous γ-globin

Brendel et al. (2016) J Clin Invest PMID 27599293
Combination therapy with RNAi

“less means more”
**IVS I-110 β-thalassaemia**

Intronic mutation of β-globin (HBB)

Creation of aberrant splice acceptor site (GG>AG)

Partial dominance: reduced β-globin from normal loci

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**Exon 1**

+1 normal SD

**Exon 2**

+110 aberrant SA

+131 normal SA

**Exon 3**

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60% aberrant mRNA

40% normal mRNA

10% of the β-globin levels of healthy controls

**trans action of aberrant HBB mRNA?**

Degradation
Correction of endogenous β-globin

Using TALEN and CRISPR/Cas9 (RGEN)