Fostering Responsible Research with Genome Editing Technologies: a European perspective

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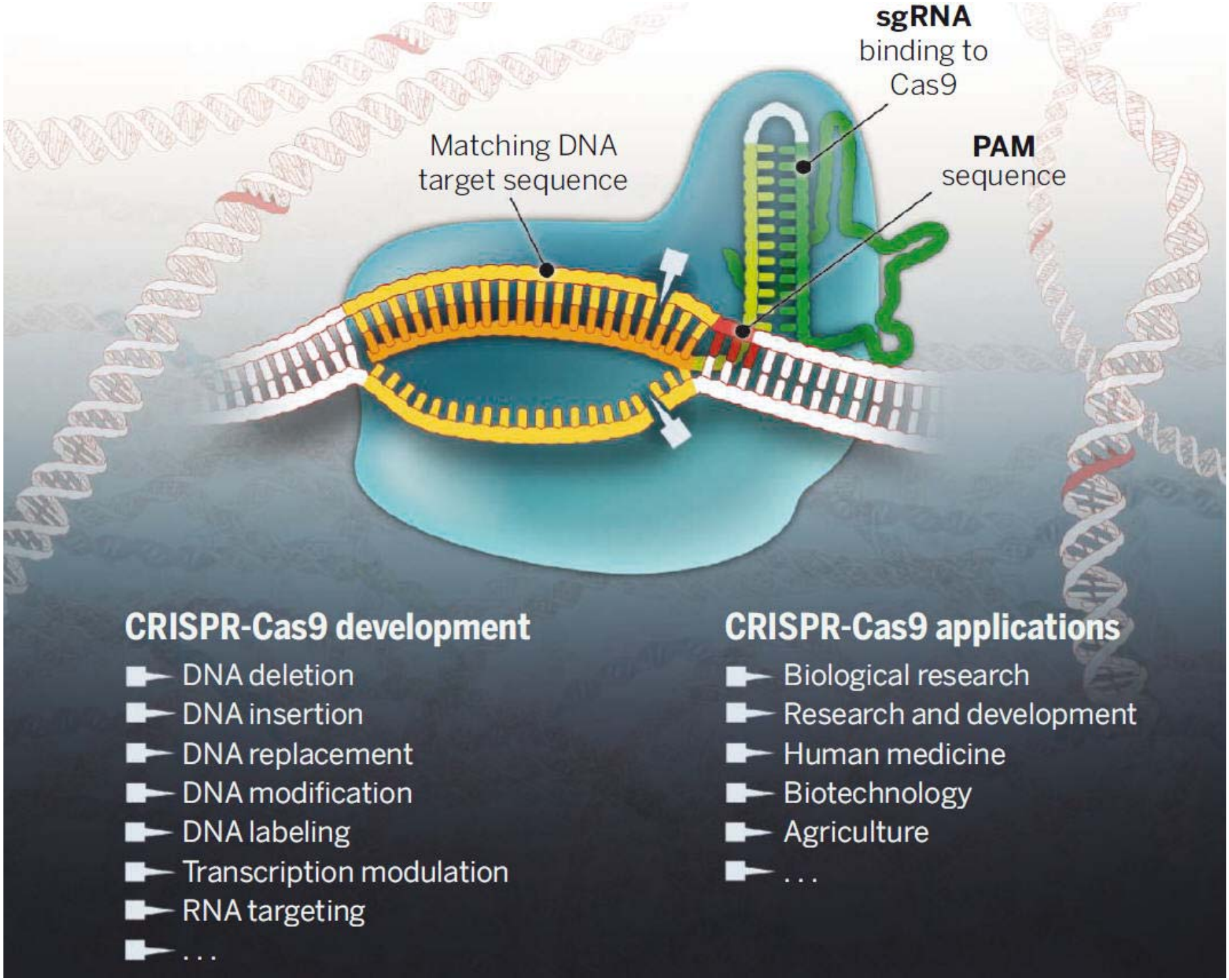
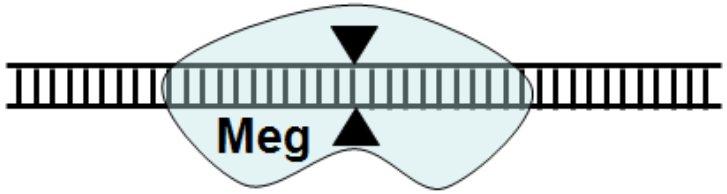
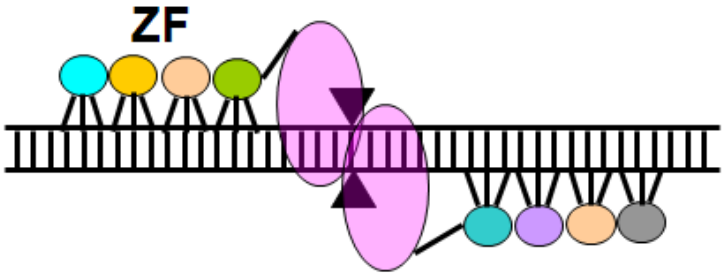
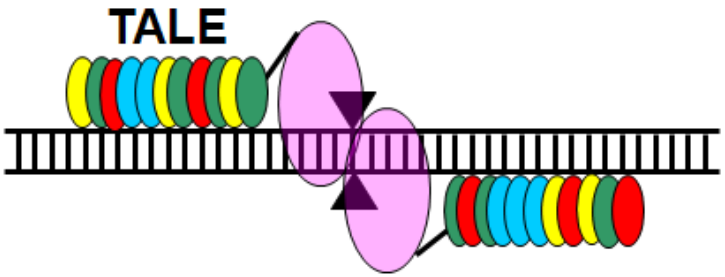

sgRNA
binding to
Cas9
Matching DNA
target sequence
PAM
sequence
CRISPR-Cas9 development
DNA deletion
DNA insertion
DNA replacement
DNA modification
DNA labeling
Transcription modulation
RNA targeting
. . .
CRISPR-Cas9 applications
Biological research
Research and development
Human medicine
Biotechnology
Agriculture
. . .



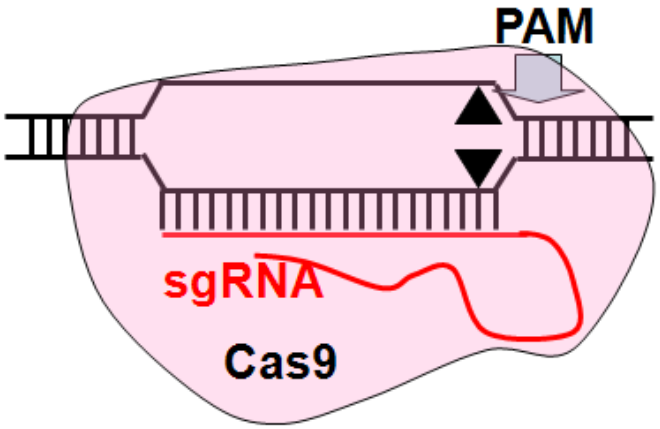
Meganuclease 20-40 bp/Enzyme



ZFN 3 bp/Finger



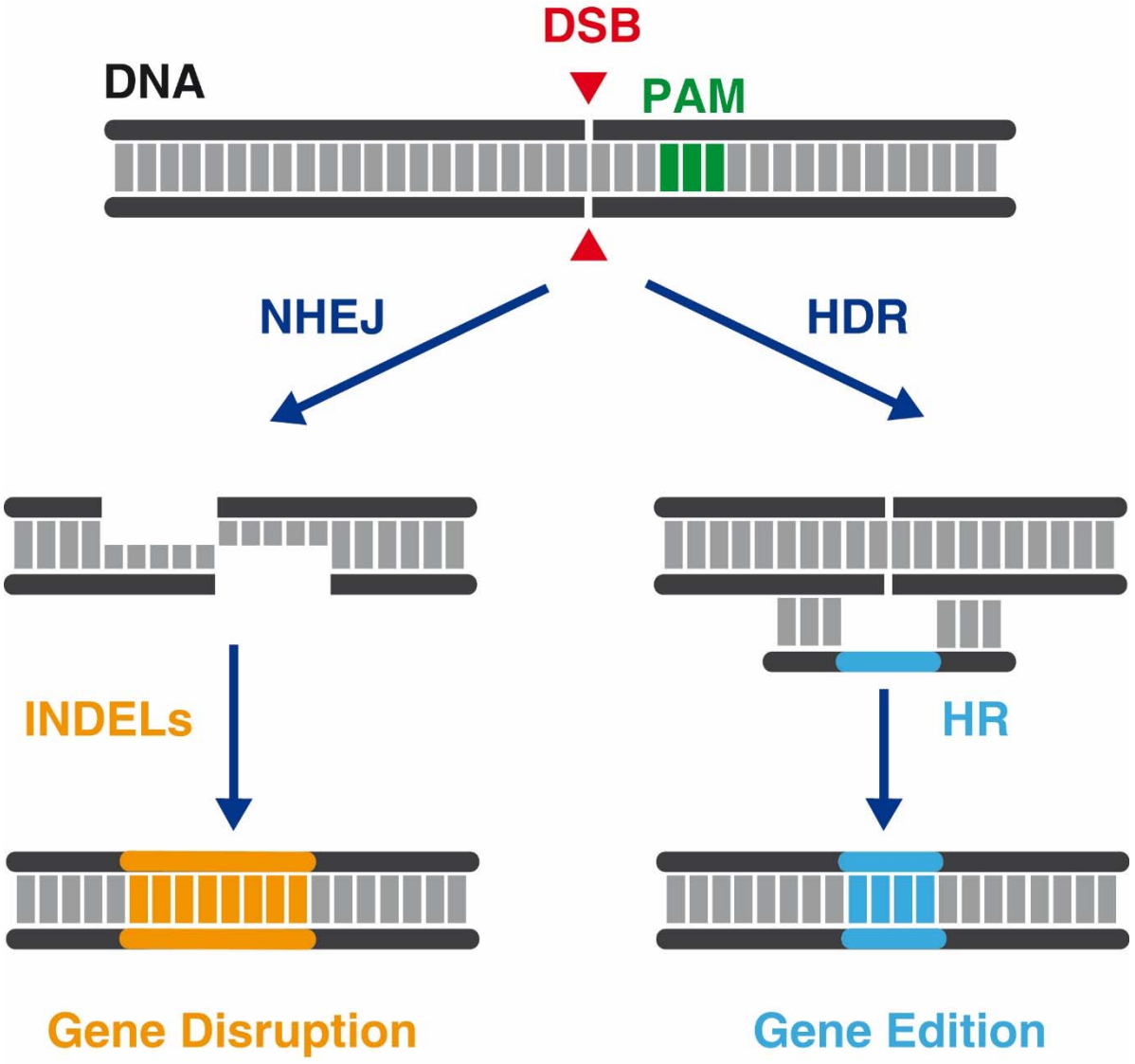
TALEN 1 bp/Module



CRISPR-Cas 1 bp/Base

Easier, cheaper, faster

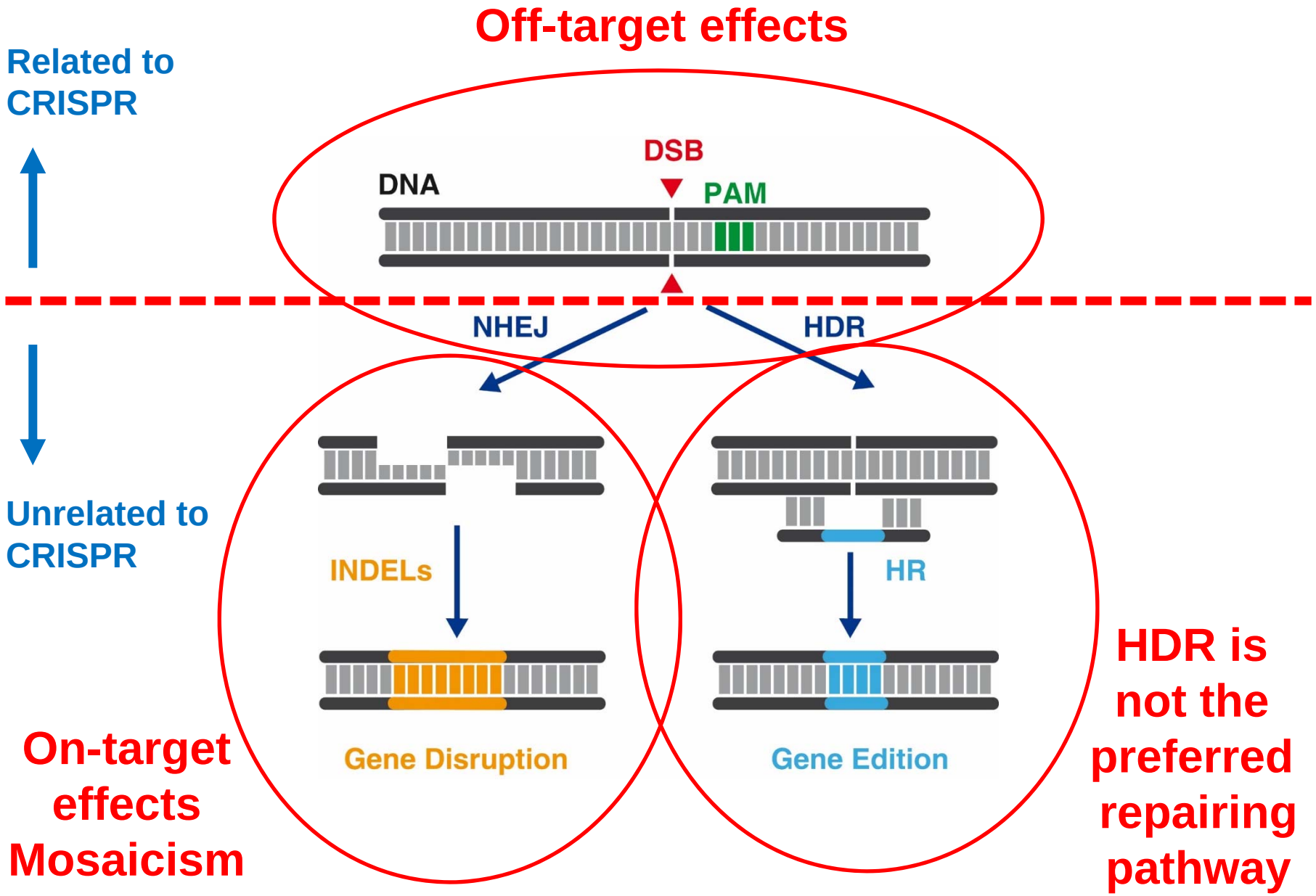
Fixing the DSB: NHEJ vs HDR



Current limitations of CRISPR

- **On-target uncertainty**: many alleles are generated through NHEJ
- Most/all founder edited-organisms are **mosaic**
- Error-prone NHEJ is the default repairing pathway
- Donor template-specific HDR is not the preferred repairing pathway
- **Off-targets**: similar target sequences can be altered
- Reaching a significant number of target cells (viral & non-viral delivery systems)

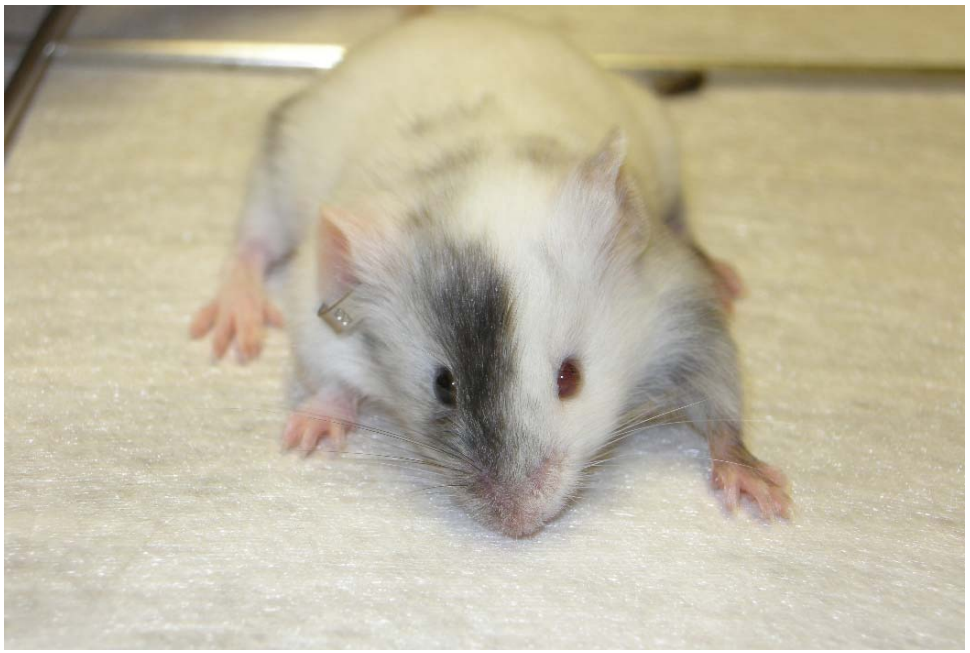
Current limitations of genome editing


Off-target effects
Related to CRISPR
DSB
DNA
PAM
NHEJ
HDR
Unrelated to CRISPR
INDELs
HR
Gene Disruption
Gene Edition
On-target effects Mosaicism
HDR is not the preferred repairing pathway

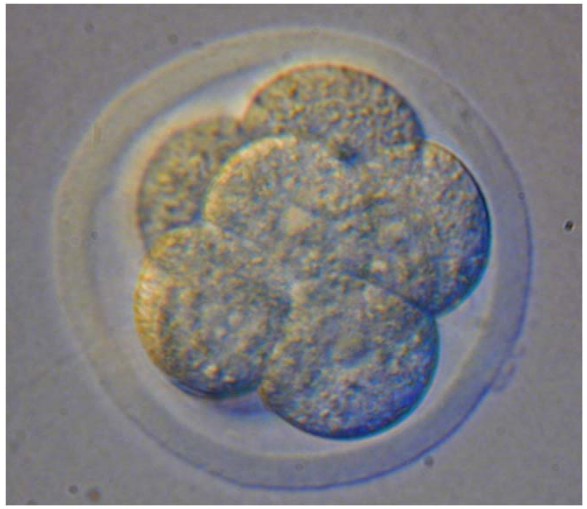
Off-targets: we can deal with them

- Off-targets depend mainly on the selected guide RNA and, to a lesser extent, on the Cas (different Cas have different properties)
- New algorithms developed for selecting optimal guide RNAs (Breaking-Cas, CRISPOR, Crispr-GOLD…)
- Can be reduced to an acceptable minimum by reducing the amount (Cas protein, not RNA or DNA) and the time of action of Cas nucleases (inhibitors)

On-targets: the real problem



- Founder animals are nearly always complex mosaic
- Many different alleles can be present
- Not all of them might transmit through germline



One 8-cell embryo = 16 possible alleles

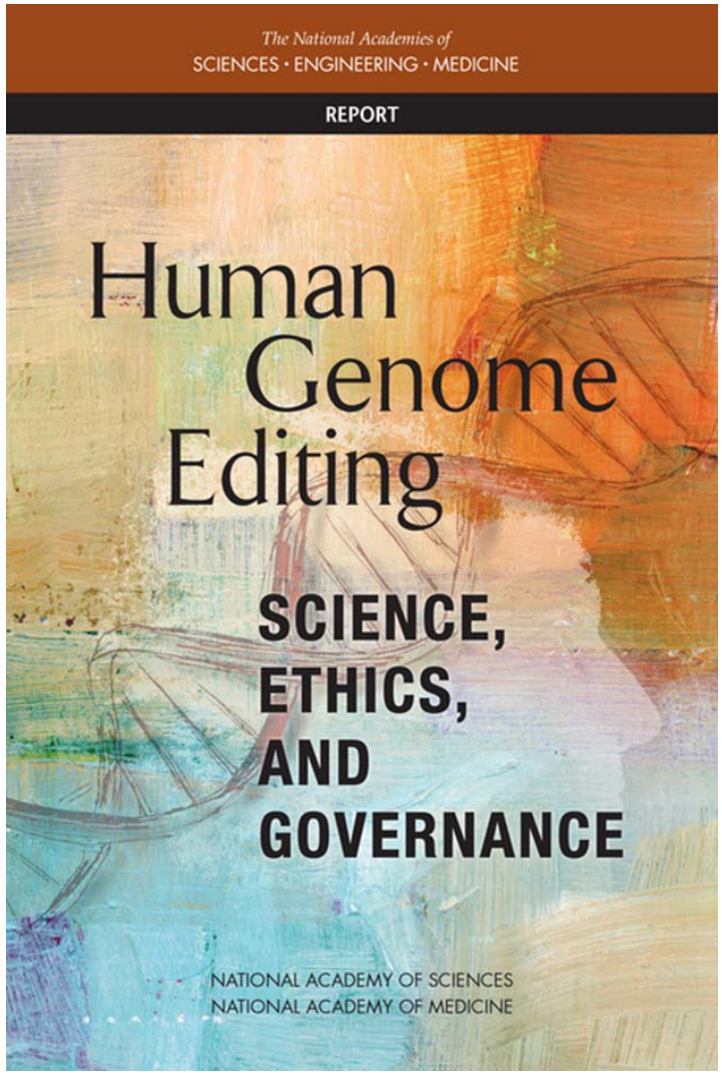
CRISPR-mediated gene therapy of a human rare disease: chronic granulomatous disease (CGD) in human iPS cells

Challenge: multiple alleles are generated

Flyn et al. (2015) Experimental Hematology

Numerous reports published on genome editing

- US NAS/NAM
- EASAC
- INSERM
- EGE
- ASHG
- Deutscher Ethikrat
- Fed. Eur. Acad. Med.
- HUGO
- Leopoldina
- Schweizer Ethikrat
- …



Regulation for genome editing

- Legislation always late and behind scientific advance
- Current legislation refers to GMO
  - Genome edition is it really GM?
- Germ-line vs Somatic gene therapy
  - Oviedo convention (art 13) not possible to modify the human genome if modifications can be transmitted to descendants
- Human reproduction techniques and research
  - Oviedo convention (art 18) the creation of human embryos for research purposes is prohibited
- No consensus for Asilomar-like 1975
- Moratorium not appropriate
- Global world
- Guidance, advice for Regulatory Authorities

Transgenic Res (2017) 26:709–713
DOI 10.1007/s11248-017-0028-z

LETTER TO THE EDITOR

20 July 2017

Fostering responsible research with genome editing technologies: a European perspective

Hervé Chneiweiss · François Hirsch · Lluis Montoliu · Albrecht M. Müller · Solveig Fenet · Marion Abecassis · Jennifer Merchant · Bernard Baertschi · Mylène Botbol-Baum · James A. Houghton · Mihalis Kritikos · Janet Mifsud · Ewa Bartnik · Johannes Rath · Christiane Druml · Bärbel Friedrich · Ana Sofia Carvalho · Dirk Lanzerath · Agnès Saint-Raymond

Received: 31 May 2017 / Accepted: 7 June 2017 / Published online: 20 July 2017

Abstract In this consensus paper resulting from a meeting that involved representatives from more than 20 European partners, we recommend the foundation of an expert group (European Steering Committee) to assess the potential benefits and draw-backs of genome editing (off-targets, mosaicisms, etc.), and to design risk matrices and scenarios for a responsible use of this promising technology. In addition, this European steering committee will contribute in promoting an open debate on societal aspects prior to a translation into national and international legislation.

Keywords CRISPR-Cas · Gene editing · Science and society · Responsible research and innovation

INSERM, CSIC, Leopoldina, EGE, EMA, ERC, UNESCO, EURORDIS, EC, Universities…

France, Spain, Germany, Portugal, Belgium, UK, Austria, Poland, Malta, Ireland, Switzerland

Fostering responsable research with genome editing: EU view

GENERAL PRINCIPLES

1. To foster research that will assess the feasibility, the efficacy and the safety of genome editing techniques, such as the benefit-to-harm balance of any potential clinical application can be evaluated.

   – Establishing a European Steering Committee (ESC)
   - Acceptable levels and types of off-target effects
   - Acceptable levels of mosaicism
   - Acceptable levels of epigenetic effects

Chneiweiss et al. 2017

Fostering responsable research with genome editing: EU view

GENERAL PRINCIPLES

2. To evaluate the potential adverse effects of gene drive applications with a thorough risk assessment analysis and mitigated before environmental trials are undertaken outside the laboratory.

Chneiweiss et al. 2017

Fostering responsable research with genome editing: EU view

GENERAL PRINCIPLES

3. To reassess the ban on all modifications of the germ line nuclear genome for clinical application in human reproduction.

Oviedo convention (1997), article 13: “An intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants.”

Supernumerary IVF human embryos can be used for research

IVF human embryos cannot be created ad hoc for research

Chneiweiss et al. 2017

Fostering responsable research with genome editing: EU view

GENERAL PRINCIPLES

4. To be pro-active to prevent this technology from being hijacked by those extremist views and to avoid misleading public expectation with overinflated promises.

Chneiweiss et al. 2017

Fostering responsable research with genome editing: EU view

GENERAL PRINCIPLES

5. To raise awareness about the distinction between the care/treatment of human diseases and human enhancement.

Chneiweiss et al. 2017

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20 July 2017

2 August 2017

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

Genome editing has potential for the targeted correction of germline mutations. Here we describe the correction of the heterozygous *MYBPC3* mutation in human preimplantation embryos with precise CRISPR–Cas9-based targeting accuracy and high homology-directed repair efficiency by activating an endogenous, germline-specific DNA repair response. Induced double-strand breaks (DSBs) at the mutant paternal allele were predominantly repaired using the homologous wild-type maternal gene instead of a synthetic DNA template. By modulating the cell cycle stage at which the DSB was induced, we were able to avoid mosaicism in cleaving embryos and achieve a high yield of homozygous embryos carrying the wild-type *MYBPC3* gene without evidence of off-target mutations. The efficiency, accuracy and safety of the approach presented suggest that it has potential to be used for the correction of heritable mutations in human embryos by complementing preimplantation genetic diagnosis. However, much remains to be considered before clinical applications, including the reproducibility of the technique with other heterozygous mutations.

Science, Art, Beauty by Josiah Zayner, Ph.D.

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The First Attempt At H 4

DIY Guide to Creating 1


How to Genetically Er 4


How to Genetically Er 2


How to Genetically Er 2


Let's start at the begir 5


Green Flourescent Pr 2

I transplanted someor 2

I transplanted someor 2

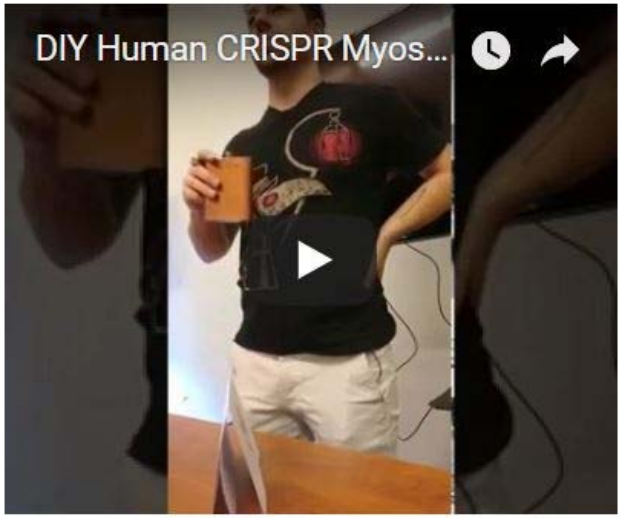
I transplanted someor 1


Science Hack Day SF 3

And the Nobel Prize g 1

The First Attempt At Human CRISPR Gene Editing

The first attempt at human CRISPR gene editing did not occur in a hospital or University or in a clinical trial by some $100 million funded company. Instead, it happened in small cramped room in San Francisco in front of 30 or so people who squeezed in to listen to a talk about how biohackers are making genetic and cellular modification accessible.



This is the first time in the history of the Earth that humans are no longer slaves to the genetics they are born with. As I write this, the FDA is in the process of approving the first human gene therapy treatment. Still it's too slow for me, clinical trials have been going on since before 2008. I want to accelerate that. I want people to have a choice about their genetics.

To push it forward I did a CRISPR experiment on myself.

Intramuscular injection of Cas9 plasmid and sgRNA to target myostatin

www.ifyoudontknownowyaknow.com

13 October 2017

News › Science

Scientists make first attempt to permanently change a person's DNA to cure a disease

A risky new treatment is being trialled in the US to reverse the effects of an incurable genetic disorder

Josh Gabbatiss | 12 hours ago |

80 shares

Associated Press, 15 Nov 2017

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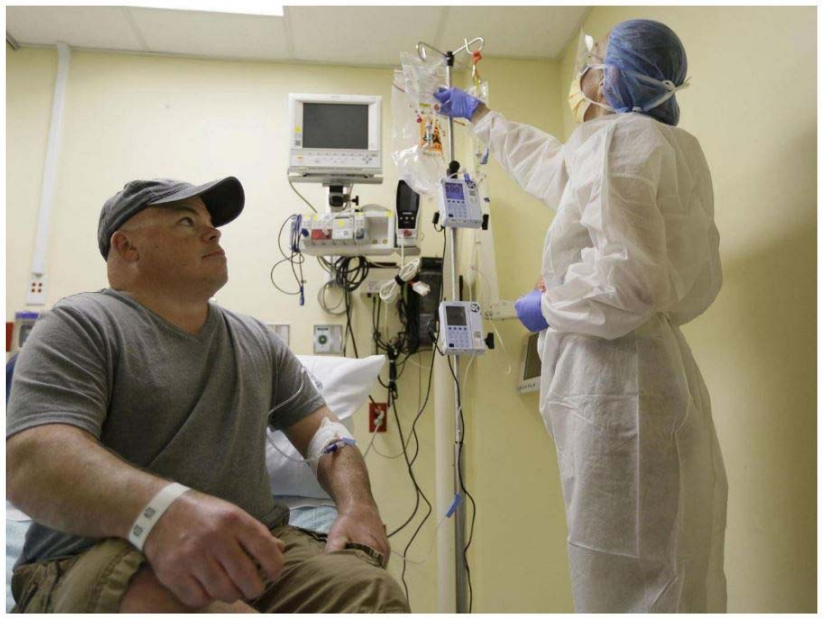

Brian Madeux, 44, looks up at nurse practitioner Jacqueline Madde while receiving the first human gene editing therapy at the UCSF Benioff Children's Hospital in Oakland, California *ASSOCIATED PRESS*

- UCSF Benioff Children's Hospital in Oakland, California
- IV injection of viral particles with ZFNs
- Approved by NIH
- Sangamo
- Hunter's syndrome (I2S gene) Mucopolysaccharidosis II (MPS II)
- Lysosomal storage disease
- Injected last Monday **13 Nov 2017**

First genome editing (driven by ZFN) somatic gene therapy in a patient IN VIVO

Fostering Responsible Research with genome editing technologies: a European perspective

Instituts thématiques

Inserm

Institut national
de la santé et de la recherche médicale

External European Experts Meeting

Paris, 13 november 2017

Ethics issues/topics

- Communicating science to the community and to the public: education
- Scientific integrity, responsibility issue
- RRI: Research Responsible and Innovation
- Medical uses versus optimization versus enhancement (Eugenics)
- Safety and security
- Access to the advances in science, social equity, justice
- Careful and case-by-case risk/benefit analysis
- Scientific freedom
- Creating versus replicating genetic variants (GMO vs GEO)
- Human relation with nature: human playing God
- Somatic versus embryo germline modification/gene therapy
- Interfering Genetic Inheritance (Gene Drive)
- Revitalizing extint species
- Reversibility and Traceability
- Biodiversity, Benefit sharing and Respecting cultural context
- Technological imperative
- Do it yourself? / Garage laboratories
- Potential dual use (civil versus military) or malevolant misuse (bioweapon)
- RNA versus DNA editing
- Manipulating animal genome/traits

Next steps

- Inviting additional Ethics Committees from European institutions
- Inviting Ethics Committees from South-America, Asia and Africa
- Considering converting this discussion group into a legal entity (association?)
- Releasing a document with the identified Ethics issues, the need for updating/modifying current legislation, need to agree on standard practices
- Next meeting to take place end of March 2018