Possibilities of therapeutic genome editing in monogenic and polygenic disease

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14.06.2019, EULAR, Madrid


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sake
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BREXIT

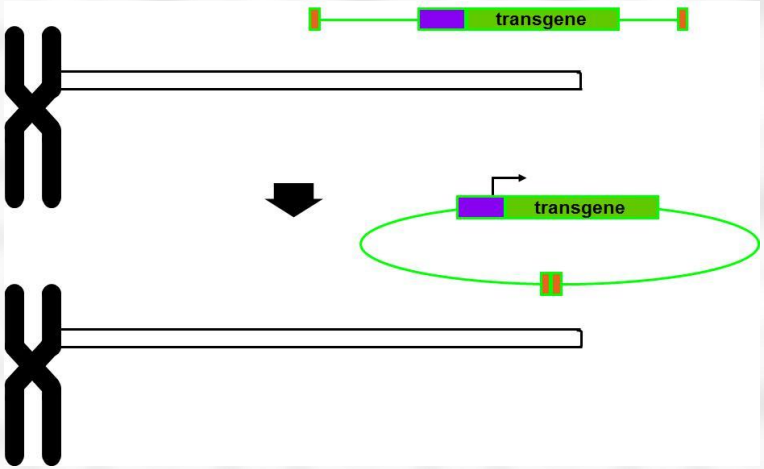
DISCLOSURE

- **Editor-in-Chief, *Gene Therapy* (Springer-Nature), stipend**

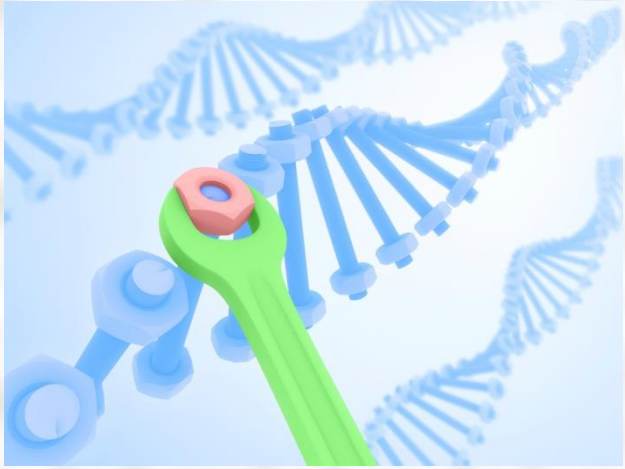
Professor of Advanced Therapy, Director of Centre of Gene and Cell Therapy,
Royal Holloway, University of London
Editor-in-Chief, *Gene Therapy*
Treasurer, *British Society for Gene and Cell Therapy*
Chair, *Genetic Alliance UK*

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

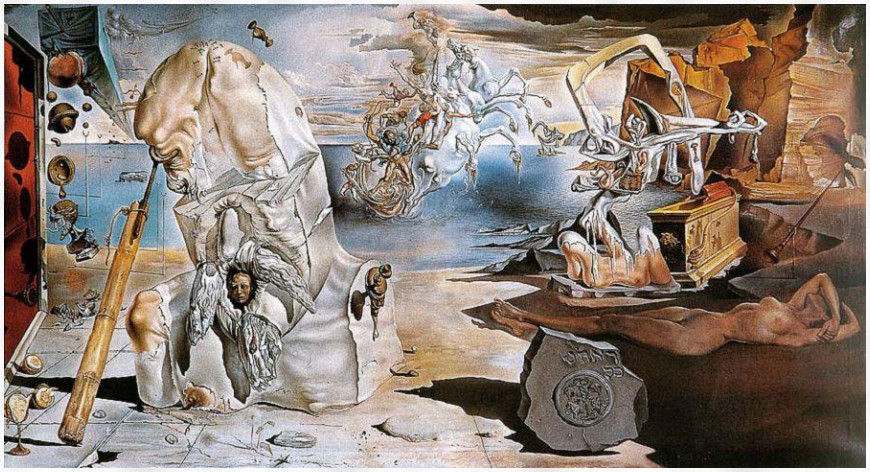
- **Strategies: Genome editing and Gene addition**
  - Site-specific designer nucleases
  - Episomal systems
  - Replicating episomes
  - Induced pluripotent stem cells
  - *In utero* gene delivery

- **Vector systems:**
  - Lentiviral (HIV-1, integration-deficient)
  - Adeno-associated viral
  - Retroviral
  - Adenoviral
  - Non-viral

What is he talking about?

- 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?



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Keeping it simple, please get back to me if interested

Why are Rare Diseases important?

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.

Why are Rare Diseases important?

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?

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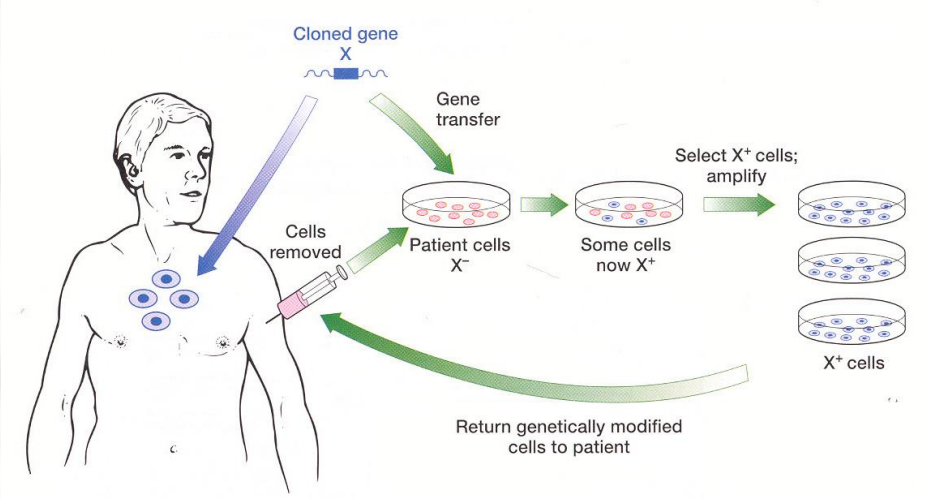
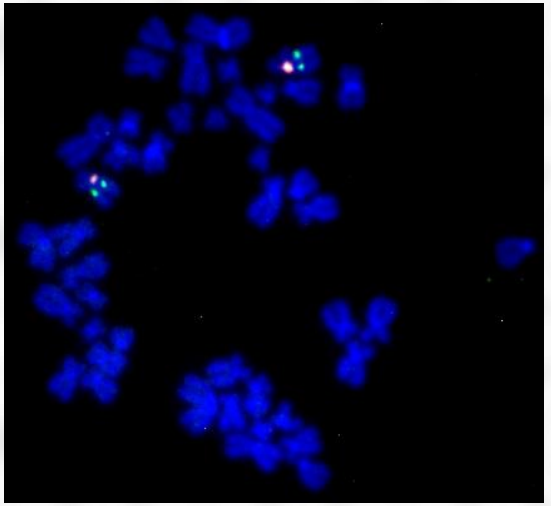
CGCT
Centre of Gene and Cell Therapy

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?

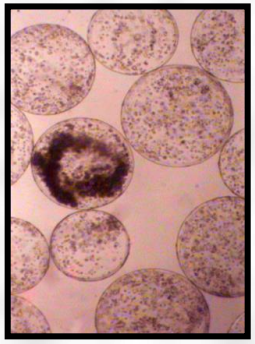
- Introduce a minigene
- Make a gene produce more (or less) protein
- Kill cells
- Vaccinate
- Stop a gene from working
- Repair a gene

Gene therapy vectors

Non-Viral

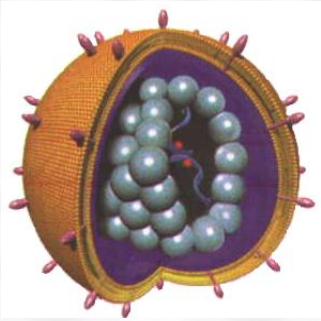
Naked DNA

Lipoplex/
Polyplex

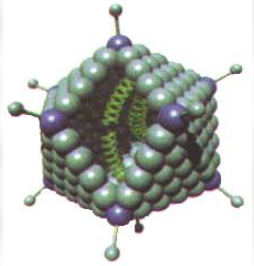


Recombinant Cells
(Microencapsulation)

Viral



Retroviral
vectors



Adenoviral
vectors

Adeno-Associated
Virus vectors

Approved gene therapy products

https://bit.ly/2Kf9OWo

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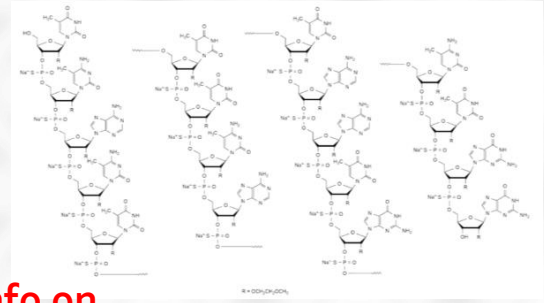
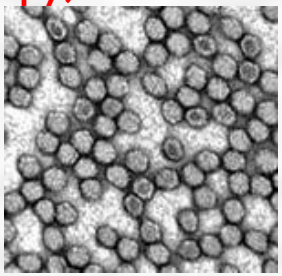
[Antisense oligonucleotides:]



- 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)

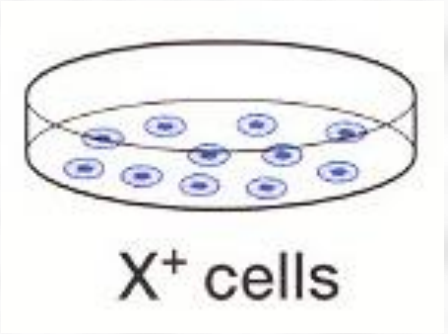
See hyperlink for up-to-date info on approved gene therapy (and cell therapy) products, provided by ISSCR

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 $\beta^{A\text{-}T87Q}$-globin-treated autologous $CD34^{+}$ cells, β-thalassemia, EU)

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

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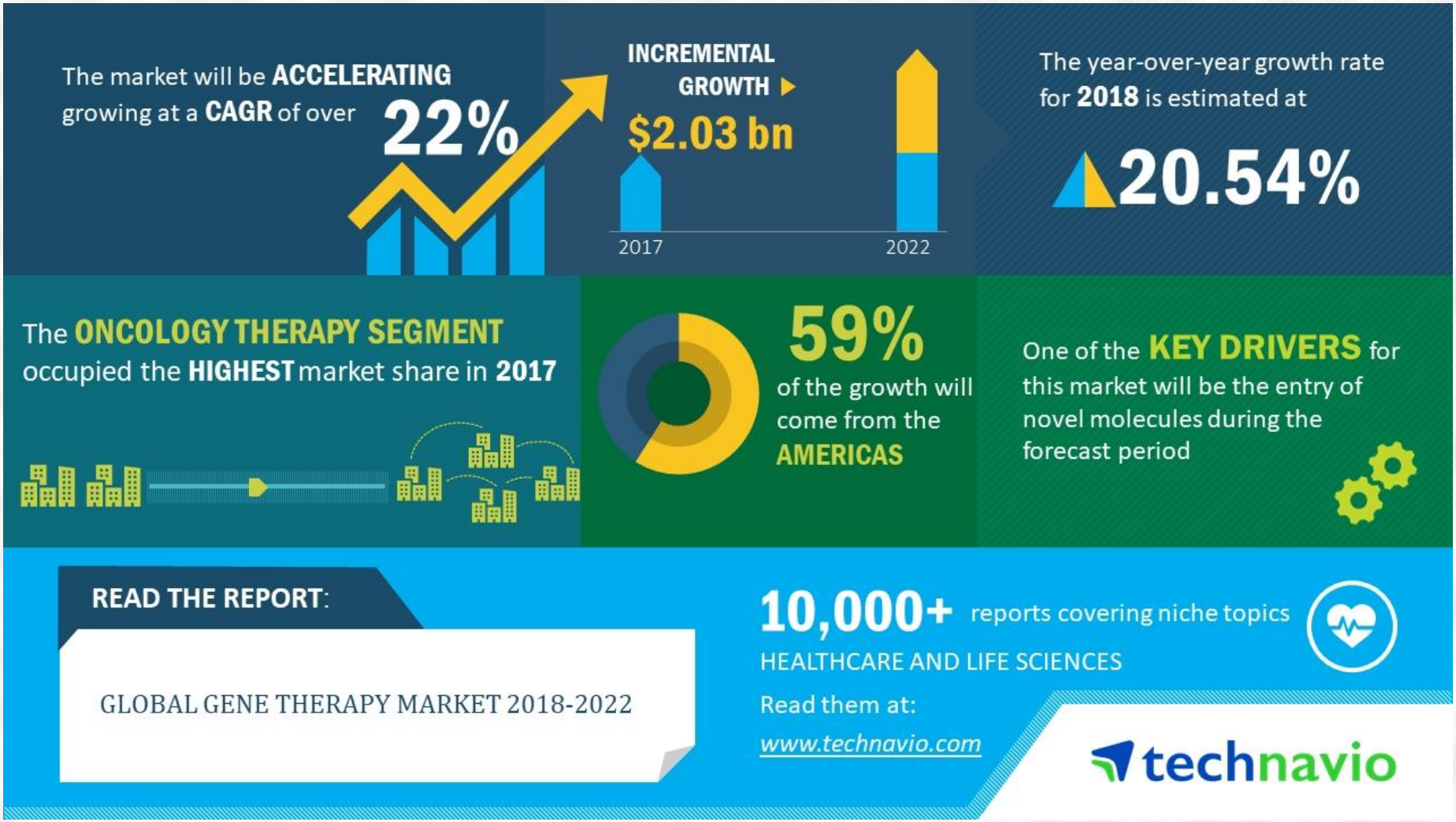
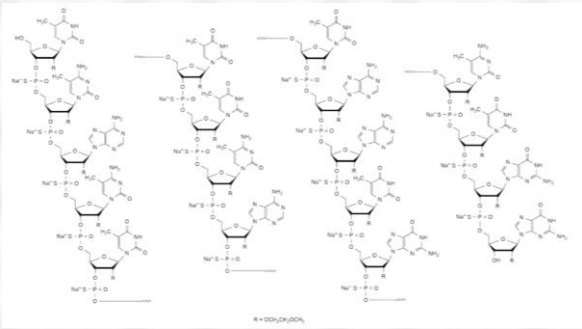
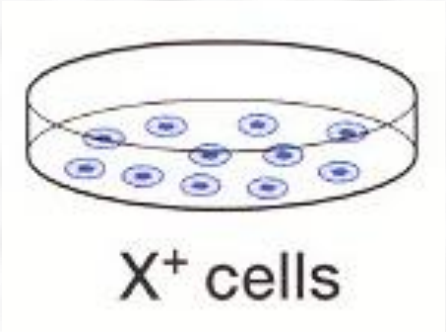


The problem with access to the gene therapy market

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

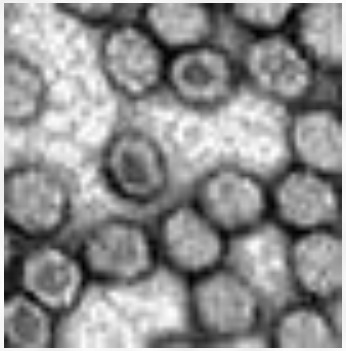


Strimvelis: EUR594,000

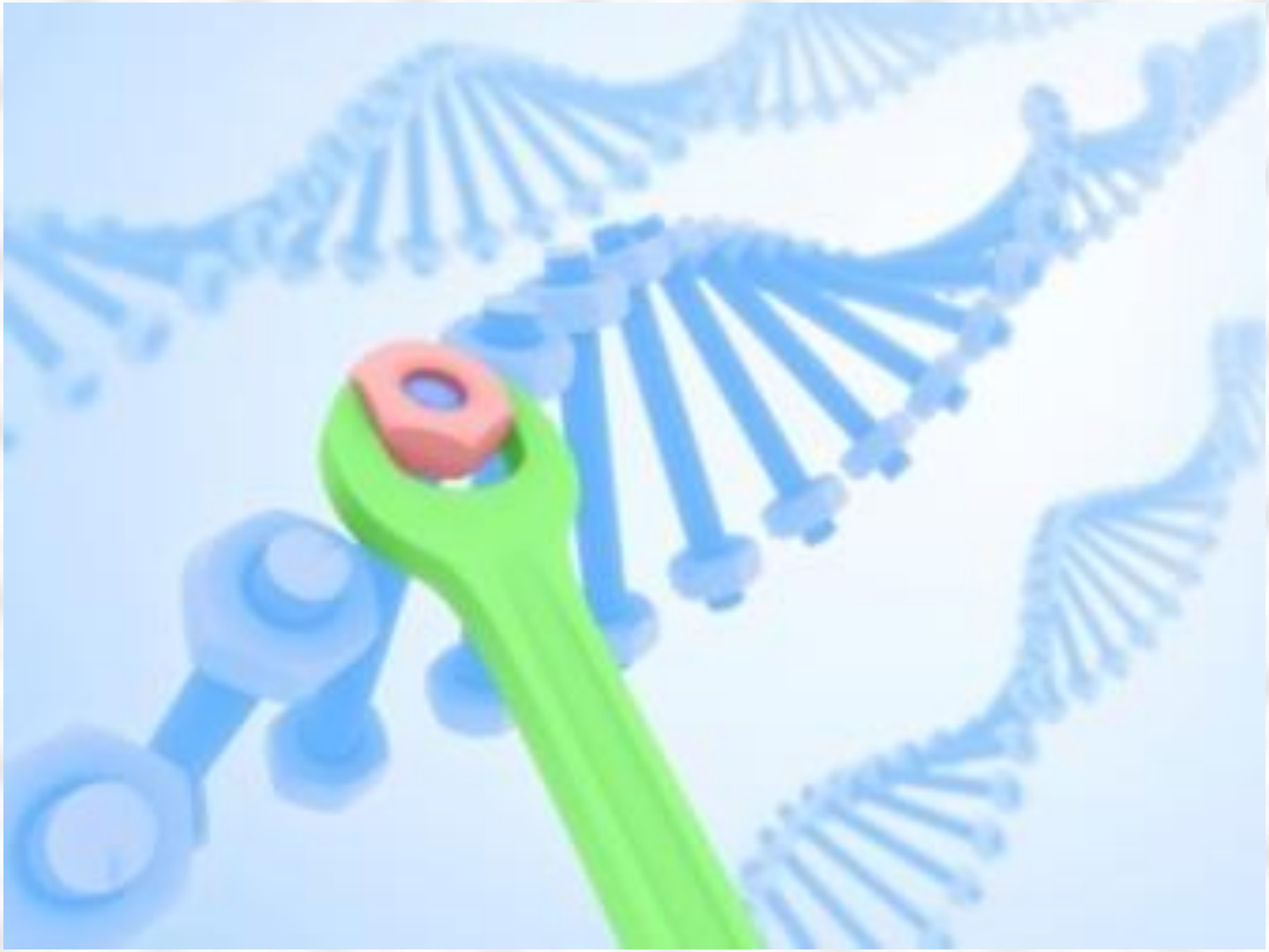


Luxturna: $425,000 (per eye)

Zolgensma: $2,100,000



Genome editing is a form of gene therapy that allows the introduction of defined modifications



The future is CRISPR…maybe

(Nature,10 Mar 2016)

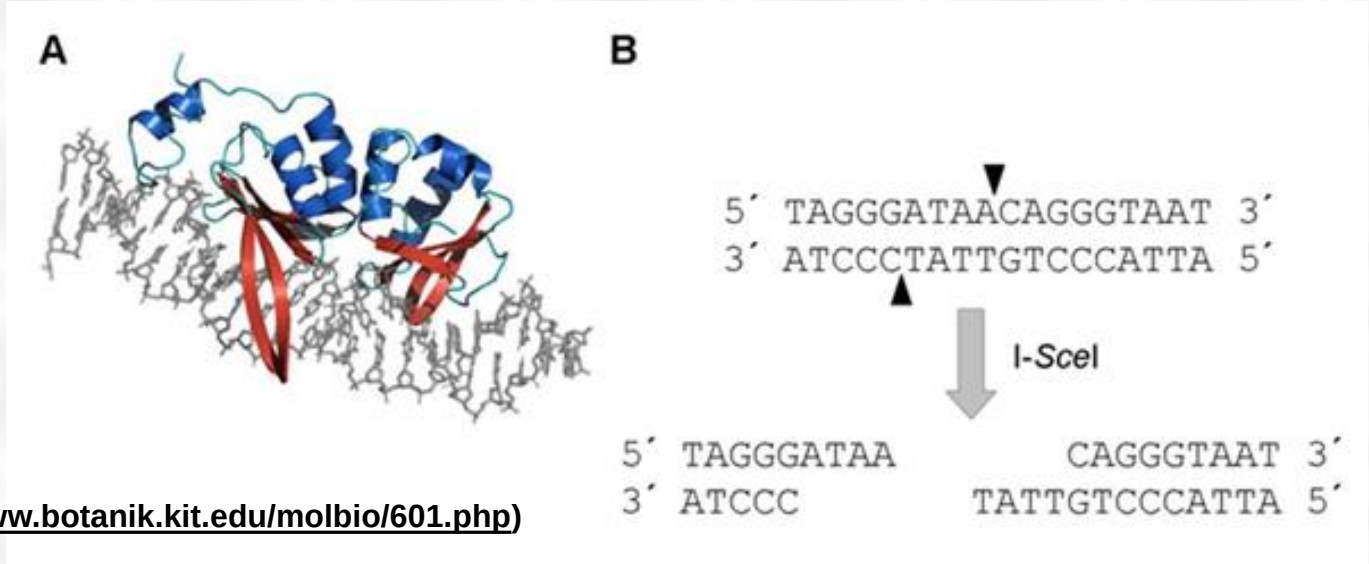
DSBs and the frequency of genome editing

5012–5019 Nucleic Acids Research, 1995, Vol. 23, No. 24

Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells

Fatima Smih, Philippe Rouet, Peter J. Romanienko[1] and Maria Jasin*

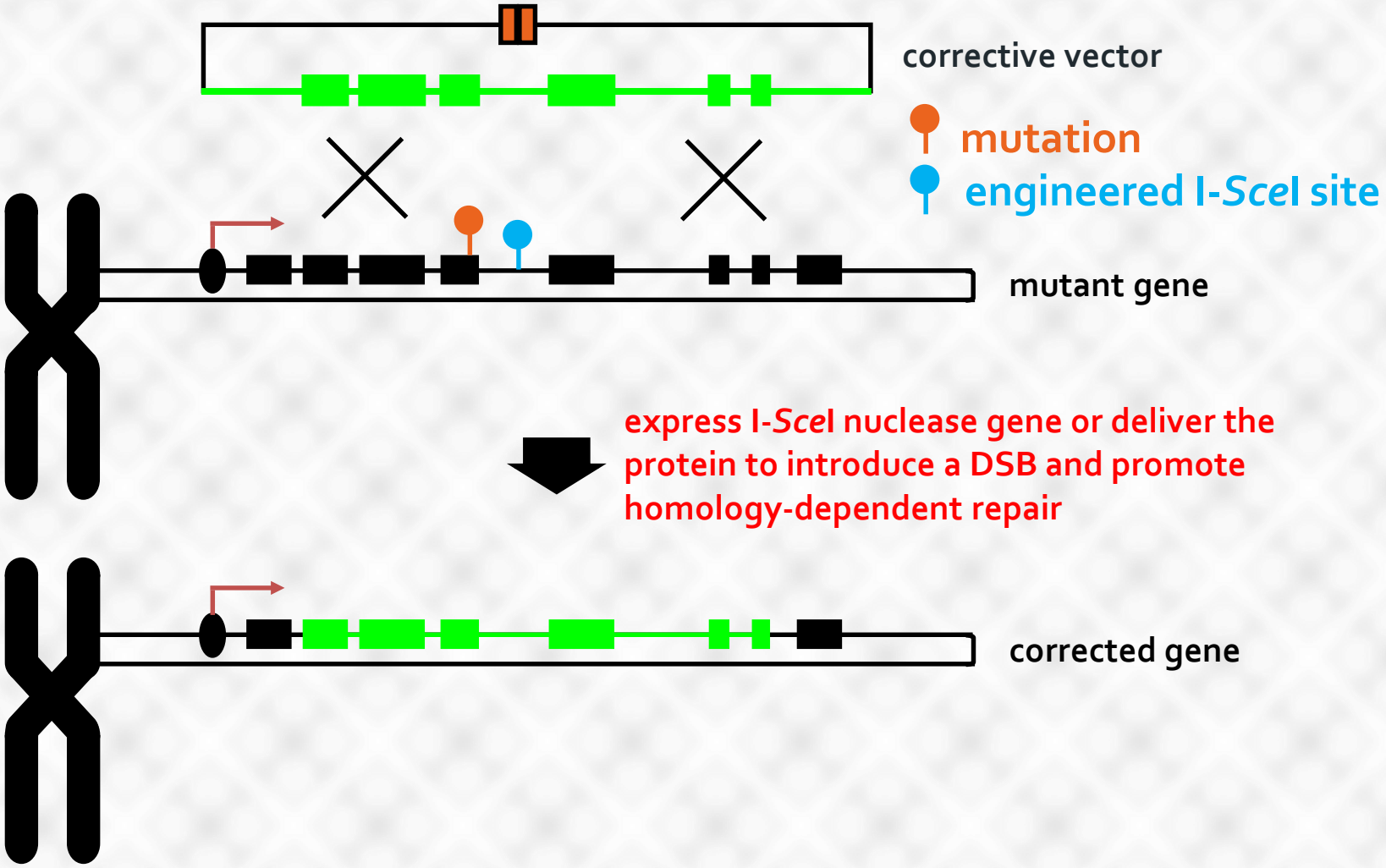
I-*Sce*I's place in the history of genome editing



(http://www.botanik.kit.edu/molbio/601.php)

- 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



Improving the frequency of genome editing

- Standard plasmid-based frequency: ≤ 0,001%
- Overexpress *RAD51/recA* - (≤ 0,001%; 2-fold increase)
- Microinject gene targeting construct - (≤ 0.8%)
- Use AAV-based vectors - (≤ 1%)
- Induce I-*Sce*I DSB in the target gene - (≤ 20%)

(% 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
Meganucleases used in mammalian cell.
Rouet et al, 1994.

1996
ZFPs fused to *Fok*1 to form ZFNs.
Kim et al, 1996.

2003
ZFNs used in mammalian cell.
Porteus and Baltimore, 2003.

2006
Artificial meganucleases created to cleave human gene sequences.
Smith et al, 2006.

2007
Nobel Prize
Capecchi, Smithies, Evans.

2009
TALEN effector code found. *Boch et al, 2009; Moscou and Bognadove, 2009.*

2010
TALEs fused to *Fok*1 to form TALENs and used in non-mammalian cell.
Christian et al, 2010.

2010
ZFNs used in first human clinical trial for genome editing.
Tebas et al, 2014.

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

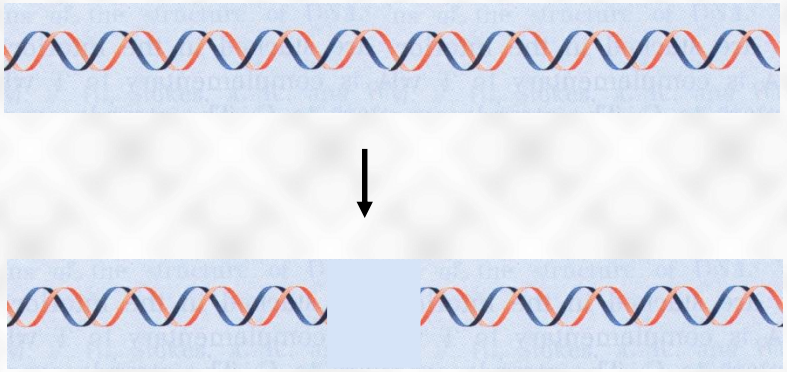
2011
Genome editing wins method of the year, awarded by Nature Methods.

2012
In vitro genome editing with CRISPR/Cas9.
Jinek at al, 2012.

2013
Multiplex genome editing with CRISPR/Cas9.
Cong et al, 2013.

(Crompton and Yáñez-Muñoz)

Generation of DNA double-strand breaks (DSBs)



Endogenous agents

Replication fork collapse
Oxidative damage
Telomere failure
Folate deficiency

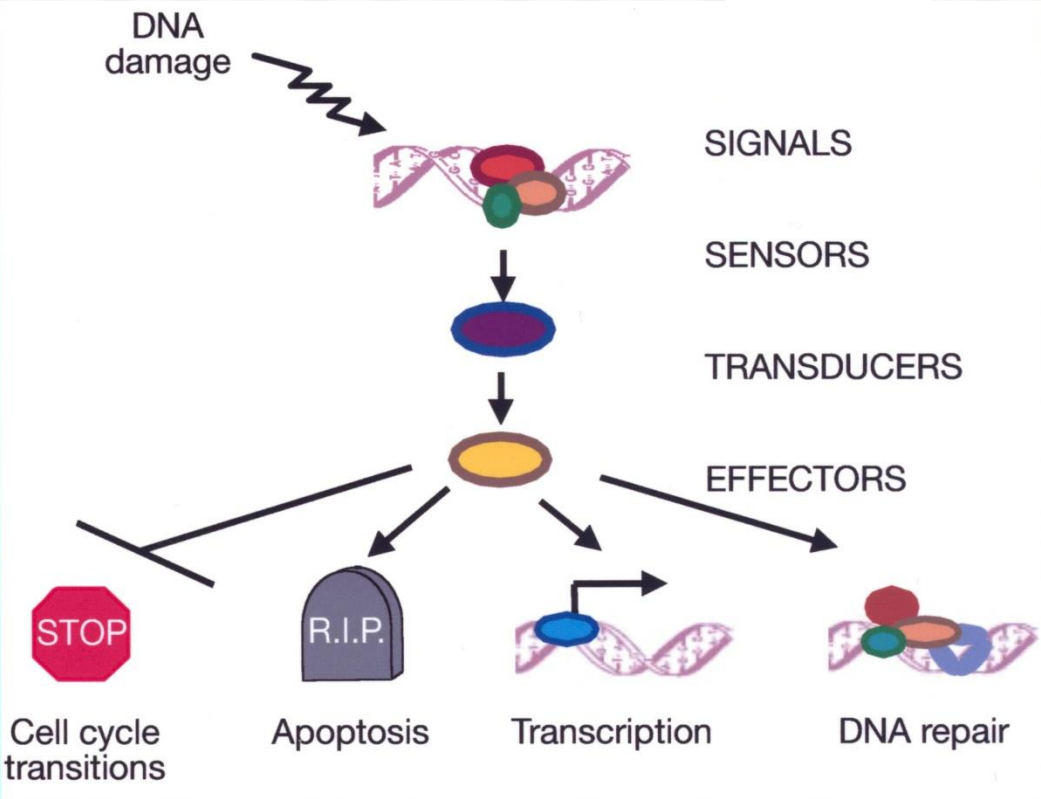
Programmed rearrangements
Meiosis

Exogenous agents

Ionising radiation
Chemotherapeutics
Chemicals

Chimeric nucleases

Complexity of the cellular response to DSBs



Zhou and Elledge (2000) *Nature* 408, 433-439

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-*Cre*I-based)

HOMING ENDONUCLEASE I-CREI / DNA SUBSTRATE COMPLEX WITH CALCIUM

1G9Y

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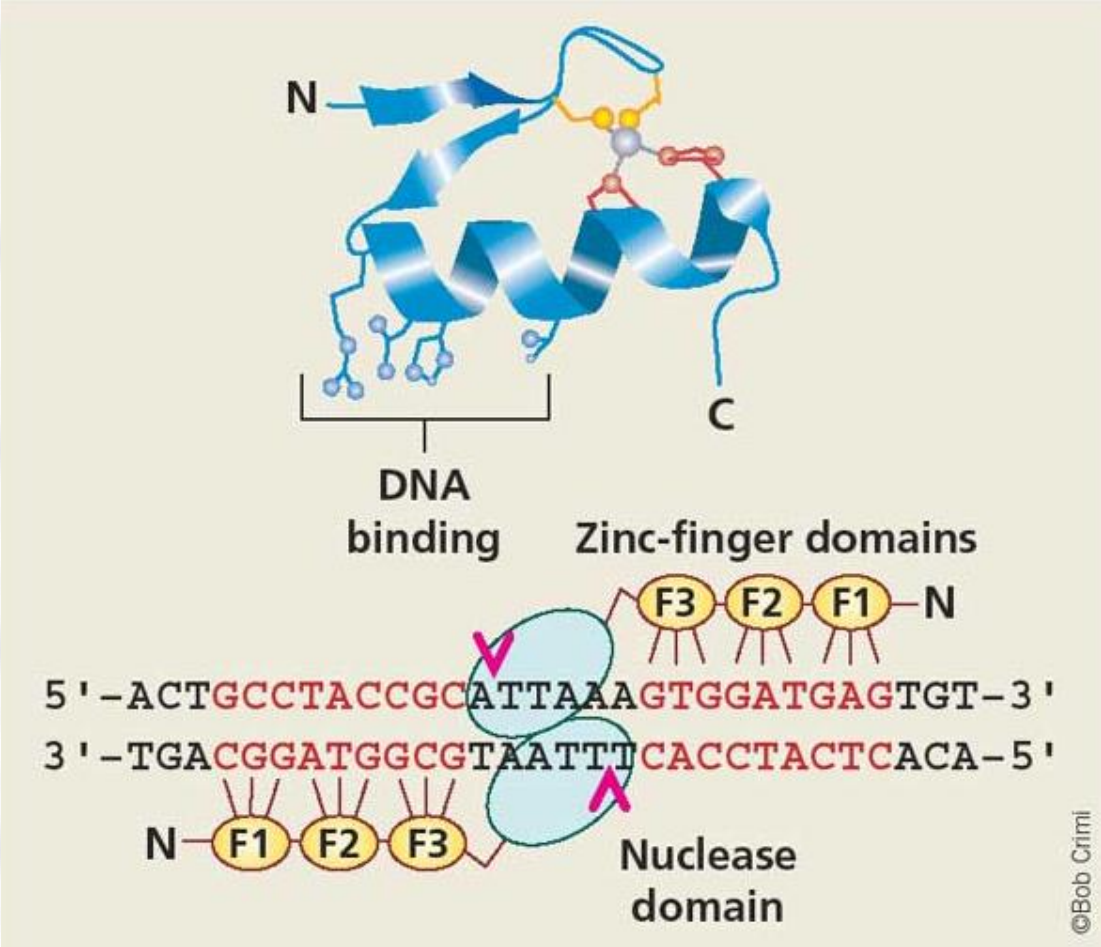
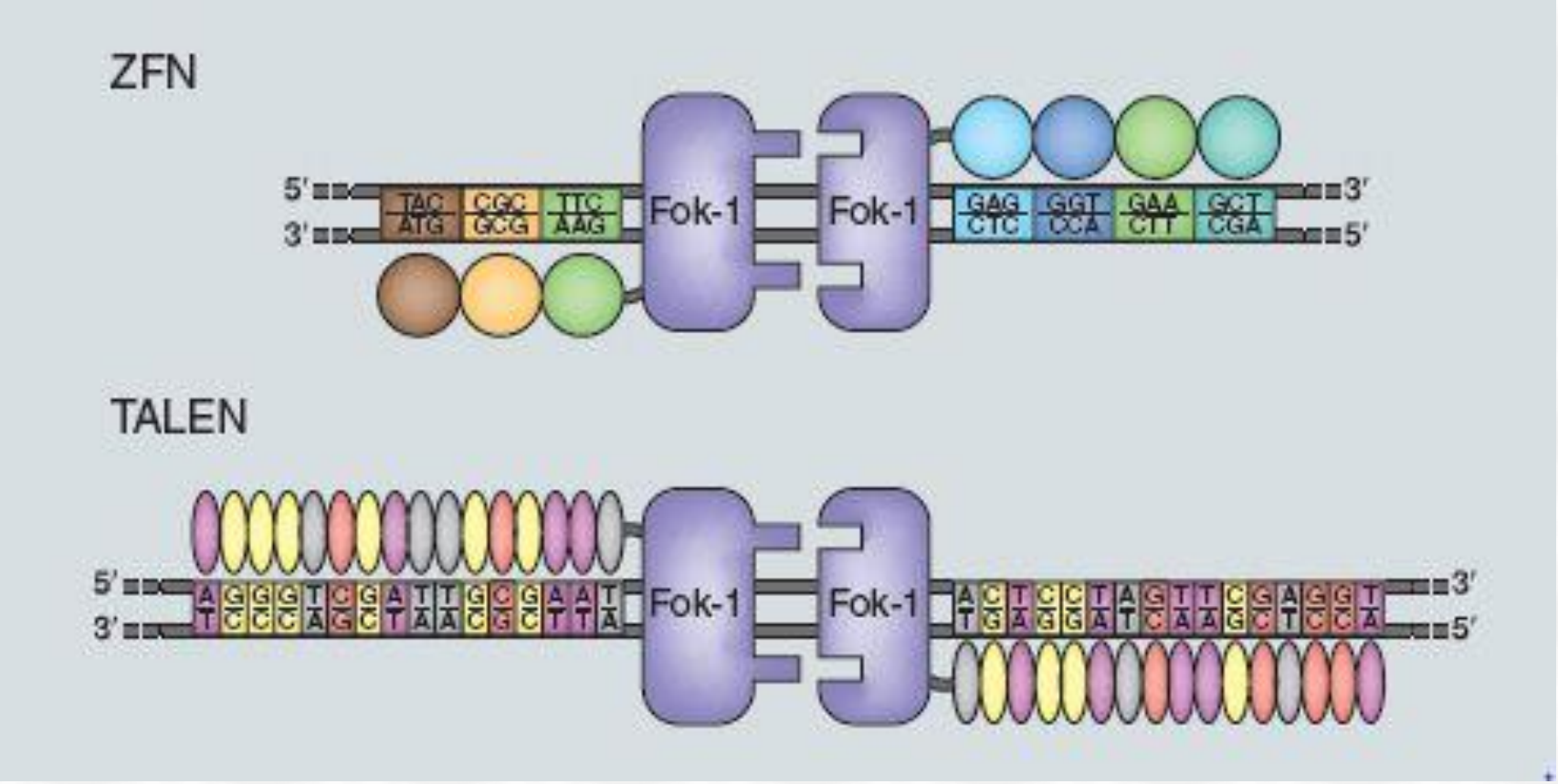
(http://www.rcsb.org/pdb/explore/jmol.do?structureId=1G9Y&bionumber=1)

Zinc-finger nucleases



(Nat Biotech 21, 759-760, 2003)

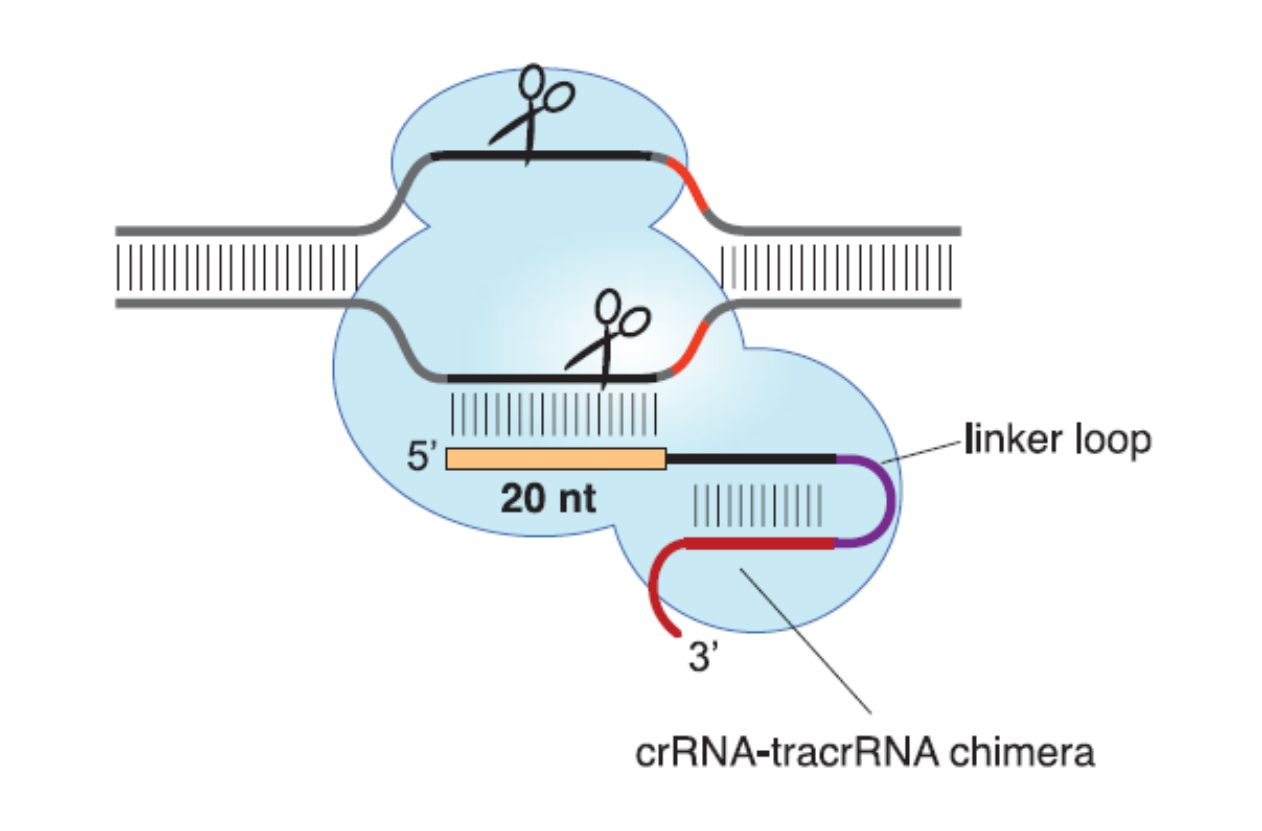
ZFNs and TALENs



ZFN: zinc-finger nuclease **TALEN: Transcription activator–like effector nuclease**

(Nat Meth 9, 27, 2012)

CRISPR/Cas



Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems

(Jinek et al., Science 337, 816-821, 2012)

Comparison of chimeric nucleases

	Meganucleases	ZFN	TALEN	CRISPR/Cas9
Flexible localisation	Complex	Limited	Average	Almost total
Nuclease construction	Laborious	Significant	Significant	Simple
In vitro testing	Laborious	Significant	Significant	Simple
Targeting efficiency	Not reported	Limiting factor	Average	Good
Off-target effects	Low	High	High	High
Multiplexing	No	No	No	Yes
Time investement	High	Moderate	Moderate	Low
Cost	High	Average	Average	Low

(https://www.genoway.com/services/crispr-cas9-models/nucleases.htm)

CRISPR has made genome editing democratic

Pole dancing vaults towards Olympics

News

Sean O'Neill

One has its origins in Ancient Greece when long spears were used for vaulting the walls of besieged cities, the other traces its roots to the strip club boom of the 1980s.

Yet within a decade it looks possible that pole dancing could join pole vaulting as an Olympic sport.

The Global Association of International Sports Federations (GAISF) confirmed yesterday that it has given observer status to the International Pole Sports Federation (IPSF) in a move which sets out a "clear pathway" towards full Olympic recognition.

Patrick Baumann, president of GAISF said it was an "exciting time" for pole sports and added: "We will do everything within our remit to help them realise their full potential and... one day, maybe become part of the Olympic programme".

With skateboarding making its debut at Tokyo 2020, Katie Coates, president of the IPSF, is cautiously optimistic that pole sports could make the grade by 2024. She held her first meeting with the International Olympic Committee, in February and described it as encouraging.

"I'm not saying yes we will be there, but I'm not saying no either — there is a good opportunity for us and the sporting bodies are interested in young, trendy sports being recognised because they get people involved," said Ms Coates.

"We're proving everybody wrong. We've been told again and again by the traditional sports that it will be very difficult for us to be recognised as a sport, difficult that just spurs me on to achieve

Pole dancing has come a long way since it appeared at 1980s strip clubs and has an accepted scoring system. Katie Coates, right, has helped take it towards Olympic recognition, which it may gain by 2024

Given a sporting chance

The Global Association of International Sports Federations (GAISF) gave observer status to six other nascent sporting bodies this month, opening the door to possible Olympic glory.

The World Armwrestling Federation The playground and bar table staple has been dressed up as a sport that the GAISF says tests "power, strength, endurance, technique, strategy, experience and passion".

World Dodgeball Association As in the film — two teams of players throw balls at each other and try to avoid being hit themselves.

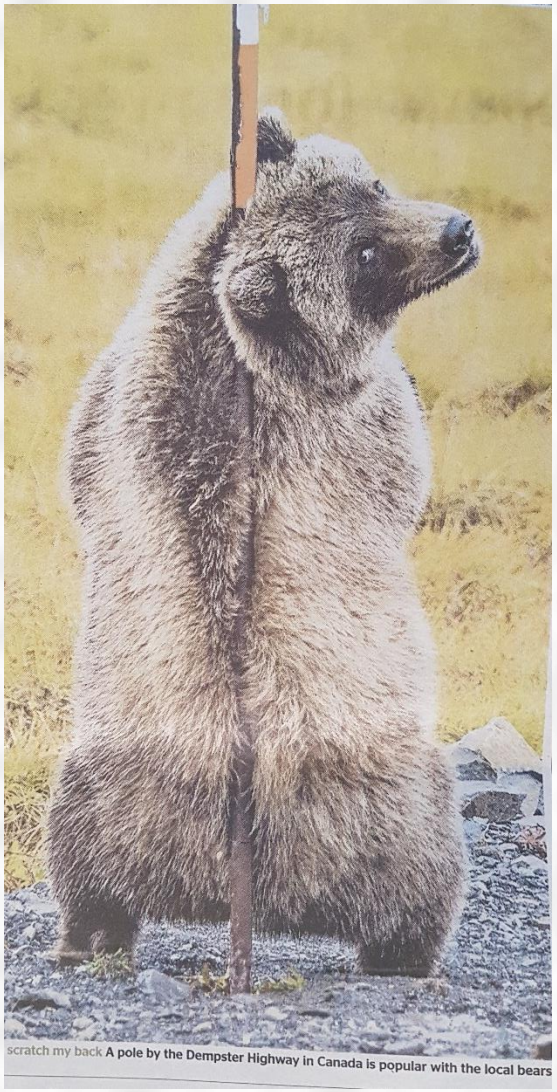
Federation for International FootGolf Players kick a football into a hole in the manner of golf, just like golf but with bigger balls, no clubs and no requirement for terrible clothes.

International Union of Kettlebell Lifting A body dedicated to trying to turn a tedious gym activity into a sport.

International Federation of Match Poker A variation on the classic card game, but without gambling, thereby removing most of the fun.

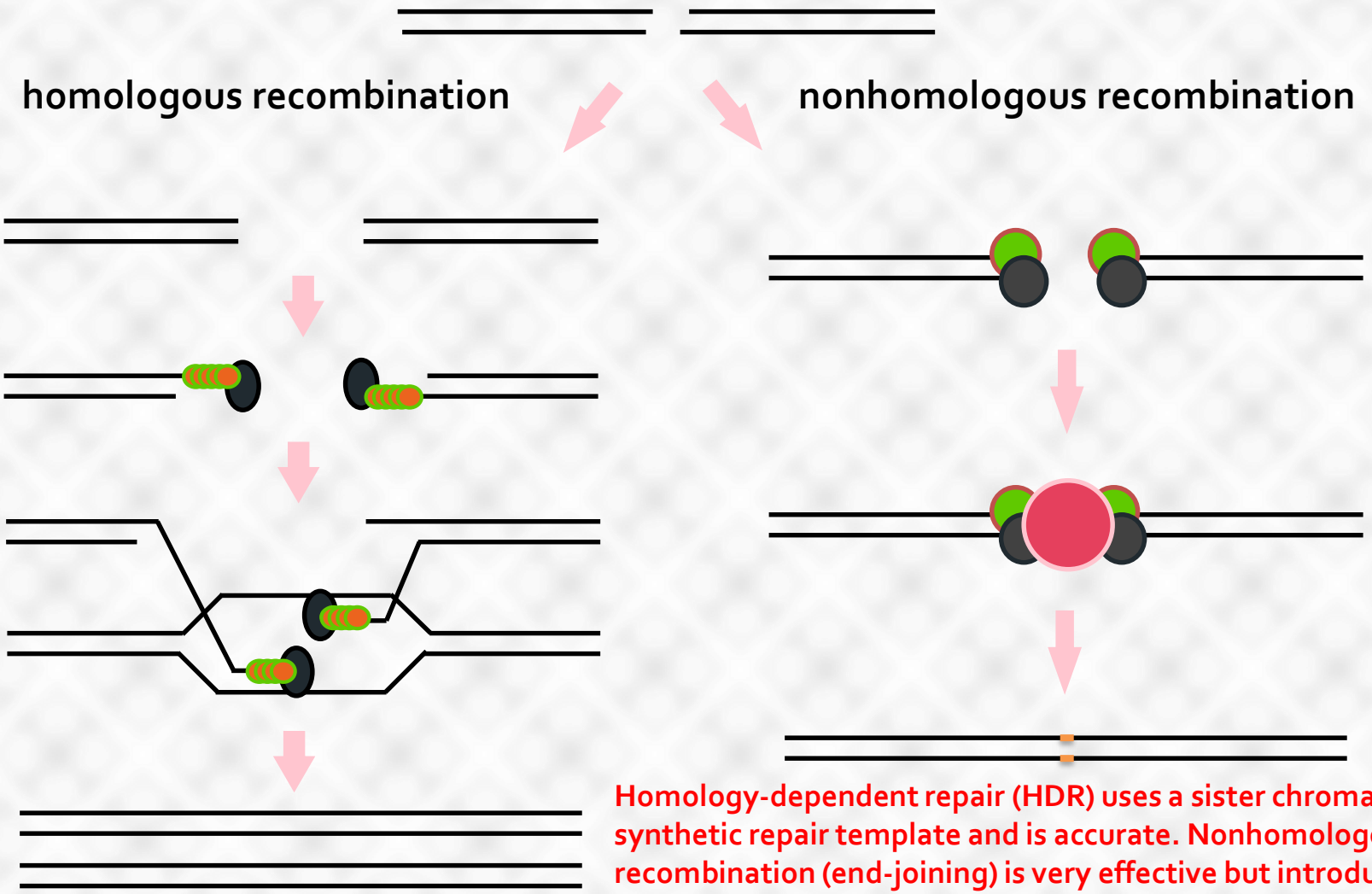
International Table Soccer Federation Yes, the age-old manual arcade game of table football; the GAISF says it helps to "build social cohesion" and is "an extraordinary vector of exchange". Really.

Before CRISPR it was possible but technically very demanding; now anyone can use it



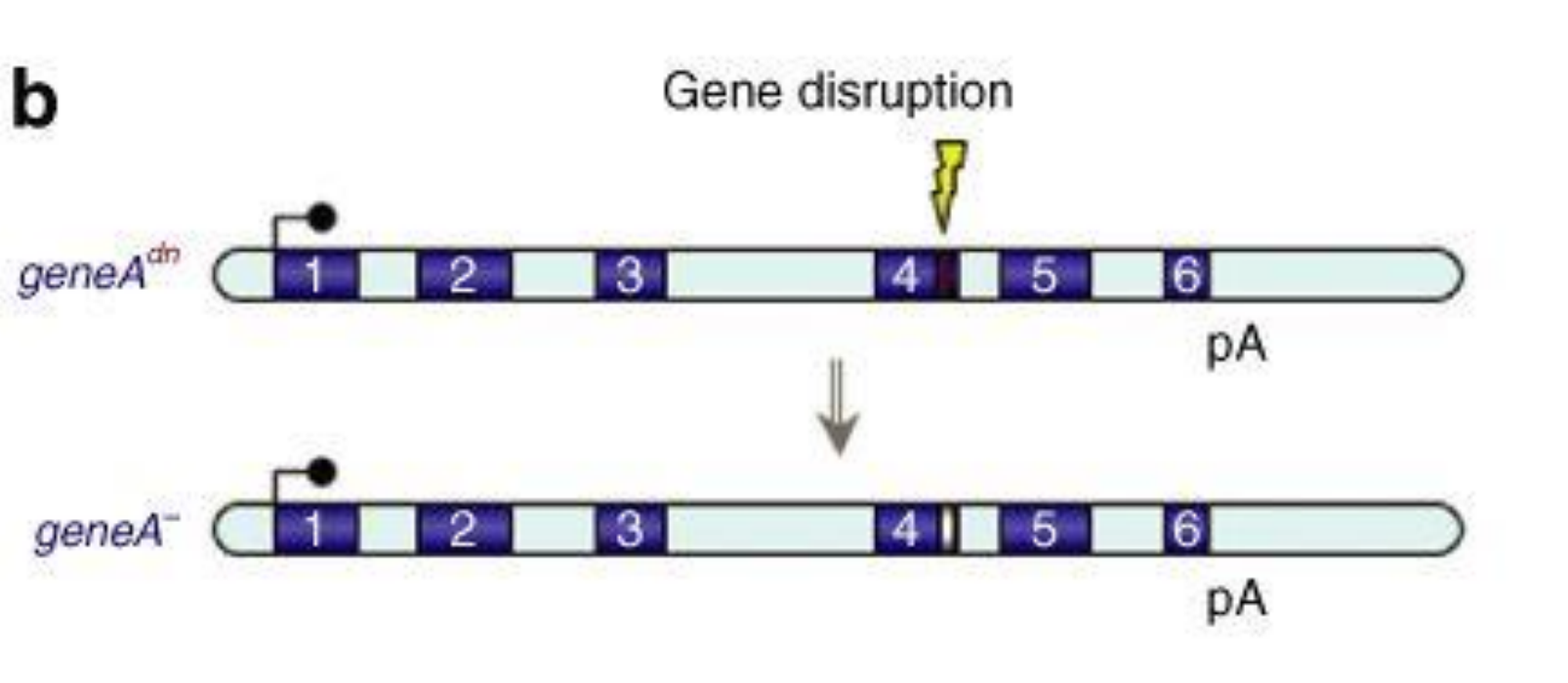
scratch my back A pole by the Dempster Highway in Canada is popular with the local bears

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)

Genome editing methods: gene disruption for HIV control

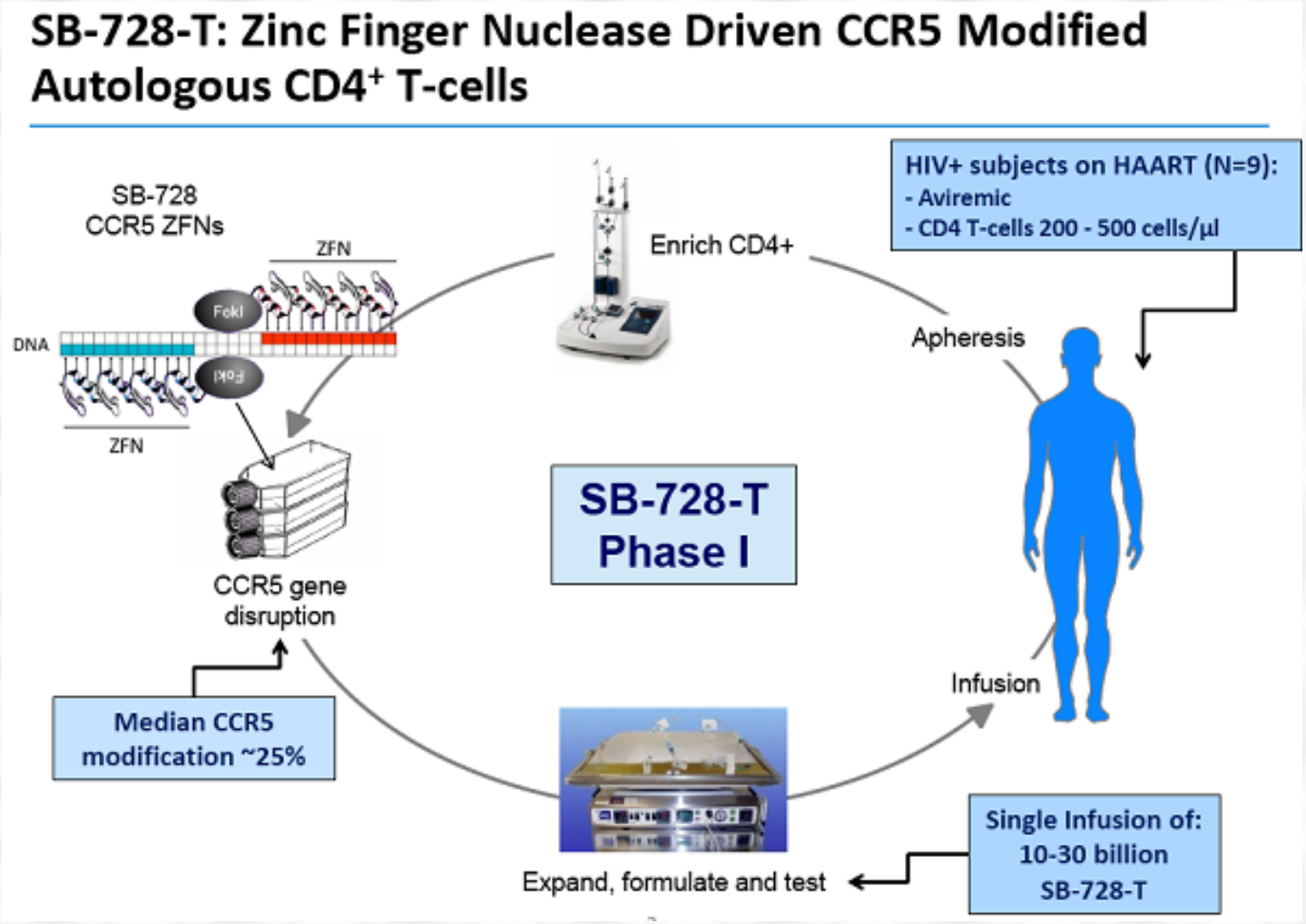
Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to *CCR5* control HIV-1 *in vivo*

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

CCR5 is the major HIV-1 co-receptor, and individuals homozygous for a 32-bp deletion in *CCR5* are resistant to infection by CCR5-tropic HIV-1. Using engineered zinc-finger nucleases (ZFNs), we disrupted *CCR5* in human CD34$^+$ 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/IL2rγ^{null} mice and gave rise to polyclonal multi-lineage progeny in which *CCR5* was permanently disrupted. Control mice receiving untreated HSPCs and challenged with CCR5-tropic HIV-1 showed profound CD4$^+$ T-cell loss. In contrast, mice transplanted with ZFN-modified HSPCs underwent rapid selection for *CCR5*$^{-/-}$ cells, had significantly lower HIV-1 levels and preserved human cells throughout their tissues. The demonstration that a minority of *CCR5*$^{-/-}$ HSPCs can populate an infected animal with HIV-1-resistant, *CCR5*$^{-/-}$ progeny supports the use of ZFN-modified autologous hematopoietic stem cells as a clinical approach to treating HIV-1.

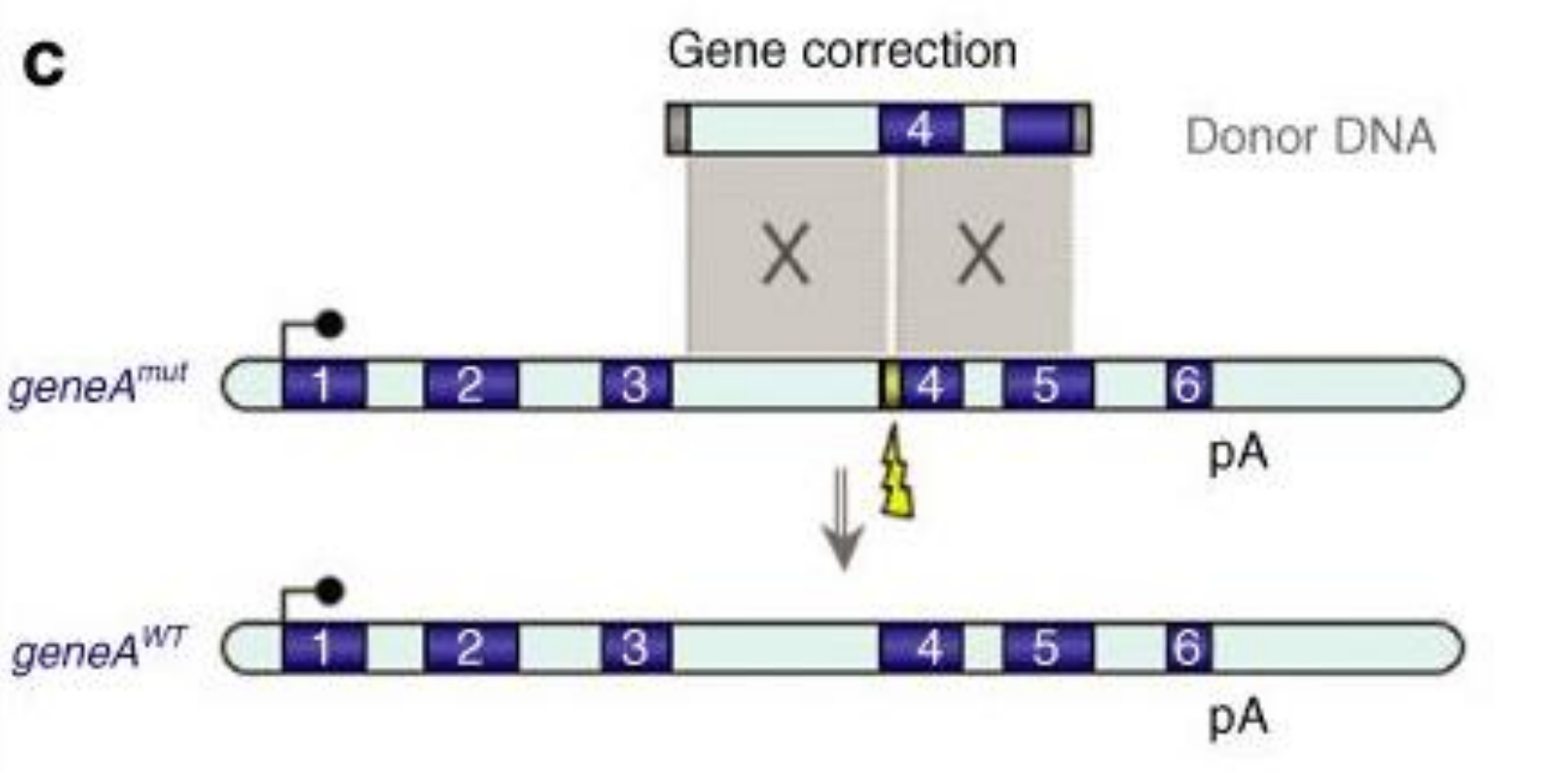
(Nat Biotech 2010, doi:10.1038/nbt.1663)

Sangamo's AIDS clinical trial



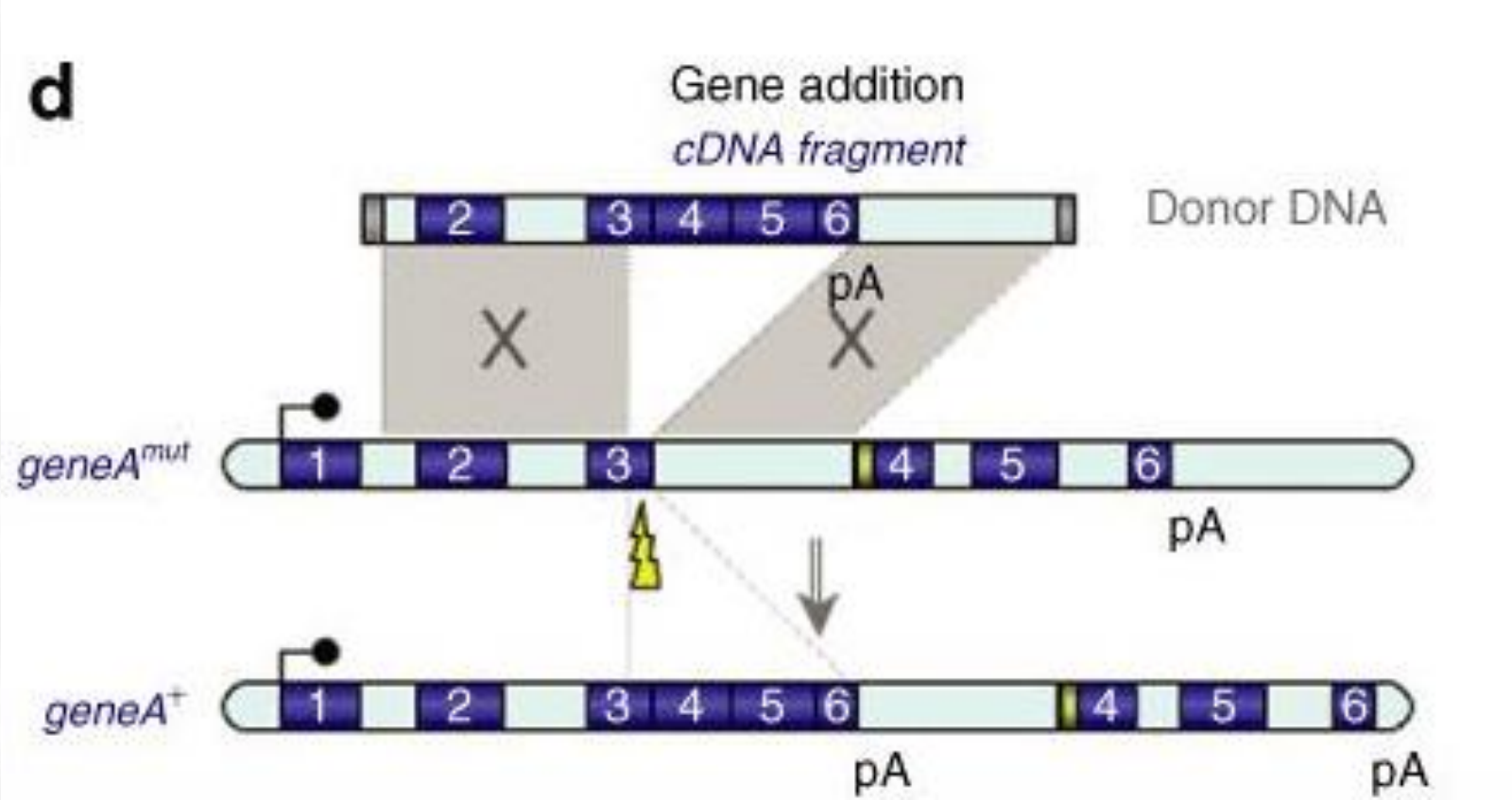
Overall, successful engineering of *CCR5* knockout in either T-cells or haematopoietic stem cells in clinical trials

Genome editing methods: gene correction



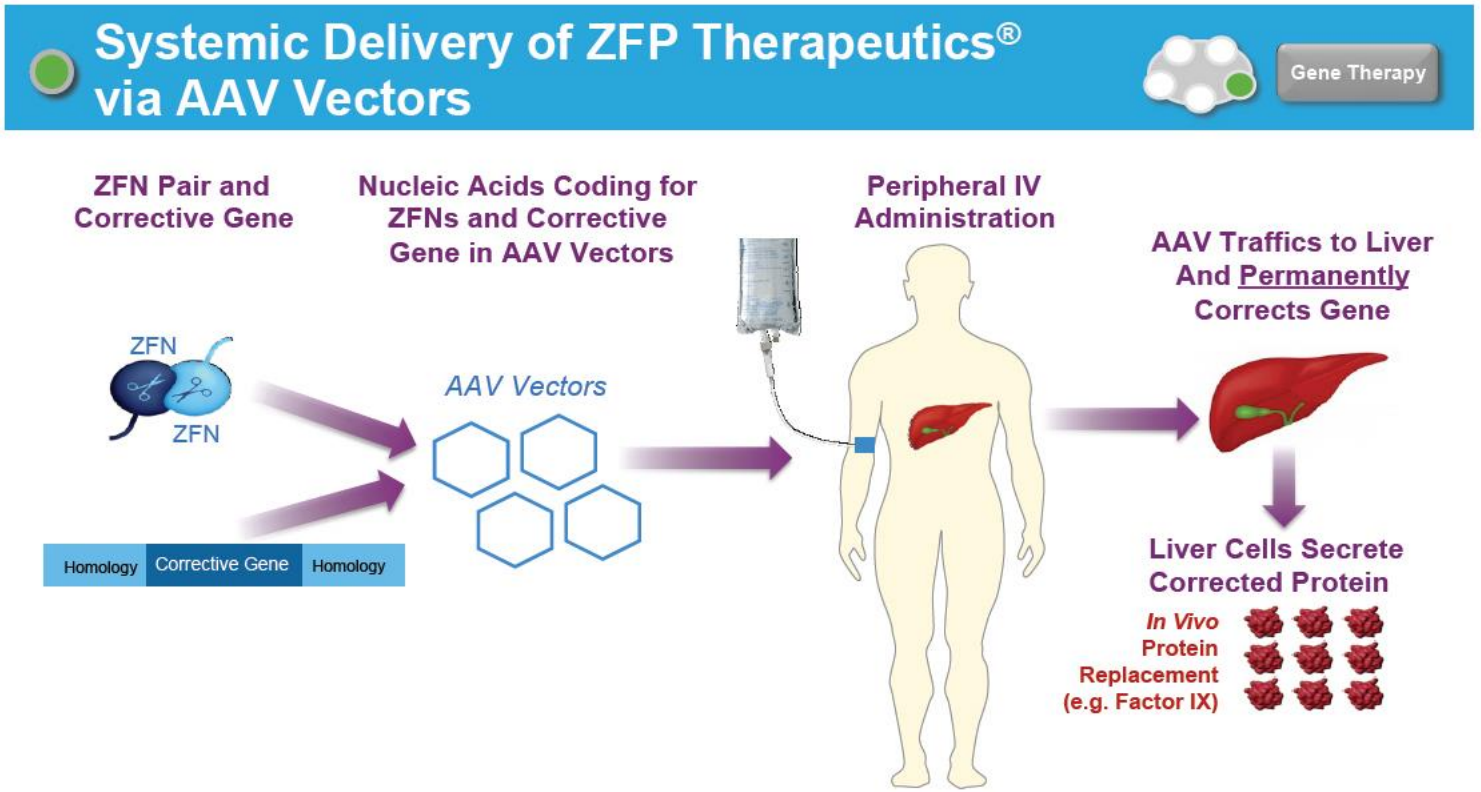
(Cathomen and Joung, Mol Ther. 16, 1200-7, 2008)

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)

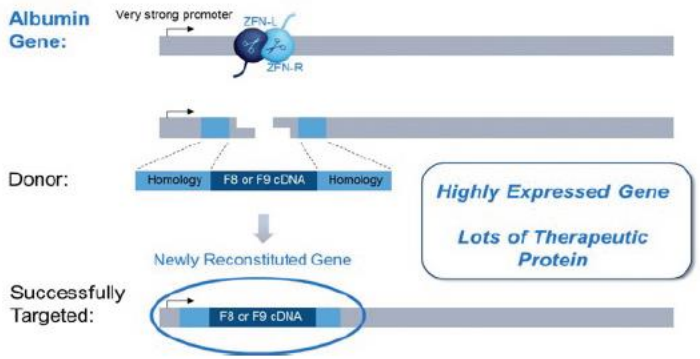
Gene Therapy in Albumin Safe Harbor Locus: Factor VIII & Factor IX

Gene Therapy

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



Shire

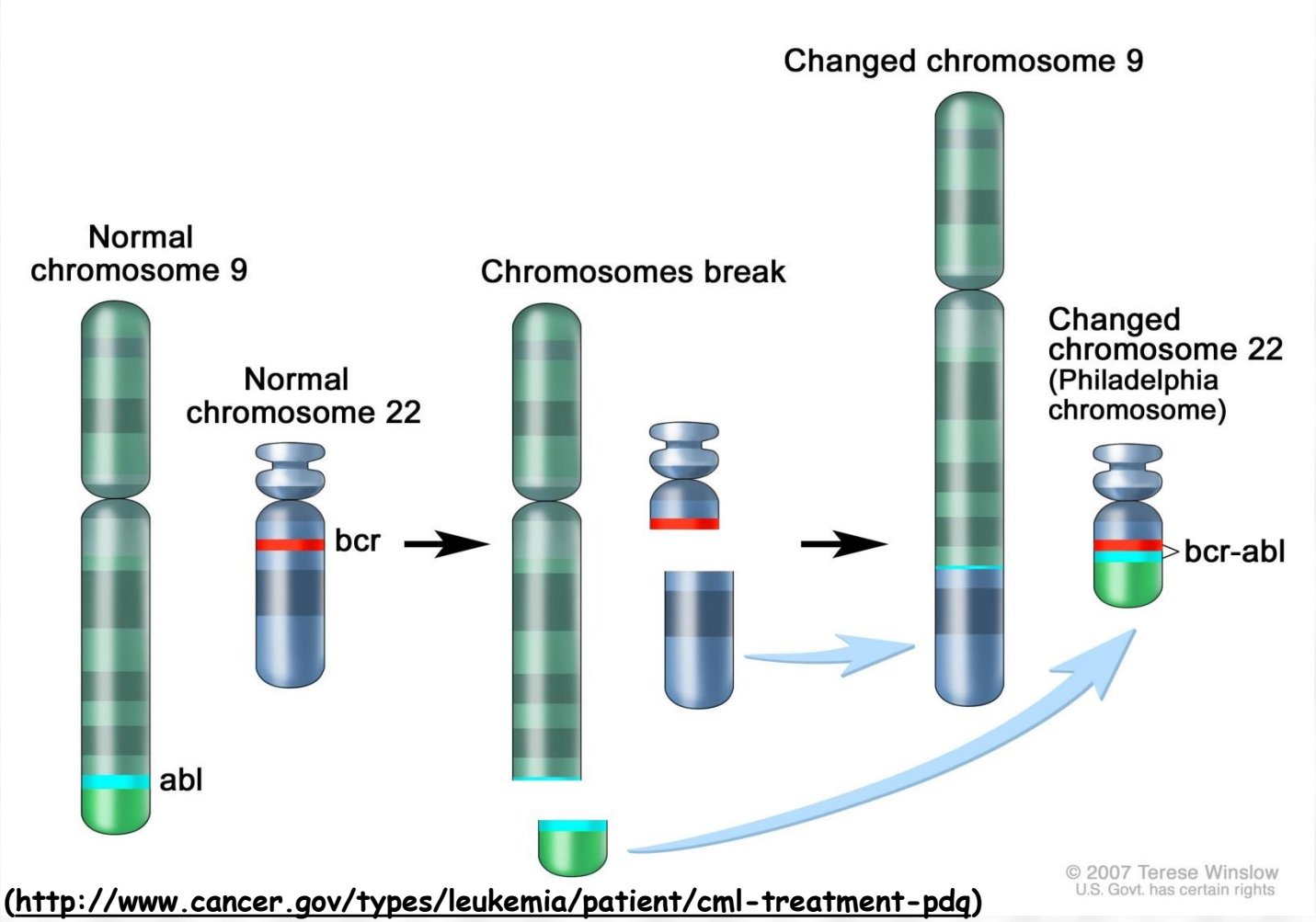
46

http://www.sec.gov/Archives/edgar/data/936402/000095010314008732/dp51789_ex9901.htm

Major issues in genome editing

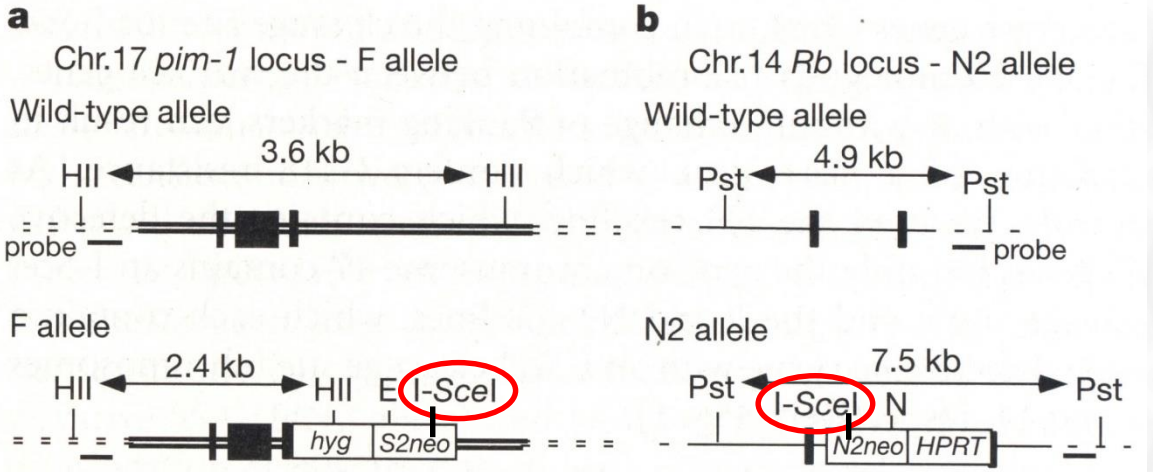
- Delivery (effectiveness is cell- and/or tissue-dependent)
- Efficiency (cell-type dependent)
- Fidelity (on-target; to be improved, not all changes are intended one)
- Specificity (off-target; to be improved, non-target sites can be cut)
- Translocation risk (multiplexing requires sequential editing)

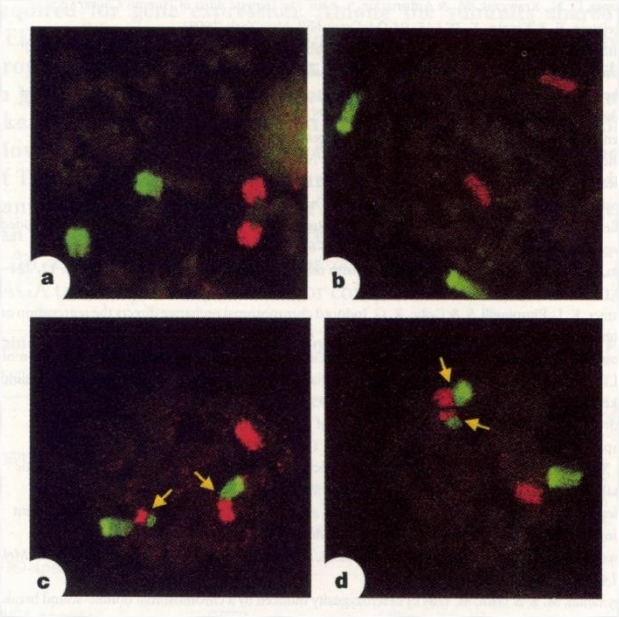
Chronic myeloid leukaemia



(http://www.cancer.gov/types/leukemia/patient/cml-treatment-pdq)

Simultaneous double-strand breaks cause translocations

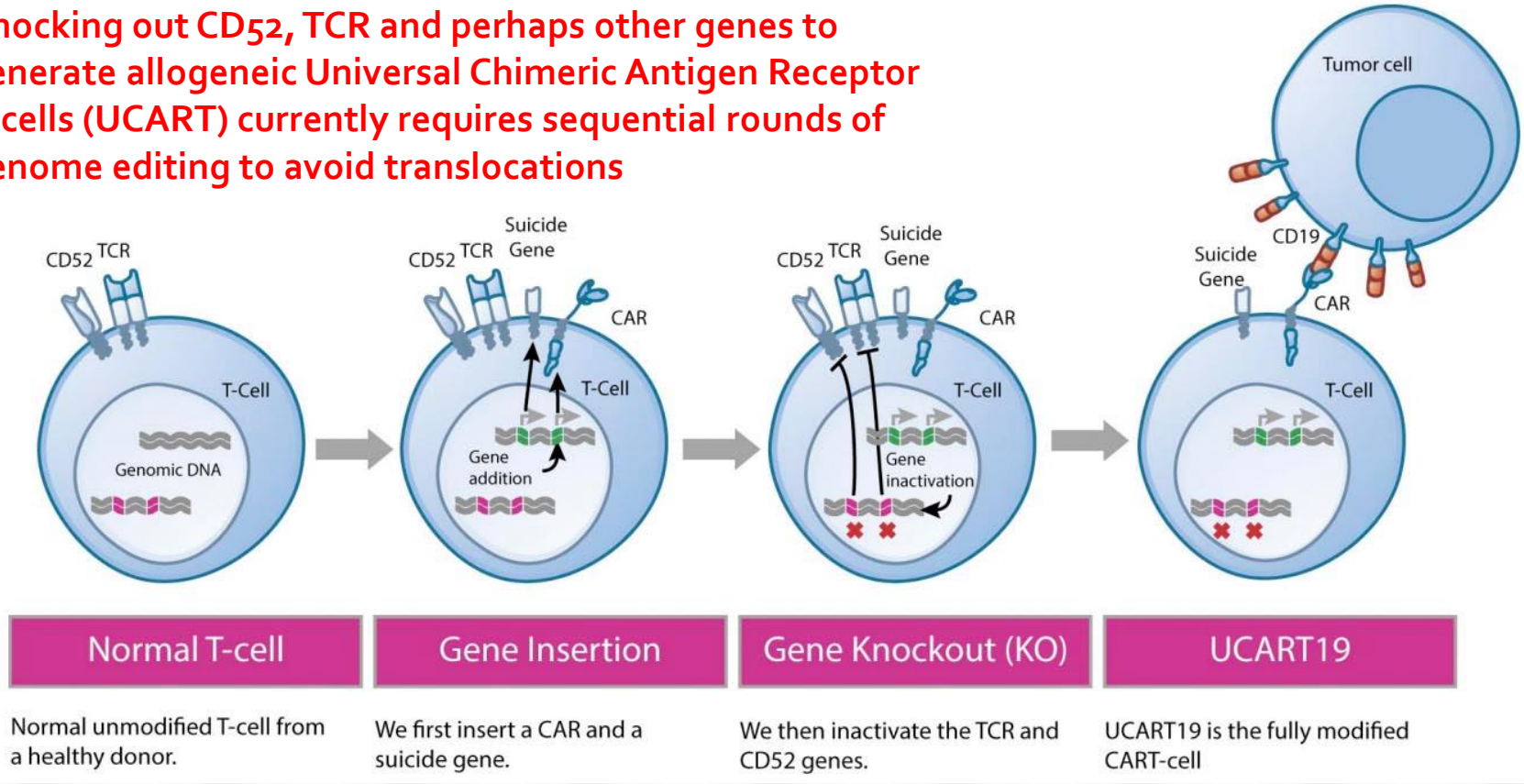




Richardson and Jasin (2000) *Nature* 405, 697-700

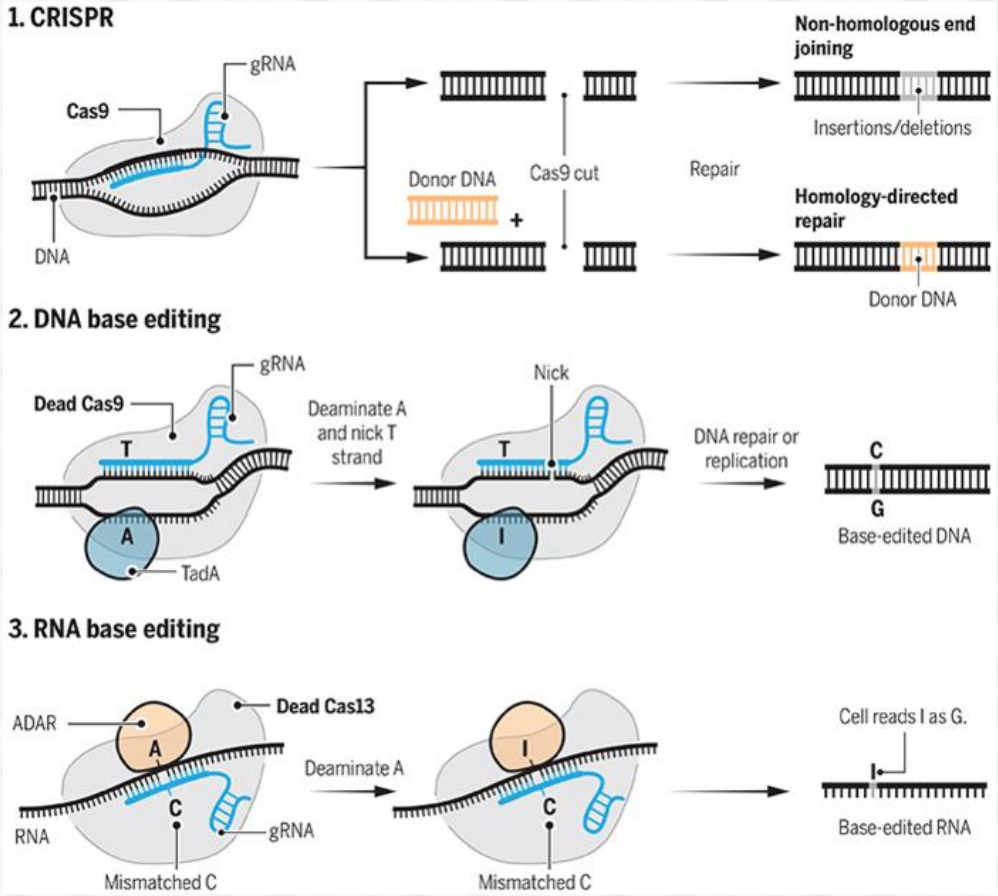
Cellectis off-the-shelf double-knockout UCARTs

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



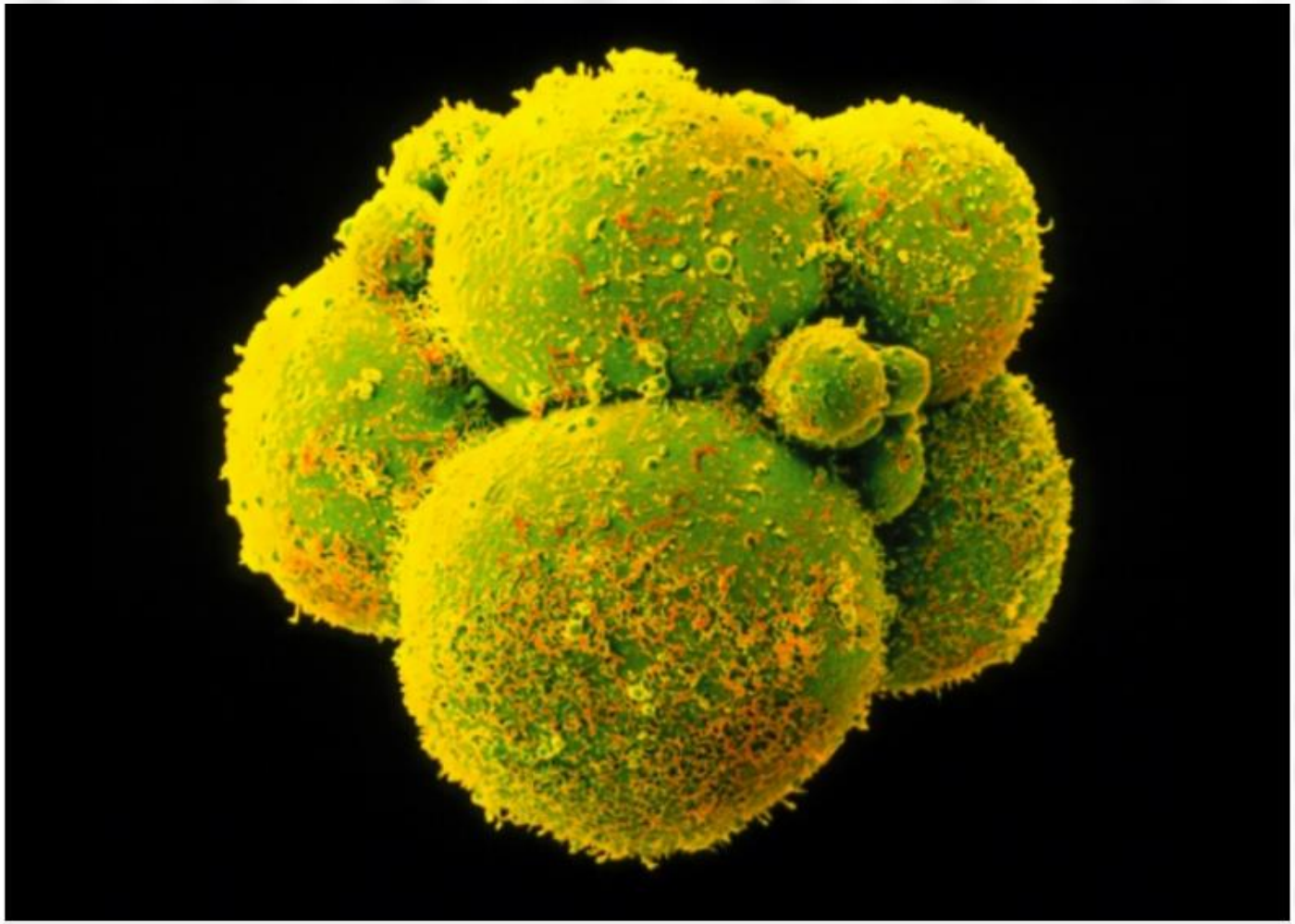
https://labiotech.eu/medical/ucart19-universal-car-t-given-to-another-baby-gosh-cellectis-leukemia/

Base Editing: no DSB required



http://www.sciencemag.org/news/2017/10/novel-crispr-derived-base-editors-surgically-alter-dna-or-rna-offering-new-ways-fix

Human embryos…the ultimate frontier

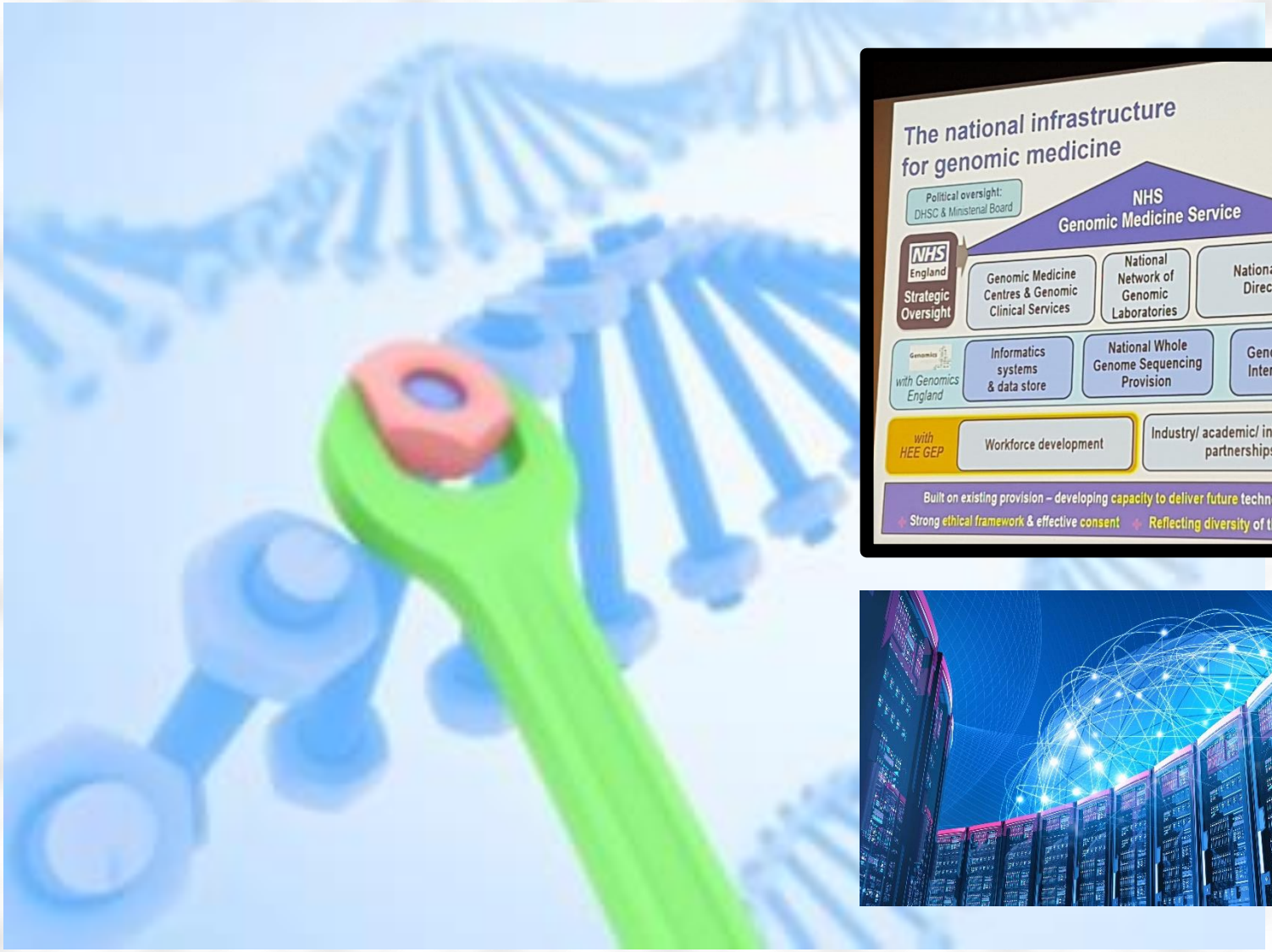


Dr. Yorgos Nikas/SPL

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

(Nature,22 Apr 2015)

Join the genomic medicine revolution!



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