wellcome centre human genetics

An update on the worldwide controversy on germline gene editing

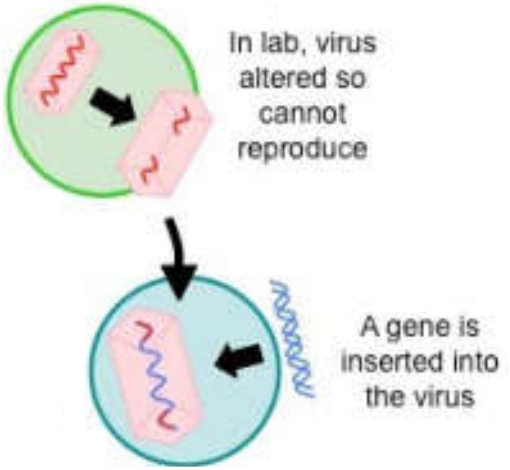
Dr. Ben Davies

Gene Therapy - replacing a broken gene

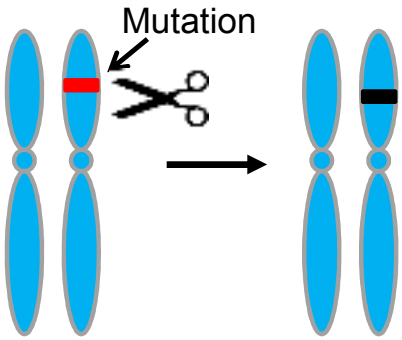
Sickle cell anaemia

- Mutation in a gene encoding one of the proteins that make up haemoglobin

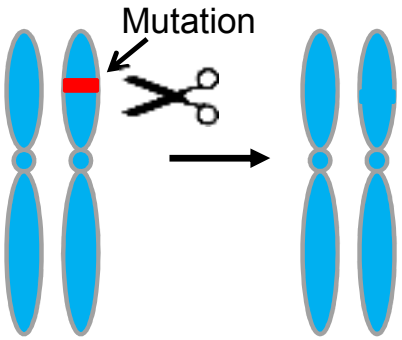
- Currently gene therapy is focussed on delivering extra copies of healthy genes to patients with mutations



Could repairing the actual diseased genes be a better strategy?



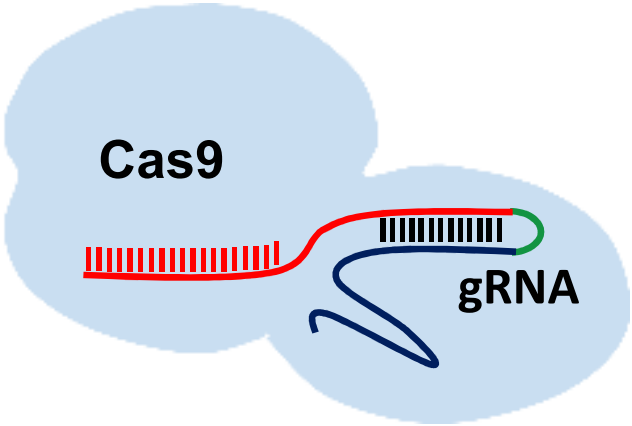
Mutant gene is inactivated

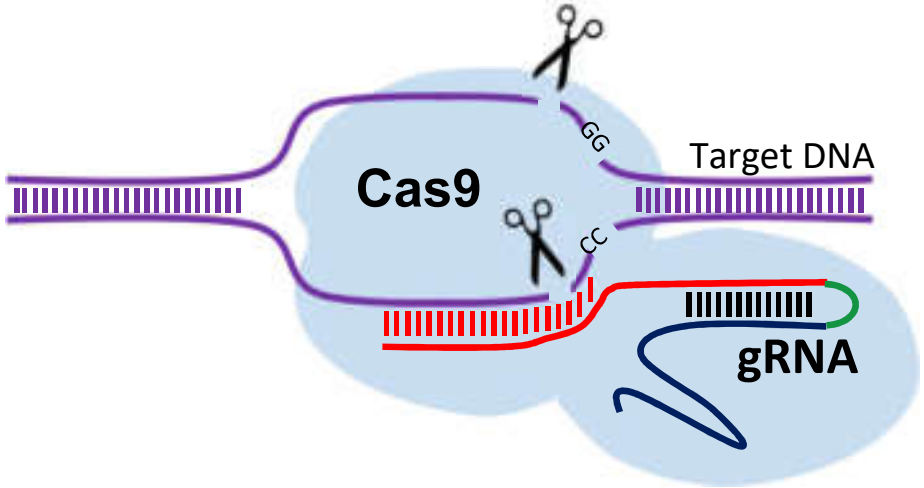


Mutant gene is repaired

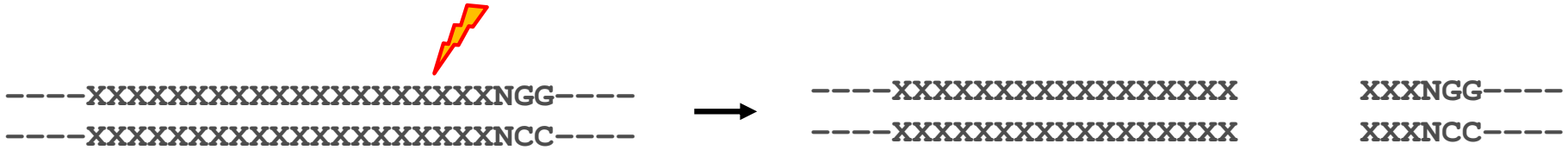
- So we need some molecular scissors
- This was, until recently, science fiction!

The Present – CRISPR/Cas9
(Clustered Regularly Interspaced Short Palindromic Repeats)

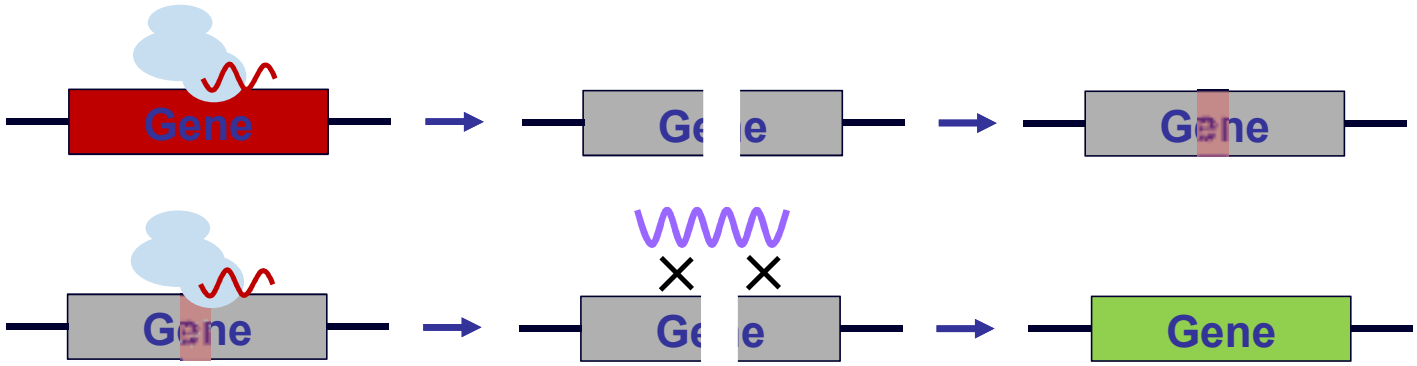




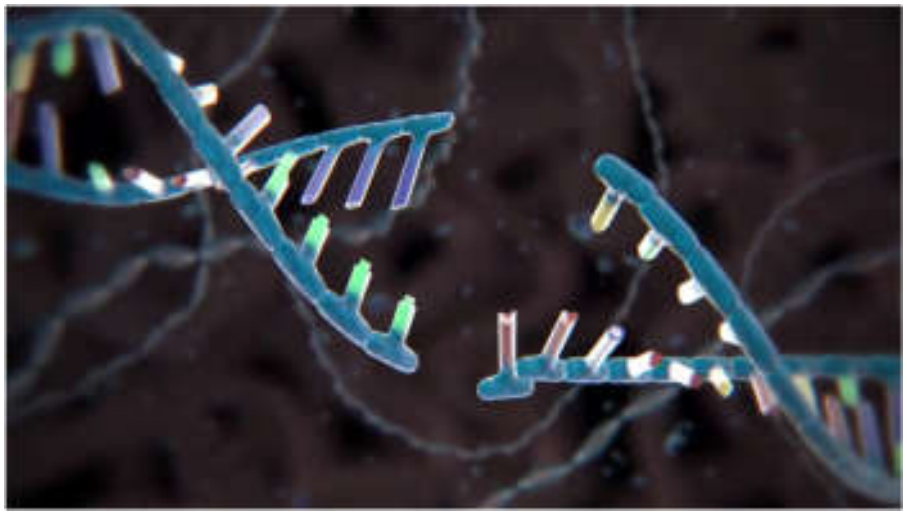
- Cas9 is the nuclease which cleaves its double stranded target site
- A guide-RNA (crRNA:tracrRNA) defines where the nuclease cleaves
- The target site can be any 20 nt sequence followed by -NGG

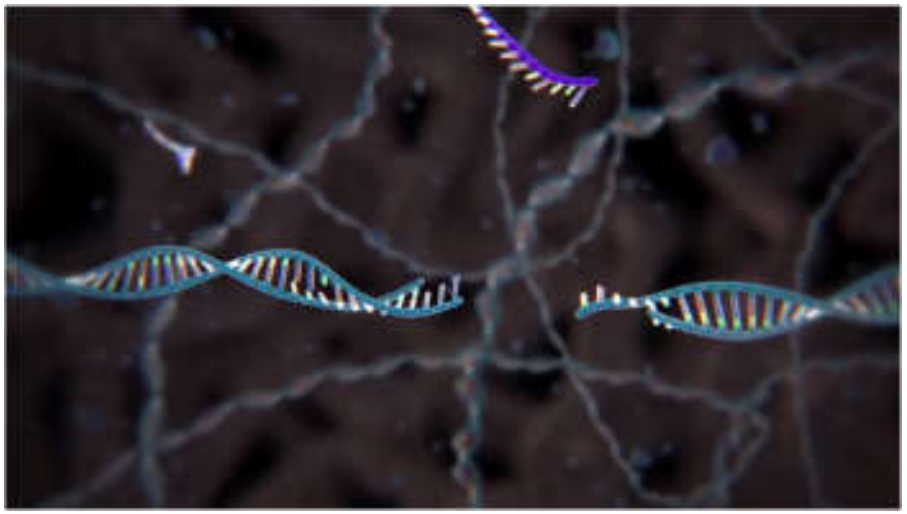


How cutting DNA can be used therapeutically



Mutant gene repair



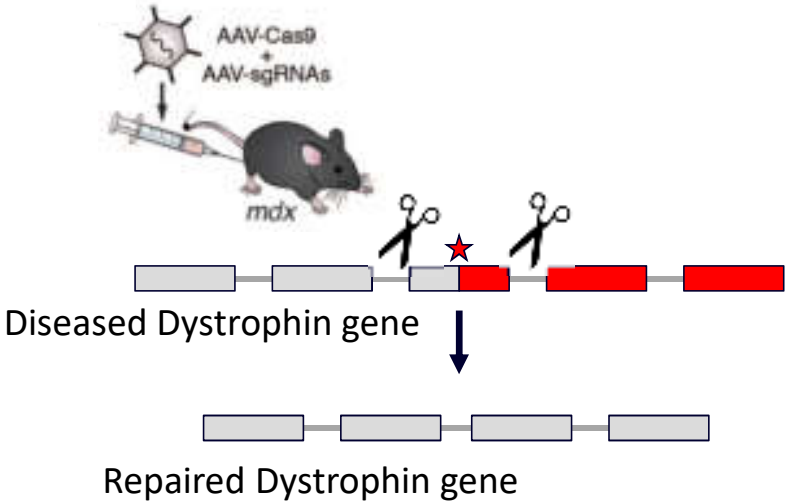


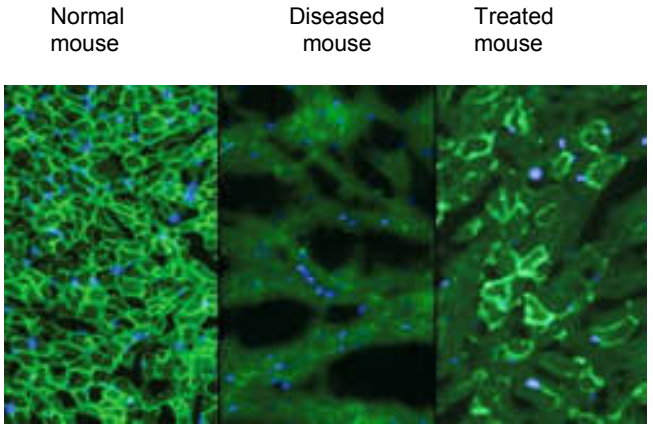
CRISPR is providing a therapeutic approach for muscular dystrophy in preclinical models

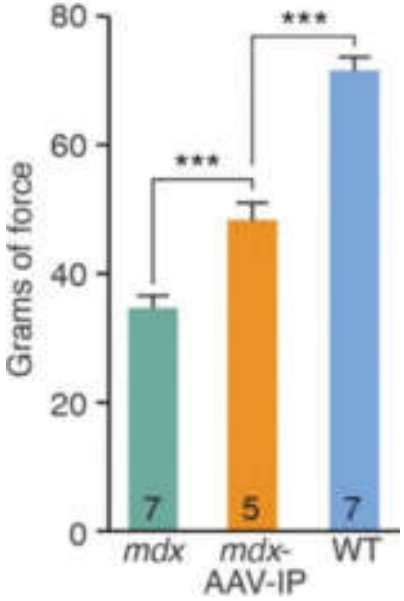
Diseased Dystrophin gene

Long et al., Science 2016
Nelson et al., Science 2016

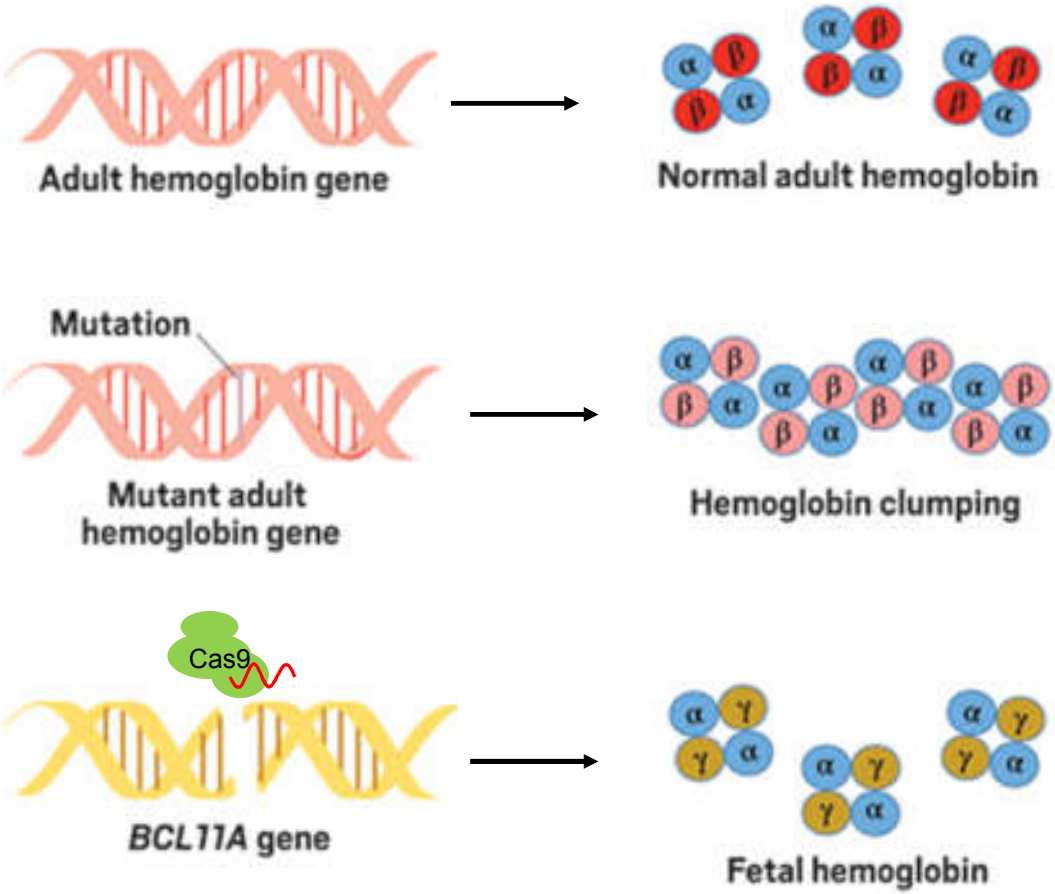
- Gene editing techniques using CRISPR-Cas9 have recently been used to correct this mutation in a mouse model

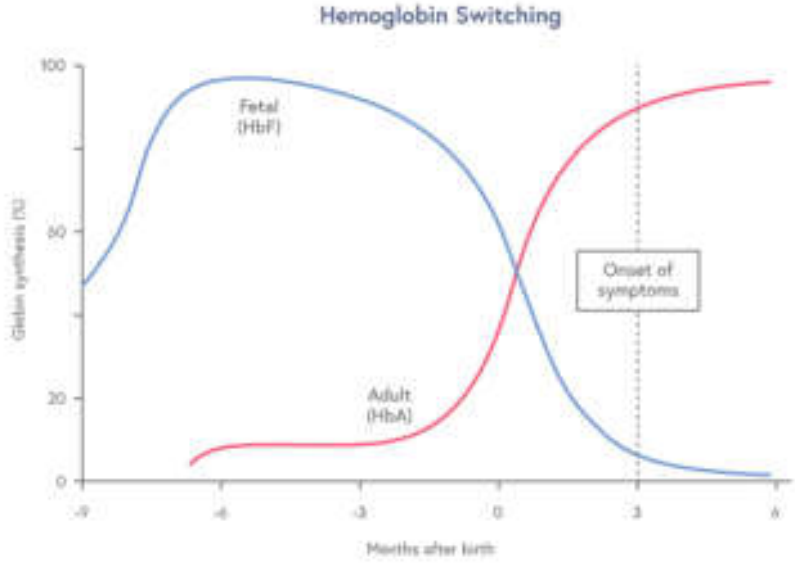






CRISPR is providing a therapeutic approach for haemoglobinopathies in human patients – Phase I/II



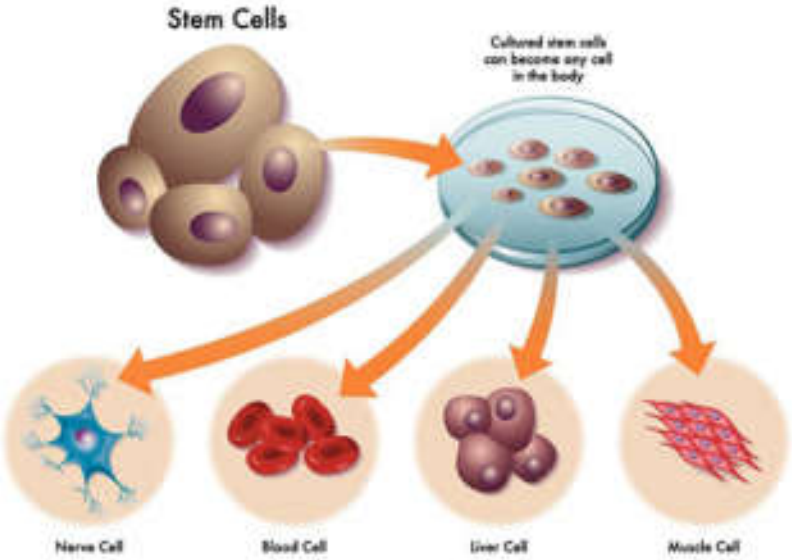


CRISPR gene editing in humans appears safe, and potentially effective

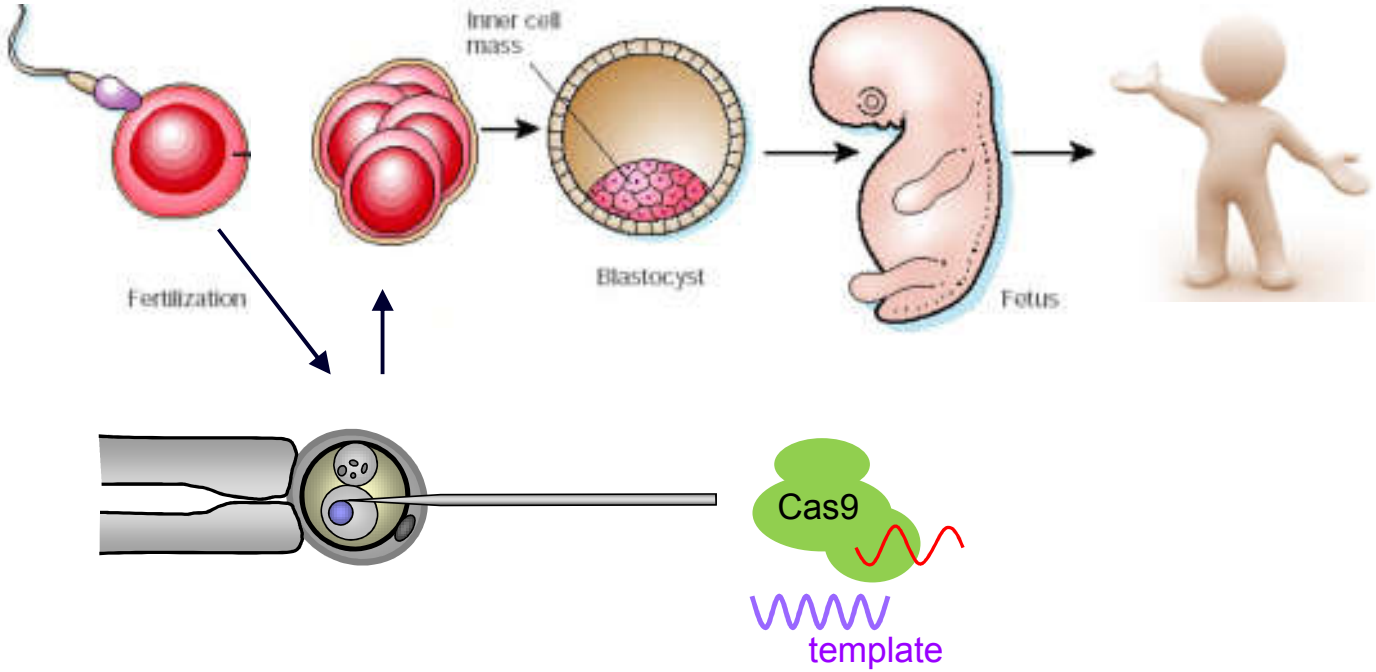
Crispr Therapeutics and Vertex Pharmaceuticals release first data from sickle cell disease and β-thalassemia clinical trial

Which cells to treat?

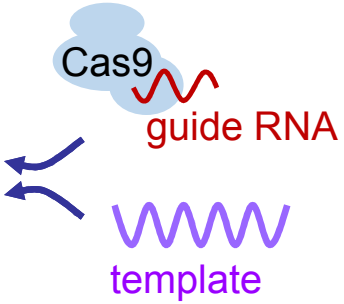
- A frequent problem with gene therapy is that the therapeutic gene delivery has to keep being applied to the diseased organ
- If, however, we target stem cells which can repopulate a particular tissue or organ, this could be a permanent fix.



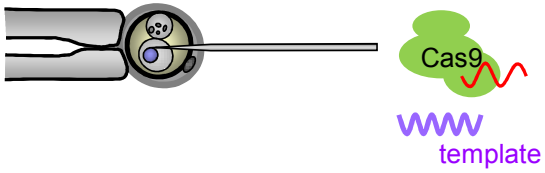
Can we / should we fix genes in the embryo?

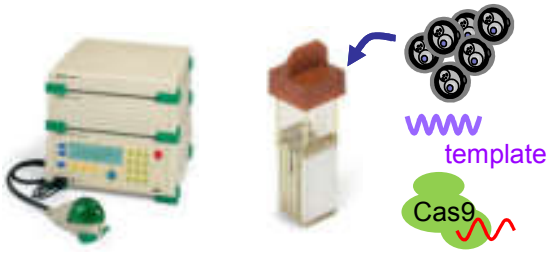


CRISPR/Cas9 Mutagenesis within the zygote

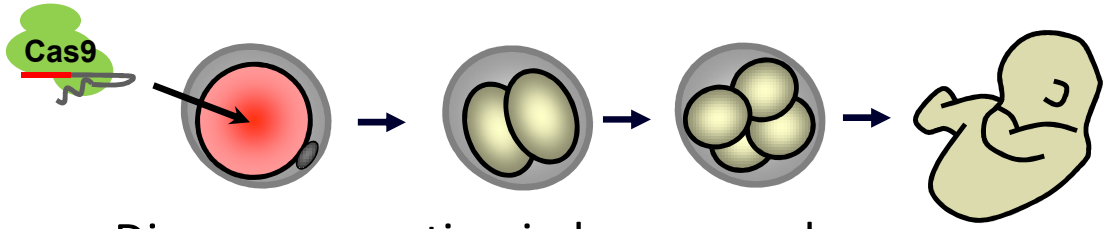


Alternative methods for delivery to the zygote





What works in mouse, works in human



- Disease correction in human embryos

ARTICLE

Correction of a pathogenic gene mutation in human embryos

ARTICLE

Genome editing reveals a role for OCT4 in human embryogenesis

Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

ORIGINAL ARTICLE

CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein

Lichun Tang · Yanting Zeng · Hongzi Du · Mengmeng Gong · Jin Peng · Buxi Zhang · Ming Lei · Fang Zhao · Weihua Wang · Xiaowei Li · Jianqiao Liu

Tild-CRISPR Allows for Efficient and Precise Gene Knockin in Mouse and Human Cells

Problems – off-target mutation

- The specificity of CRISPR is defined by only 20 nt
- (near) identical sequences may be present elsewhere
- …..and Cas9 nuclease tolerates certain mismatches
- How real is this problem when applying CRISPR/Cas9 nucleases in the single cell embryo

PLOS | GENETICS

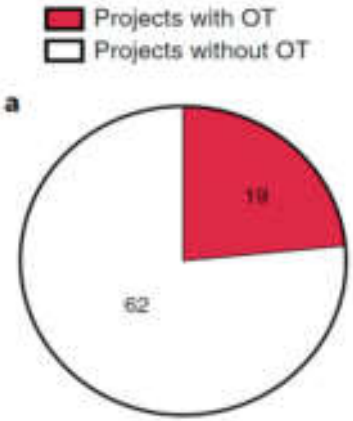
No unexpected CRISPR-Cas9 off-target activity revealed by trio sequencing of gene-edited mice

Vivek Iyer, Katharina Boroviak, Mark Thomas, Brendan Doe, Laura Riva, Edward Ryder, David J. Adams

nature methods | BRIEF COMMUNICATION

CRISPR off-target analysis in genetically engineered rats and mice

Keith R. Anderson, Maximilian Haeussler, Colin Watanabe, Vasantharajan Janakiraman, Jessica Lund, Zora Modrusan, Jeremy Stinson, Qixin Bei, Andrew Buechler, Charles Yu, Sobha R. Thamminana, Lucinda Tam, Michael-Anne Sowick, Tulja Alcantar, Natasha O'Neil, Jinjie Li, Linda Ta, Lisa Lima, Merone Roose-Girma, Xin Rairdan, Steffen Durinck and Søren Warming



Off-targets – solutions?

ARTICLE

High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects

SpCas9-HF

Rationally engineered Cas9 nucleases with improved specificity

Ian M. Slaymaker, Linyi Gao, Bernd Zetsche, David A. Scott, Winston X. Yan, Feng Zhang

eSpCas9

Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing

Benjamin P. Kleinstiver, Alexander A. Sousa, Russell T. Walton, Y. Esther Tak, Jonathan Y. Hsu, Kendell Clement, Moira M. Welch, Joy E. Horng, Jose Malagon-Lopez, Irene Scarfò, Marcela V. Maus, Luca Pinello, Martin J. Aryee and J. Keith Joung

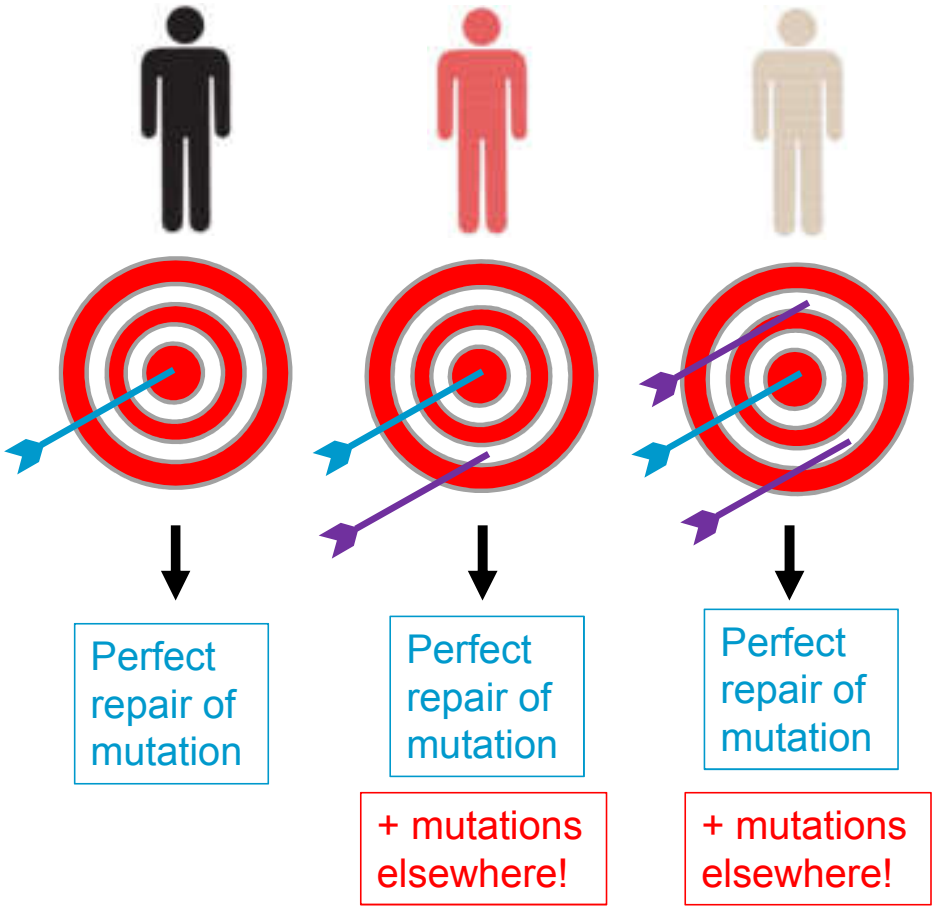
- Better algorithms predicting accuracy
- Engineered Cas9s with high accuracy
- Other CRISPR/Cas family members show higher levels of accuracy
- Accuracy is also vital for discriminating a mutant copy of a gene from a healthy copy

Real off-target prediction demands a personalized genome

Mutations found	Average per individual
Total variants	3,776,362
SNVs	3,579,423
Indels	196,940
Coding synonymous	11,742
Coding non-synonymous	11,468
Coding STOP/Splicing	478

Taliun et al bioRxiv2019

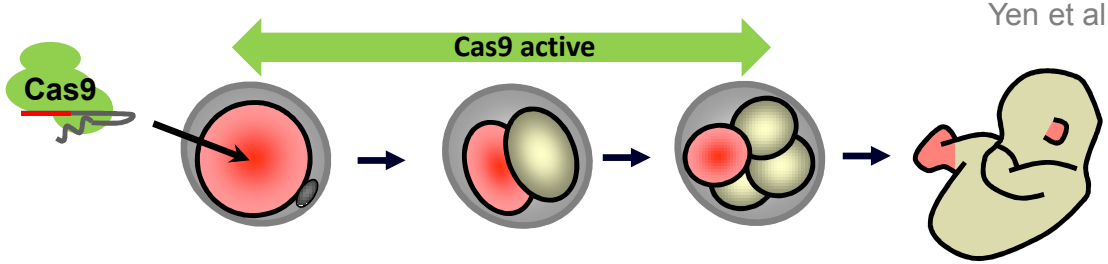
- Each of us differs on average at 1 nucleotide every 1000
- How can we predict off-target mutagenesis when we don't have the genome sequence of our patients



Problems – mosaicism

- Founder mice generated with CRISPR are almost invariably mosaic
- Not all cells are mutated/corrected!

Yen et al., 2014



Problems – large deletions and rearrangements

nature biotechnology

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley

nature COMMUNICATIONS

ARTICLE

CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome

nature COMMUNICATIONS

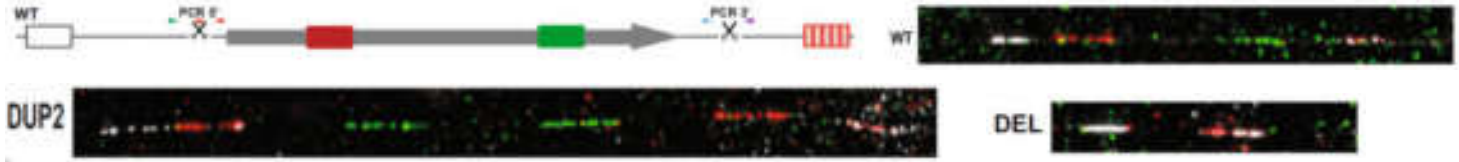
ARTICLE

CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations

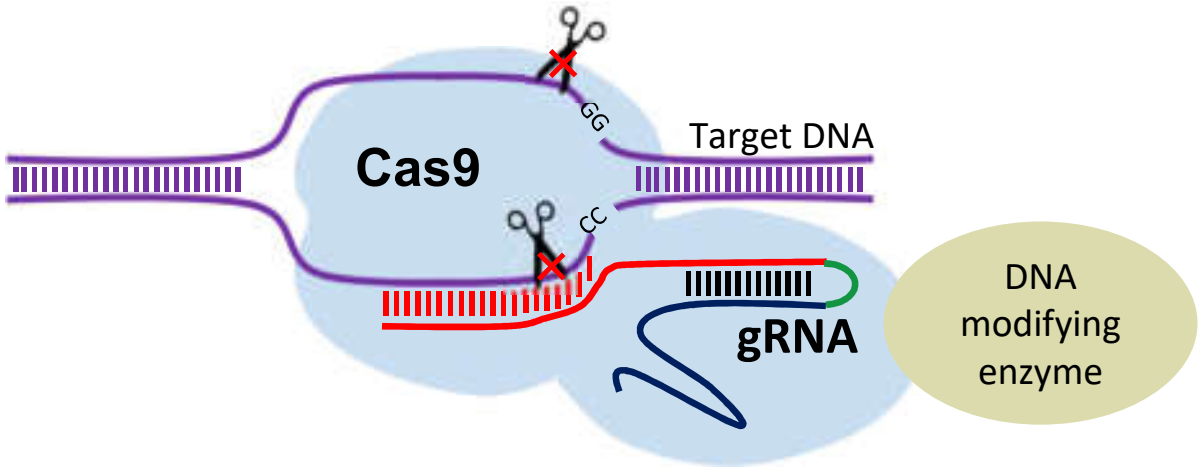
SCIENTIFIC REPORTS

OPEN

Revealing hidden complexities of genomic rearrangements generated with Cas9

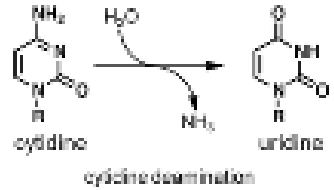


Solutions – avoid cutting the genome in the first place

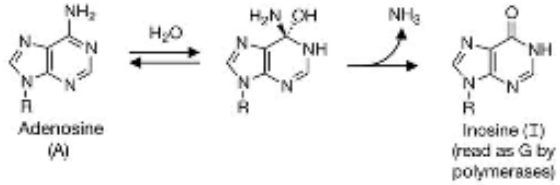


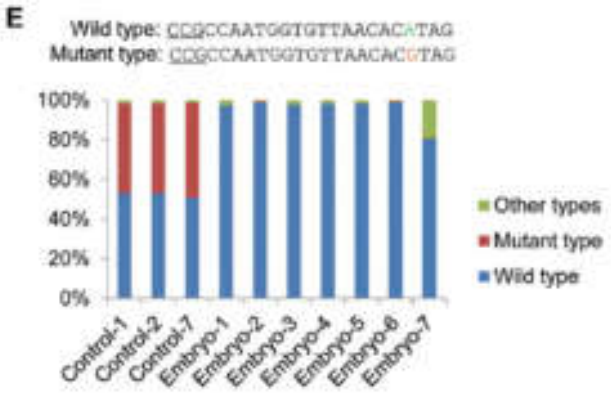
DNA modifying enzyme

- C to T

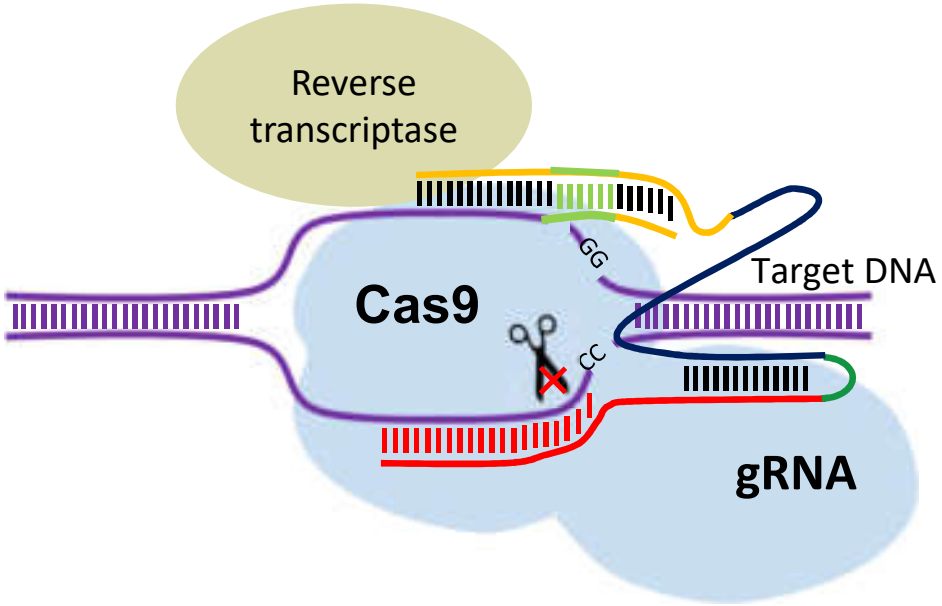


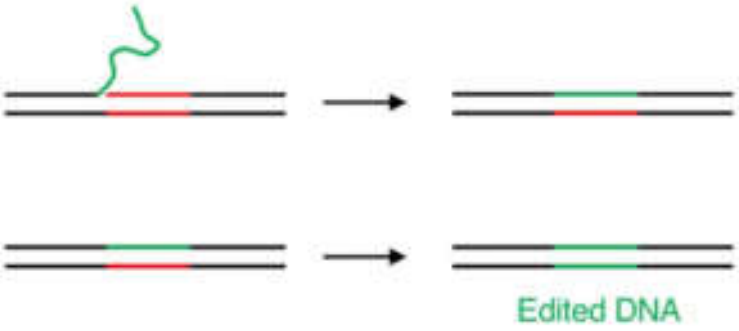
- A to G





Prime editing – a new copy/paste technology





Article

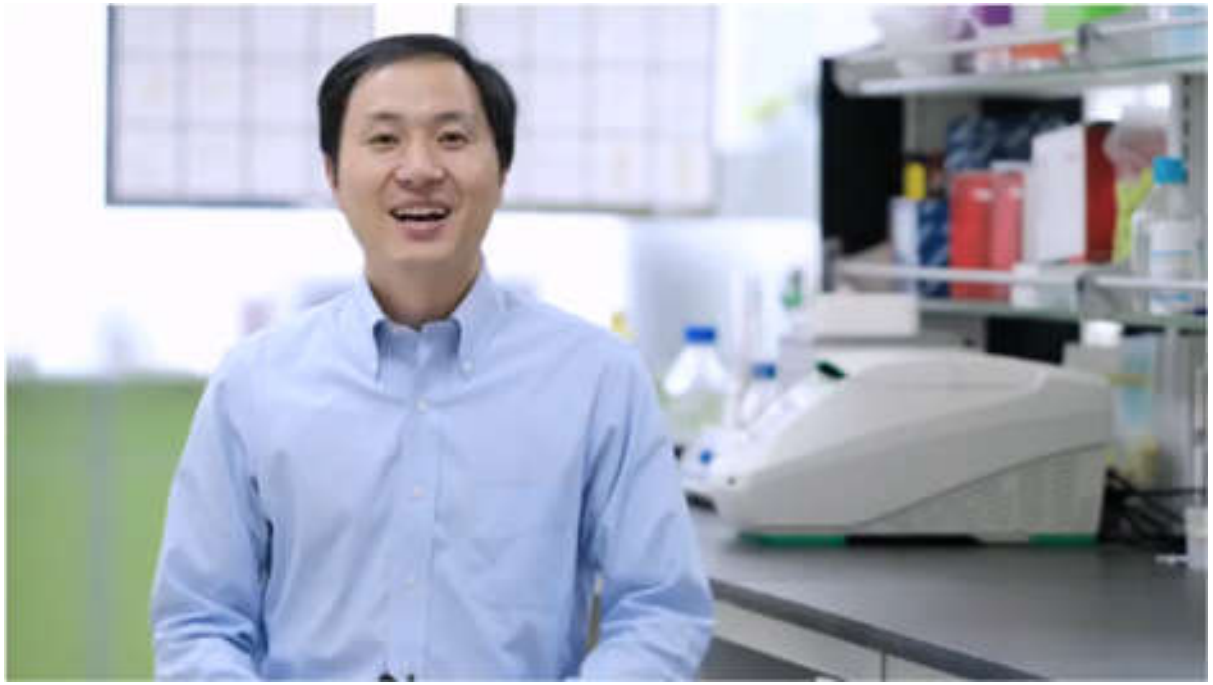
Search-and-replace genome editing without double-strand breaks or donor DNA

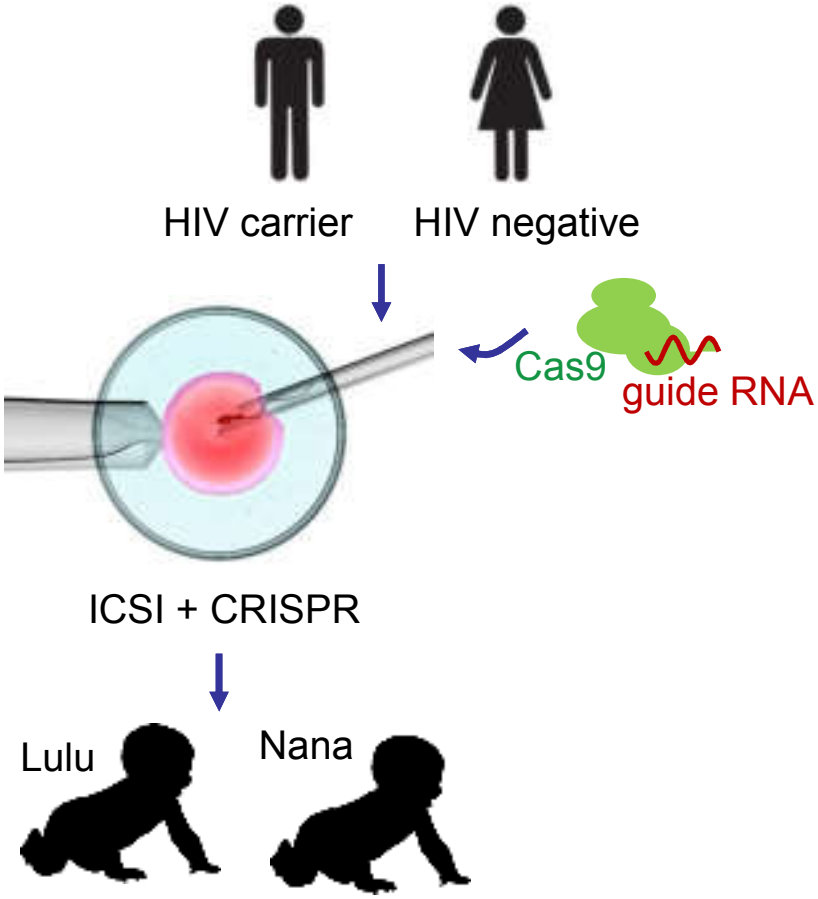
https://doi.org/10.1038/s41586-019-1711-4
Received: 26 August 2019
Accepted: 10 October 2019

Andrew V. Anzalone[1,2], Peyton B. Randolph[1,2], Jessie R. Davis[1,2], Alexander A. Sousa[1,2], Luke W. Koblan[1,2], Jonathan M. Levy[1,2], Peter J. Chen[1,2], Christopher Wilson[1,2], Gregory A. Newby[1,2], Aditya Raguram[1,2] & David R. Liu[1,2]*

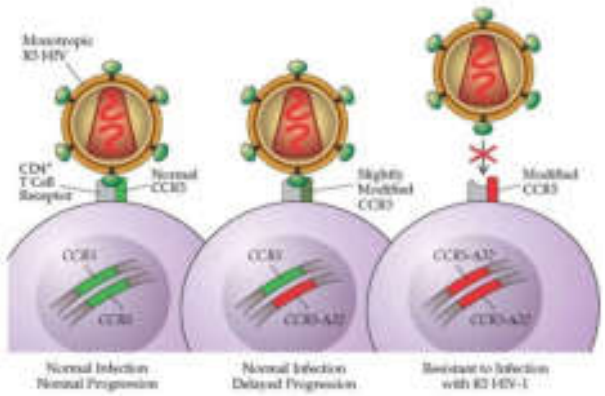
Meet Dr. He Jiankui

wellcome centre human genetics





Some people in the population are resistant to HIV



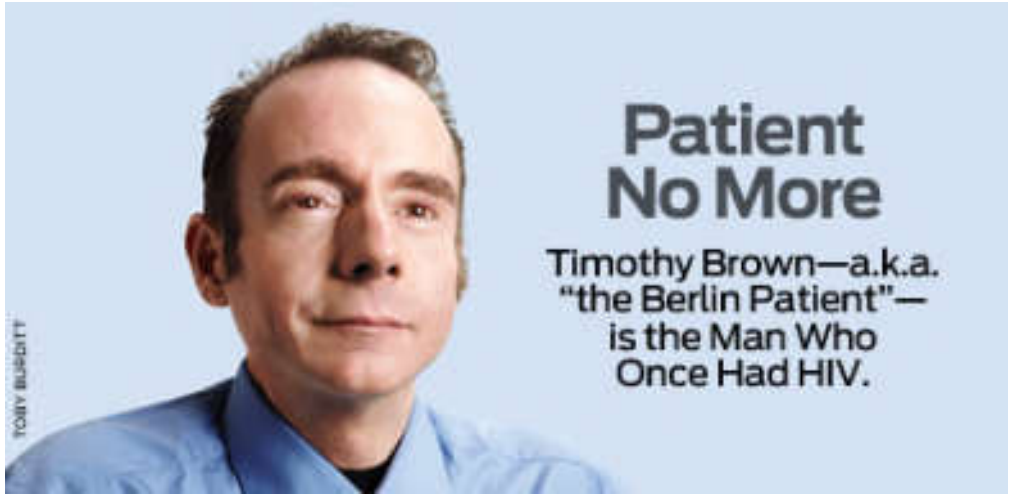
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Long-Term Control of HIV by *CCR5* Delta32/Delta32 Stem-Cell Transplantation

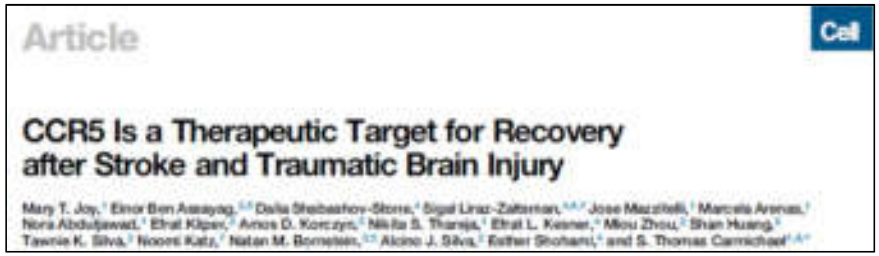
Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müllig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

- Naturally occurring mutation in a protein receptor, CCR5, stops HIV entry into cells
- If donor cells from a naturally HIV resistant patient are infused into an HIV patient, can they be cured?



Dangerous and nonsensical use of the technology

- No clinical need for CCR5 gene editing
- The consequences of CCR5 knock-out throughout life in humans is unclear.
- CCR5 loss in mouse leads to increased susceptibility to other viral infections, such as influenza and West Nile virus.
- CCR5 loss has been associated with reduced longevity and altered response to neuronal injury


Article

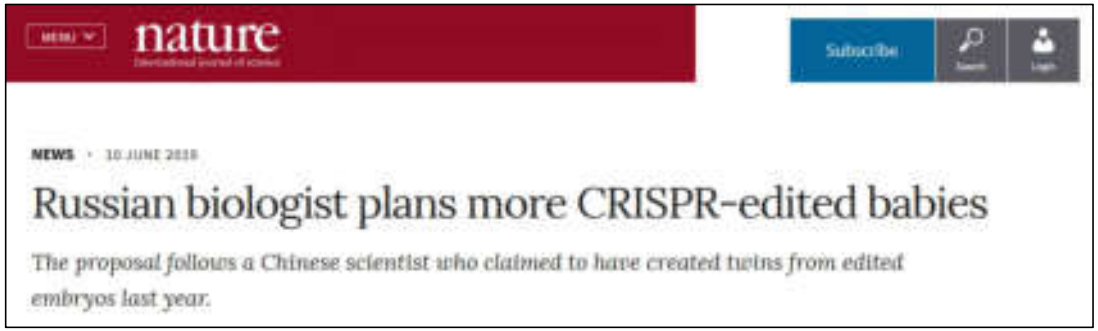
Cell

CCR5 Is a Therapeutic Target for Recovery after Stroke and Traumatic Brain Injury

- Heterozygous embryo knowingly implanted – one normal copy of CCR5 remains.
- Biological activity of Nana's de novo alleles (15 bp deletion = 5 amino acids deletion) of CCR5 completely unclear.

A call for heightened regulation and a moratorium

wellcome centre human genetics



Alliance for Regenerative Medicine

Statement of Principles on Genome Editing – August 27, 2019

"We assert that germline gene editing is currently inappropriate in human clinical settings"

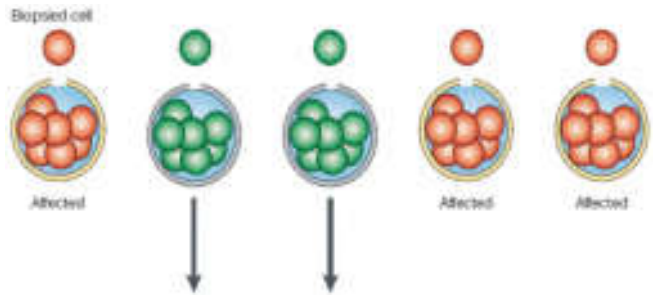
INTERNATIONAL COMMISSION ON THE CLINICAL USE OF HUMAN GERMLINE GENOME EDITING

World Health Organization

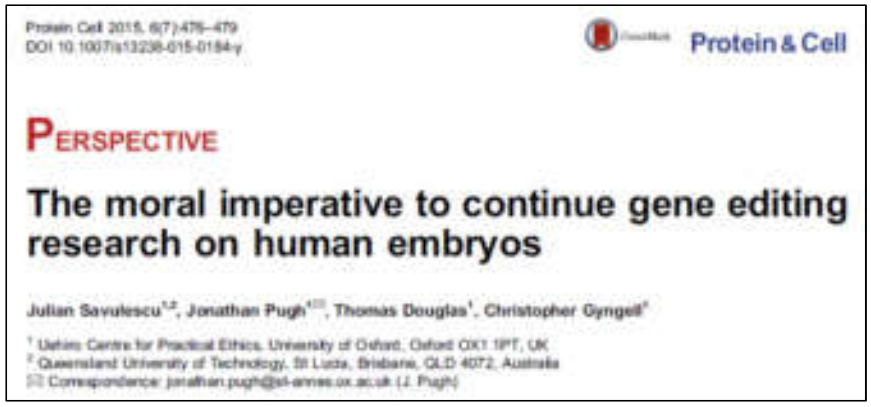
WHO expert advisory committee on Developing global standards for governance and oversight of Human Genome editing

But, is there a need for germline editing?

- Preimplantation Genetic Diagnosis (PGD) can be used to determine which embryos are healthy



- But PGD reduces the chances of success of ART
- The number of embryos obtained may be too low to allow the effective use of PGD
- Certain genetic situations aren't appropriate for PGD


Protein Cell 2015, 6(7):476–479
DOI 10.1007/s13238-015-0184-y

Protein & Cell

PERSPECTIVE

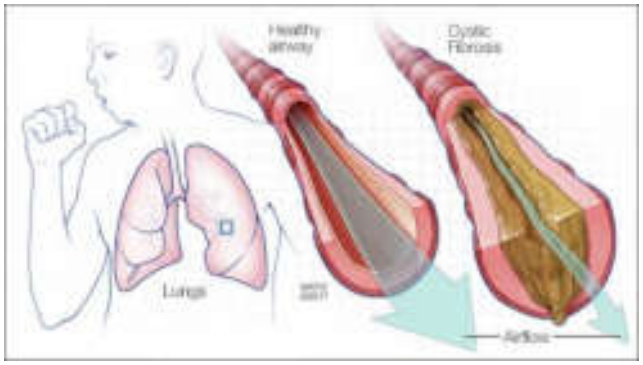
The moral imperative to continue gene editing research on human embryos

Julian Savulescu[1,2], Jonathan Pugh[1], Thomas Douglas[1], Christopher Gyngell[2]

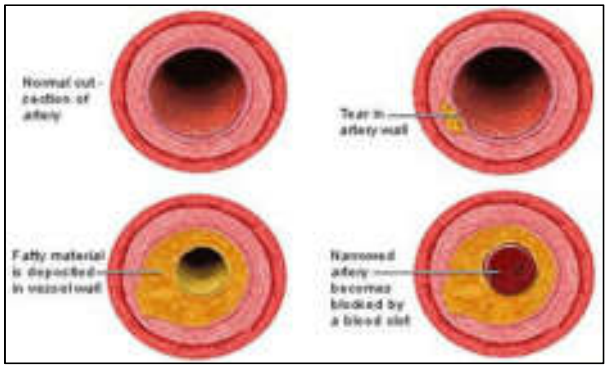
[1] Uehiro Centre for Practical Ethics, University of Oxford, Oxford OX1 1PT, UK
[2] Queensland University of Technology, St Lucia, Brisbane, QLD 4072, Australia
Correspondence: jonathan.pugh@st-annes.ox.ac.uk (J. Pugh)

- Intentionally refraining from engaging in life-saving research is not morally defensible.
- Research is needed to ascertain and address any risks

What should we use genome editing for?



Genetic disease – which ones?



Reducing risk of heart / vascular disease

Perfect eyesight

Improved intelligence

Fitness and strength

Longevity

Summary

- Current status
  - CRISPR/Cas site-specific nuclease can be easily programmed to address virtually any genomic sequence, enabling gene editing
  - Their introduction to fertilized zygotes leads to efficient mutagenesis
  - Lack of precision and associated non-specific DNA damage is a concern
  - Next generation tools being rapidly developed to counter these concerns
- The near future
  - Greater understanding of DNA repair outcomes
  - Mining the bacterial kingdom to identify new enzymes and evolution of existing ones
  - More information about clinical trail safety where CRISPR is being used in somatic tissues ex vivo (and also now in vivo).
  - Optimization and thorough safety assessment of base editing
  - A therapeutic application in human embryos

Acknowledgements

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Polinka Pliego-Hernadez

Samy Alghadban

Amine Bouchareb

British Heart Foundation

MRC Medical Research Council

wellcome

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

wellcome centre human genetics

UNIVERSITY OF OXFORD