Gene therapy and gene editing: from trailblazer to gamechanger

Prof. Dr. Thierry VandenDriessche
With this profound new knowledge, humankind is on the verge of gaining immense, new power to heal. It will revolutionize the diagnosis, prevention and treatment of most, if not all, human diseases.”

President Bill Clinton 26 June 2000
Nature 405, 983–984; 2000
GENETIC DISEASES

Just 1 point-mutation in 3000.000.000 bp can cause a potentially life-threatening disorder (e.g.: hemophilia, Duchenne muscular dystrophy, cystic fibrosis, …)

Equivalent to 1 typing error in 15 Encyclopaedia Britannicae
Can these ‘broken genes’ be fixed by gene therapy?

**GENE ADDITION**

Introducing a functional normal copy of the gene that compensates for or complements the mutated gene

**RECESSIVE GENETIC DISORDERS**

**GENE EDITING**

Correcting the mutation directly *in situ* within the defective gene itself ("molecular Typex")

**DOMINANT AND RECESSIVE GENETIC DISORDERS**
Gene Editing

- Mutated ‘broken’ gene
  - GENE ADDITION
  - Corrected gene
  - Normal functional gene

- Mutated ‘broken’ gene
  - GENE EDITING
  - Corrected gene
GENE THERAPY: PROVEN EFFICACY IN PATIENTS

Anno 2018

HEREDITARY BLINDNESS (LEBER AMAUROSIS)
approved for US market

CANCER (MELANOMA, LYMPHOMA)
approved for US market

IMMUNE DEFECTS (SCID)
approved for EU market

ANEMIA (β-THALASSEMIA)

NEURODEGENERATION (ADRENOLEUKODYSTROPHY)

BLOOD CLOTTING DEFECTS (HEMOPHILIA)

METABOLIC DISORDERS (LIPOPROTEIN LIPASE DEFICIENCY)
approved for EU market

GRAFT vs. HOST DISEASE
approved for EU market
FDA announces first US gene therapy approval for cancer treatment

By Michael Nedelman, CNN

The New York Times

F.D.A. Approves Second Gene-Altering Treatment for Cancer

By DENISE GRADY  OCT. 18, 2017
FDA-approved cancer gene therapy

T cells

vector

cancer-specific receptor (CAR)

genetically modified “KILLER” T cells

cancer cell
Effective treatment of lymphoid cancers by immuno-gene therapy

CAR-T cell

Emily Whitehead (U. Penn. C. June)
“Tonight I’m launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes.

And to give us all access to the personalized information we need to keep ourselves and our families healthier.”

President Barack Obama
2015 State of the Union Address | January 20, 2015
RESEARCH PRIORITIES

GENE THERAPY FOR HEREDITARY DISEASES AND CANCER

- DUCHENNE MUSCULAR DYSTROPHY
- POMPE
- MYOTUBULAR MYOPATHY
- MYOTONIC DYSTROPHY (DM1)
- CANCER

HEMOPHILIA A & B
HEMOPHILIA

• **CAUSE:**
defect in gene encoding coagulation factor (FVIII, FIX)

• **CONSEQUENCE:**
Uncontrolled bleeding, arthropathy
Potentially life-threatening (e.g. brain, internal organs)

• Gene therapy could provide a cure
Optimizing gene therapy & genome engineering

- Improving gene transfer
- Promoter engineering
- Vector engineering
- Cas9 engineering

- Improving expression
- Improving activity & specificity
POTENT HEPATOCYTE-SPECIFIC
CIS-REGULATORY MODULE (CRM)

“MOLECULAR TURBOCHARGER” AS EXPRESSION BOOSTER
HOW TO INCREASE GENE EXPRESSION AND SPECIFICITY?
**DE NOVO & IN SILICO DESIGN OF ROBUST LIVER-SPECIFIC PROMOTERS**

Compare promoters of genes that are highly and exclusively expressed in liver with those that are highly expressed in other tissues but NOT in the liver.

Evolutionary conserved CIS-REGULATORY MODULES (CRM) containing TFBS that are common among potent liver-specific promoters.
IDENTIFICATION OF HEPATOCYTE-SPECIFIC CRM (HS-CRM)
HS-CRM8: MAINTAINING LIVER-SPECIFICITY
CRMs: ROBUST HEPATOCYTE SPECIFIC FIX EXPRESSION

HSP + HS-CRM8 hepatocyte-specific promoter

hyperactive HSP FIX

AAV9

<table>
<thead>
<tr>
<th>CRM</th>
<th>FIX (ng/ml)</th>
<th>HEPATOCYTE SPECIFIC</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP-1 (strong, TTR)</td>
<td>5x10^9 gc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP-2 (weak, Palm)</td>
<td>5x10^11 gc</td>
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</tr>
</tbody>
</table>
THERAPEUTIC FIX LEVELS IN RHESUS MONKEYS
no toxicity

From ‘in silico’ to ‘in macaco’

Chuah et al.
Mol. Ther.,
2014

Computationally designed liver-specific transcriptional modules and hyperactive factor IX improve hepatic gene therapy

Nisha Nair,¹ Melvin Y. Rincon,¹,² Hanneke Evens,¹ Shilpita Sarcar,¹ Sumitava Dastidar,¹ Emira Samara-Kuko,¹ Omid Ghandeharian,¹ Hiu Man Viecelli,³ Beat Thöny,³ Pieter De Bleser,⁴ Thierry VandenDriessche,¹,² and Marinee K. Chuah¹,²

**Molecular Therapy** Sep;22(9):1605-13, 2014.

Liver-Specific Transcriptional Modules Identified by Genome-Wide In Silico Analysis Enable Efficient Gene Therapy in Mice and Non-Human Primates

Marinee K Chuah¹,², Inge Petrus², Pieter De Bleser³, Caroline Le Guiner⁴,⁵, Gwladys Gernoux⁴,⁵, Oumeya Adjali⁴,⁵, Nisha Nair¹, Jessica Willems¹, Hanneke Evens¹, Melvin Y Rincon¹,², Janka Matrajt¹,⁶,⁷, Mario Di Matteo¹,², Ermira Samara-Kuko¹, Bing Yan⁶,⁷, Abel Acosta-Sanchez⁶,⁷, Amine Meliani⁸,⁹, Ghislaine Cherel¹⁰,¹¹, Véronique Blouin⁴,⁵, Olivier Christophe¹⁰,¹¹, Philippe Moullier⁴,⁵, Federico MingoZZi⁸,⁹, Thierry VandenDriessche¹,²
X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua)

Paolo Simioni, M.D., Ph.D., Daniela Tormene, M.D., Ph.D., Giulio Tognin, M.D., Sabrina Gavasso, Ph.D., Cristiana Bulato, Ph.D., Nicholas P. Iacobelli, B.A., Jonathan D. Finn, Ph.D., Luca Spiezia, M.D., Ph.D., Claudia Radu, Ph.D., and Valder R. Arruda, M.D., Ph.D.

SYNTHEtic TRANSGENES: CODON-OPTIMIZATION + HYPERACTIVATING MUTATIONS

Nair et al. Blood, 2014
Hyper-functional coagulation factor IX improves the efficacy of gene therapy in hemophilic mice

Alessio Cantore, Nisha Nair, Patrizia Della Valle, Mario Di Matteo, Janka Mátrai, Francesca Sanvito, Chiara Brombin, Clelia Di Serio, Armando D'Angelo, Marinee Chuah, Luigi Naldini and Thierry VandenDriessche

Liver-directed lentiviral gene therapy in a dog model of hemophilia B

Alessio Cantore,1,2,∗ Marco Ranzani,1,2,† Cynthia C. Bartholomae,3 Monica Volpin,1,2 Patrizia Della Valle,4 Francesca Sanvito,5 Lucia Sergi Sergi,1 Pierangela Gallina,1 Fabrizio Benedicenti,1 Dwight Bellinger,6 Robin Raymer,7 Elizabeth Merricks,6 Francesca Bellintani,7 Samia Martin,6 Claudio Doglioni,6 Armando D’Angelo,6 Thierry VandenDriessche,9,10 Marinee K. Chuah,9,10 Manfred Schmidt,7 Timothy Nichols,6∗∗ Eugenio Montini,9 Luigi Naldini1,2,∗∗

Vol 7 Issue 277 277ra28, 2015
2 independent clinical trials (Pfizer, Shire) in hemophilia patients confirm our findings: more robust therapy!
RESEARCH PRIORITIES

GENE THERAPY FOR HEREDITARY DISEASES AND CANCER

- DUQUEHNE MUSCULAR DYSTROPHY
- POMPE
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HEMOPHILIA A & B

CANCER
EU-Horizon 2020 - New therapies for rare diseases

Development of next-generation gene therapy to cure hereditary muscle disorders: from bench to bedside

Orphan Drug Designation: Pompe’s disease
myotubular myopathy

Progress toward the goal of having 200 new medicinal products in 2020
Finding the right ‘molecular key’ to enter the target cells ...
Next-generation gene therapy: DMD

- Improving gene transfer
  - VECTOR AAV9
  - PROMOTER
  - GENE

- 40 to 400-fold increase in muscle

- Improving expression
- Improving activity
- Combination therapy
follistatin = myostatin inhibitor

Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child

Markus Schuelke, M.D., Kathryn R. Wagner, M.D., Ph.D., Leslie E. Stolz, Ph.D., Christoph Hübner, M.D., Thomas Riegel, M.D., Wolfgang Kömen, M.D., Thomas Braun, M.D., Ph.D., James F. Tobin, Ph.D., and Se-Jin Lee, M.D., Ph.D.


Belgian blue

myostatin mutation
Next-generation gene therapy: DMD
GENE-SPECIFIC EDITING: THE NEXT FRONTIER?

Science Breakthrough of the Year

The CRISPR Craze

A bacterial immune system yields a potentially revolutionary genome-editing technique

J. Doudna
E. Charpentier
F. Zhang
SOMATIC TISSUE-SPECIFIC GENE INACTIVATION: CRISPR/Cas9

obviates need to make transgenic KO

AAV9-CRM8-HSP-Cas9

Streptococcus pyogenes Cas9 (~4.2kb)

wt mice

hemo B mice

AAV9-U6-gRNA-FIX

Cas9

PAM

FIX

20bp complementary region
(trugRNA: 17 bp)
Efficient \textit{in Vivo} Liver-Directed Gene Editing using CRISPR/CAS9

Kshitiz Singh#, Hanneke Evens#, Nisha Nair#, Melvin Y. Rincón, Shilpita Sarcar, Ermira Samara-Kuko, Marinee K. Chuah, Thierry VandenDriessche

# joint first authors
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DOI: https://doi.org/10.1016/j.ymthe.2018.02.023
Somatic inactivation of endogenous mouse FIX

Low dose

High dose
SOMATIC TISSUE-SPECIFIC GENE INACTIVATION: CRISPR/Cas9

Deep sequencing confirmation of gene editing of FIX gene: NHEJ
SOMATIC TISSUE-SPECIFIC GENE INACTIVATION: CRISPR/Cas9

Deep sequencing analysis: on-target & off-target analysis

<table>
<thead>
<tr>
<th>Guide RNA ID Injected</th>
<th>Target ID</th>
<th>Gene</th>
<th>Genomic DNA</th>
<th>Guided RNA target (17 NT)</th>
<th>Percentage of reads supporting an indel</th>
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<tbody>
<tr>
<td>ON-target exon 1</td>
<td>Exon 1</td>
<td>mF9</td>
<td>Liver</td>
<td>GACACCTGAACACCCGTCATGGG</td>
<td>56%</td>
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<tr>
<td></td>
<td>17bp-trugRNA-</td>
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<td>mF9-Exon1</td>
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<tr>
<td></td>
<td>Exon1-OT1</td>
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<td>Liver</td>
<td>GACACTGAACACGGTCAGAG</td>
<td>Not Detected</td>
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<tr>
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<td>GACCTGAAC ACCCTCATGG</td>
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<td>GTACCTGAAC ACCCTCTGAG</td>
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<tr>
<td>ON-target exon 6</td>
<td>Exon 6</td>
<td>mF9</td>
<td>Liver</td>
<td>GACCTCACTCGAG TTGTTGG</td>
<td>19%</td>
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<td>17bp-trugRNA-mF9-Exon6</td>
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<tr>
<td></td>
<td>Exon6-OT1</td>
<td>Grin2b</td>
<td>Liver</td>
<td>CACTCTAACTCGAG GGGGG</td>
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<td>Exon6-OT2</td>
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<td>Liver</td>
<td>CACTCTACTAGAG TTGGTAG</td>
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<tr>
<td></td>
<td>Exon6-OT3</td>
<td></td>
<td>Liver</td>
<td>TACCTACACTGGAG TTGTTGG</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

NO OFF-target (in top 3 in silico predicted sites)

56%

19%
DNA double-strand breaks
- Ionizing radiation
- Oxidative damage
- Spontaneous events

Double-strand break rejoining
- Non-homologous end-joining (NHEJ)
- Homologous recombination (HR)

NHEJ: fast & error-prone
HR: slow & precise
LIVER-SPECIFIC CRM

SOMATIC TISSUE-SPECIFIC GENE INACTIVATION: CRISPR/Cas9

AAV9-CRM8-HSP-Cas9
AAV9-U6-gRNA-FIX
wt mice
HR
NHEJ
hemo B mice
Gene therapy & gene editing: no “magic bullet”
Optimal therapeutic window

Safety

Efficacy

subtherapeutic  toxicity

vector potency  vector dose
Gene therapy & gene editing: no “magic bullet”
Optimal therapeutic window

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subtherapeutic

vector potency

vector dose

toxicity
Dank u - thank you - धन्यवाद - 謝謝 – ขอบคุณ
falemnderit – grazie - gracias - danke

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EUFP6, EUP7, EU Horizon 2020, FWO, IWT, EHA, AFM, WILLY GEPTS FONDS, KONING BOUDEWIJN STICHTING (WALTER PYLEMAN FONDS), VUB IOF GEAR, VUB “STRATEGIC RESEARCH PROJECT” GROWER STK, VUB POC IOF