Possibilities of therapeutic genome editing in monogenic and polygenic disease

Rafael Yáñez, rafael.yanez@royalholloway.ac.uk
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DISCLOSURE

- Editor-in-Chief, *Gene Therapy* (Springer-Nature), stipend
Professor of Advanced Therapy, Director of Centre of Gene and Cell Therapy, Royal Holloway, University of London
Editor-in-Chief, Gene Therapy
Treasurer, British Society for Gene and Cell Therapy
Chair, Genetic Alliance UK
Yáñez lab: Developing safer gene and cell therapy methods

Episomal vectors

Genome editing
Advanced Gene and Cell Therapy Lab

Disease models:
- Spinal muscular atrophy
- Ataxia telangiectasia
- Severe combined immunodeficiency
- Duchenne MD (with G. Dickson and L. Popplewell)
- Spinal injury
- Parkinson
- Stroke

Strategies: Genome editing and Gene addition
- Site-specific designer nucleases
- Episomal systems
- Replicating episomes
- Induced pluripotent stem cells
- In utero gene delivery

Vector systems:
- Lentiviral (HIV-1, integration-deficient)
- Adeno-associated viral
- Retroviral
- Adenoviral
- Non-viral
The importance of rare diseases
The current status of gene therapy
The limiting factor: DNA double-strand breaks (DSBs)
Gene targeting becomes genome editing
Chimeric nuclease families
DSB repair pathways
Genome editing methods
Genome editing in clinical trials
Latest development: CRISPR base editors
Generic limitations
Ethical issues
How is he going to do that?

rafael.yanez@royalholloway.ac.uk
twitter: @ryanezmunoz

Keeping it simple, please get back to me if interested
In Europe, a disease is rare if fewer than 1 in 2,000 people are affected...

...6,000-8,000 rare diseases, 7% of people, 20% of Health budget...

...most rare diseases affect children and 30% of people affected will die before their 5th birthday...

...but 80% of rare diseases are inherited (genetic)...

...and many are potentially amenable to gene and stem cell therapies.
In many monogenic genetic diseases the therapeutic target has been defined and validated.

Most gene therapy technology has been developed and tested on rare diseases, but will also be applied to common diseases.
What is gene therapy?

Deliberate alteration of the genome or its function to produce a therapeutic benefit. Sometimes cells are modified outside the body, resulting in gene cell therapy.
What can you do with gene therapy?

- Introduce a minigene
- Make a gene produce more (or less) protein
- Kill cells
- Vaccinate
- Stop a gene from working
- Repair a gene
Gene therapy vectors

Non-Viral
- Naked DNA
- Lipoplex/Polypelex
- Recombinant Cells (Microencapsulation)

Viral
- Retroviral vectors
- Adenoviral vectors
- Adeno-Associated Virus vectors
Approved gene therapy products

[Antisense oligonucleotides:] 
- Exondys 51 (antisense oligonucleotide, Duchenne muscular dystrophy, US)
- Spinraza (antisense oligonucleotide, Spinal muscular atrophy, US, EU...)
- Onpattro (siRNA in lipid nanoparticle, hereditary ATTR amyloidosis, US, EU)
- Tegsedi (antisense oligonucleotide, hereditary ATTR amyloidosis, EU)

Viral vectors: 
- Gendicine (adenovirus vector, Cancer, China)
- [Glybera (adeno-associated virus vector, LPL deficiency, EU)]
- Imlygic (herpesvirus vector, Cancer, EU and US)
- Luxturna (adeno-associated virus vector, RPE65 deficiency, US, EU)
- Zolgensma (adeno-associated virus vector, Spinal muscular atrophy, US)

Genetically modified cells: 
- Strimvelis (ADA retrovirus vector-treated autologous HSCs, ADA deficiency, EU)
- Zalmoxis (HSV-TK retrovirus vector-treated allogeneic T-cells, HSCT, EU)
- Kymriah (CAR lentivirus vector-treated autologous T-cells, leukemia, US)
- Yescarta (CAR retrovirus vector-treated autologous T-cells, leukemia, US)
- Zynteglo (lentivector βA-T87Q-globin-treated autologous CD34+ cells, β-thalassemia, EU)

See hyperlink for up-to-date info on approved gene therapy (and cell therapy) products, provided by ISSCR

https://bit.ly/2Kf9OWo
Nusinersen-treated Spinal muscular atrophy

Natural history of type 1 Spinal muscular atrophy: death by age 2. After nusinersen (Spinraza) treatment children are not cured, but they can thrive and achieve developmental milestones unheard of in this severe type.
Value and growth of gene therapy market

The market will be **ACCELERATING** growing at a **CAGR** of over **22%**

**INCREMENTAL GROWTH**

2017: $2.03 bn
2022:

The year-over-year growth rate for **2018** is estimated at **20.54%**

The **ONCOLOGY THERAPY SEGMENT** occupied the **HIGHEST** market share in **2017**

**59%** of the growth will come from the **AMERICAS**

One of the **KEY DRIVERS** for this market will be the entry of novel molecules during the forecast period

READ THE REPORT:

**GLOBAL GENE THERAPY MARKET 2018-2022**

**10,000+** reports covering niche topics

**HEALTHCARE AND LIFE SCIENCES**

Read them at:

[www.technavio.com](http://www.technavio.com)
The problem with access to the gene therapy market

Spinraza: EUR90,000/dose (EUR540,000 first year, EUR270,000 thereafter)

Strimvelis: EUR594,000

Luxturna: $425,000 (per eye)

Zolgensma: $2,100,000
Genome editing is a form of gene therapy that allows the introduction of defined modifications.
The future is CRISPR...maybe

RIDING THE CRISPR WAVE

Biologists are embracing the power of gene-editing tools to explore genomes.

When ever a paper about CRISPR-Cas9 hits the press, the staff at Addgene quickly finds out. The non-profit company is where study authors often deposit molecular tools that they used in their work, and where other scientists immediately turn to get them. “We get calls within minutes of a hot paper publishing,” says Joanne Kamens, executive director of the company in Cambridge, Massachusetts.

Addgene’s phones have been ringing a lot since early 2013, when researchers first reported that they had used the CRISPR-Cas9 system to slice the genome in human cells at sites of DNA cutting. “It was all hands on deck,” Kamens says. Since then, molecular biologists have raced to adopt the technique.

Which can be used to alter the genetic code in organisms in mice, plants, and flies. Addgene has sent 80,000 related molecular tools — about 5,000 shipments — to researchers in 90 countries. And the company’s CRISPR watchers have viewed more than one million times.

Much of the conversation about CRISPR-Cas9 has revolved around its possible uses in treating disease or editing the genetic code of human embryos, but researchers say that its real impact is in the lab. When scientists use the tool, they have the ability to target and study particular DNA sequences in the vast expanses of the genome.

And editing DNA is just one trick that is used for. Scientists are hacking the tool to study how genes are turned on and off, and even how to design biological circuits — with the help of CRISPR.
Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells

Fatima Smih, Philippe Rouet, Peter J. Romanienko and Maria Jasin
I-Scel’s place in the history of genome editing

- Intron-encoded homing endonuclease from *S. cerevisiae* mitochondria
- Introduces a specific double-strand break in the DNA of the 21S rRNA gene and thus mediates the insertion of an intron, containing its own coding sequence (group I intron), into an intronless gene
- 18-bp recognition site, not present in mammalian genome
- Engineered by Bernard Dujon and col.
Introducing a DSB to promote genome editing

express I-SceI nuclease gene or deliver the protein to introduce a DSB and promote homology-dependent repair

corrective vector

mutation
generated I-SceI site

mutant gene

corrected gene
Improving the frequency of genome editing

- Standard plasmid-based frequency: \( \leq 0.001\% \)
- Overexpress \( RAD51/recA \) - \((\leq 0.001\%; 2\text{-fold increase})\)
- Microinject gene targeting construct - \((\leq 0.8\%)\)
- Use AAV-based vectors - \((\leq 1\%)\)
- Induce I-SceI DSB in the target gene - \((\leq 20\%)\)

\((\% \text{ of genome-edited cells})\)
Was there life before CRISPR?

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

1983
DSB induced recombination model proposed.
Szostak et al, 1983.

1994
Meganuclease used in mammalian cell.

1996
ZFPs fused to Fok1 to form ZFNs.
Kim et al, 1996.

2003
ZFNs used in mammalian cell.

2006
Artificial meganuclease created to cleave human gene sequences.

2009

2010
TALEs fused to Fok1 to form TALENs and used in non-mammalian cell.

2010
ZFNs used in first human clinical trial for genome editing.

2011
TALENs used in mammalian cell. Li et al, 2011.

2011
 genome editing with CRISPR/Cas9.

2012
Genome editing wins method of the year, awarded by Nature Methods.

(Crompton and Yáñez-Muñoz)
Generation of DNA double-strand breaks (DSBs)

Endogenous agents
- Replication fork collapse
- Oxidative damage
- Telomere failure
- Folate deficiency
- Programmed rearrangements
- Meiosis

Exogenous agents
- Ionising radiation
- Chemotherapeutics
- Chemicals
- Chimeric nucleases
Complexity of the cellular response to DSBs

Four families of engineered nucleases allow genome editing

- Meganucleases
- Zing-finger nucleases (ZFN)
- Transcription activator-like effector nucleases (TALEN)
- Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) nucleases

The first three require protein engineering for re-targeting; in CRISPR/Cas the protein component does not require engineering, and is re-targeted by a small, synthetic guide RNA. Therefore, CRISPR/Cas is very easy to engineer and use.
Meganucleases (I-CreI-based)

HOMING ENDONUCLEASE I-CREI / DNA SUBSTRATE COMPLEX WITH CALCIUM

1G9Y

(http://www.rcsb.org/pdb/explore/jmol.do?structureId=1G9Y&bionumber=1)
Zinc-finger nucleases

(Nat Biotech 21, 759-760, 2003)
ZFNs and TALENs

ZFN: zinc-finger nuclease

TALEN: Transcription activator–like effector nuclease

(Nat Meth 9, 27, 2012)
Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems

(Jinek et al., Science 337, 816-821, 2012)
Comparison of chimeric nucleases

<table>
<thead>
<tr>
<th>Feature</th>
<th>Meganuclease</th>
<th>ZFN</th>
<th>TALEN</th>
<th>CRISPR/Cas9</th>
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</thead>
<tbody>
<tr>
<td>Flexible localisation</td>
<td>Complex</td>
<td>Limited</td>
<td>Average</td>
<td>Almost total</td>
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<tr>
<td>Nuclease construction</td>
<td>Laborious</td>
<td>Significant</td>
<td>Significant</td>
<td>Simple</td>
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<td>In vitro testing</td>
<td>Laborious</td>
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<td>Simple</td>
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<tr>
<td>Targeting efficiency</td>
<td>Not reported</td>
<td>Limiting factor</td>
<td>Average</td>
<td>Good</td>
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<tr>
<td>Off-target effects</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
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<tr>
<td>Multiplexing</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Time investment</td>
<td>High</td>
<td>Moderate</td>
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<td>Low</td>
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<tr>
<td>Cost</td>
<td>High</td>
<td>Average</td>
<td>Average</td>
<td>Low</td>
</tr>
</tbody>
</table>

(https://www.genoway.com/services/crispr-cas9-models/nucleases.htm)
CRISPR has made genome editing democratic

Before CRISPR it was possible but technically very demanding; now anyone can use it.
Basic model for the repair of DSBs

Homology-dependent repair (HDR) uses a sister chromatid or synthetic repair template and is accurate. Nonhomologous recombination (end-joining) is very effective but introduces InDels (small INsertions/DELetions).
Genome editing methods: gene disruption

(Cathomen and Joung, Mol Ther. 16, 1200-7, 2008)
Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 \textit{in vivo}

Nathalia Holt\textsuperscript{1}, Jianbin Wang\textsuperscript{2}, Kenneth Kim\textsuperscript{2}, Geoffrey Friedman\textsuperscript{2}, Xingchao Wang\textsuperscript{3}, Vanessa Taupin\textsuperscript{3}, Gay M Crooks\textsuperscript{4}, Donald B Kohn\textsuperscript{4}, Philip D Gregory\textsuperscript{2}, Michael C Holmes\textsuperscript{2} & Paula M Cannon\textsuperscript{1}

CCR5 is the major HIV-1 co-receptor, and individuals homozygous for a 32-bp deletion in \textit{CCR5} are resistant to infection by CCR5-tropic HIV-1. Using engineered zinc-finger nucleases (ZFNs), we disrupted \textit{CCR5} in human CD34\textsuperscript{+} hematopoietic stem/progenitor cells (HSPCs) at a mean frequency of 17\% of the total alleles in a population. This procedure produces both mono- and bi-allelically disrupted cells. ZFN-treated HSPCs retained the ability to engraft NOD/SCID/IL2\textsuperscript{null} mice and gave rise to polyclonal multi-lineage progeny in which \textit{CCR5} was permanently disrupted. Control mice receiving untreated HSPCs and challenged with CCR5-tropic HIV-1 showed profound CD4\textsuperscript{+} T-cell loss. In contrast, mice transplanted with ZFN-modified HSPCs underwent rapid selection for \textit{CCR5}\textsuperscript{−/−} cells, had significantly lower HIV-1 levels and preserved human cells throughout their tissues. The demonstration that a minority of \textit{CCR5}\textsuperscript{−/−} HSPCs can populate an infected animal with HIV-1-resistant, \textit{CCR5}\textsuperscript{−/−} progeny supports the use of ZFN-modified autologous hematopoietic stem cells as a clinical approach to treating HIV-1.

(Nat Biotech 2010, doi:10.1038/nbt.1663)
Sangamo’s AIDS clinical trial

Overall, successful engineering of CCR5 knockout in either T-cells or haematopoietic stem cells in clinical trials.
Genome editing methods: gene correction

(Cathomen and Joung, Mol Ther. 16, 1200-7, 2008)
Genome editing methods: gene correction with superexon block

(Cathomen and Joung, Mol Ther. 16, 1200-7, 2008)
In vivo genome editing for gene correction (yet to be attempted)

http://www.sec.gov/Archives/edgar/data/936402/000095010314008732/dp51789_ex9901.htm
In vivo genome editing into albumin locus (attempted for MPS I and II)

Gene Therapy in Albumin
Safe Harbor Locus: Factor VIII & Factor IX

DISEASE
- Rare hereditary disorder in which the ability of patients’ blood to clot is impaired due to impaired FIX or FVIII production – leads to excessive and uncontrolled internal bleeding, pain and eventual permanent damage to joints and muscles
- Epidemiology: 1 / 5000 male births (~8 out of 10 people who have hemophilia have type A)
- Disease severity: severe, moderate, mild – dependent on percentage of FVIII / FIX level in blood, (<1%, 1-5%, >5%)

MECHANISM

Albumin Gene:

Donor:

Successfully Targeted:

Highly Expressed Gene

Lots of Therapeutic Protein

http://www.sec.gov/Archives/edgar/data/936402/000095010314008732/dp51789_ex9901.htm
Major issues in genome editing

- Delivery (effectiveness is cell- and/or tissue-dependent)
- Efficiency (cell-type dependent)
- Fidelity (on-target; to be improved, not all changes are intended one)
- Specificity (off-target; to be improved, non-target sites can be cut)
- Translocation risk (multiplexing requires sequential editing)
Chronic myeloid leukaemia

Simultaneous double-strand breaks cause translocations

Knocking out CD52, TCR and perhaps other genes to generate allogeneic Universal Chimeric Antigen Receptor T-cells (UCART) currently requires sequential rounds of genome editing to avoid translocations.

Base Editing: no DSB required

Human embryos...the ultimate frontier

Human embryos are at the centre of a debate over the ethics of gene editing.

(Nature, 22 Apr 2015)
Join the genomic medicine revolution!
Advanced Gene and Cell Therapy Lab-2019

Dr Versha Prakash
Dr Katie Lloyd-Jones
Miss Ellie Crompton
Ms Sahar Akbari Vala
Mr Ben Sadler

Dr Martin Broadstock
Dr Céline Rocca
Dr Klaus Wanisch
Dr Jamuna Selvakumaran
Dr Sherif Ahmed
Dr Hanna Kymäläinen
Dr Ngoc Lu-Nguyen
Dr Gaby Boza
Dr Tiziana Rossetti
Dr Hayder Hafdh Abdul-Razak
Dr Neda Ali Mohammadi Nafchi
Dr Mohammed Abdelrasul
Dr Simona Ursu
Dr Hugo Peluffo
Dr Mario Marotta
Dr Rebeca Hernández
Dr Victor Caraballo-Miralles
Dr F Javier Molina
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Manfred Schmidt
Christof von Kalle

CIEMAT, Spain
Guillermo Güenechea
F. Javier Molina-Hernández
Marina I Garin
Juan Bueren

Sangamo BioSciences, USA
Michael Holmes
Philip Gregory

University of Copenhagen
Camilla Andersen
Eric Paul Bennett

Reagents:
Lauren Naldini
Michael Sendtner
Christopher Baum
Cecilia Lundberg
Sebastian Kügler

iSTEM, France
Mathilde Girard

Netherlands Institute for Neuroscience
Joost Verhaagen

Royal Holloway, University of London
Taeyoung Koo
Linda Popplewell
George Dickson

Institute of Ophthalmology, UCL
Kamaljit Balaggan
Angus MacNeil
Alexander Smith
Prateek Buch
Yanai Duran
Robert MacLaren
Susie Barker
Robin Ali

Department of Human Genetics,
Aarhus Univ, Denmark
Brian Moldt
Jacob Mikkelsen

UK SMA Research Consortium
Kevin Talbot, Matthew Wood, Melissa Bowerman (Univ of Oxford)
Thomas Gillingwater, Caterina Becker (Univ of Edinburgh)
Ke Ning (Univ of Sheffield)