Progress in Genetic Medicine – Genome Editing

Kiran Musunuru, MD, PhD, MPH, FAHA
University of Pennsylvania

Progress in Genetic Medicine – Genome Editing

Kiran Musunuru, MD, PhD, MPH, FAHA
University of Pennsylvania
Disclosures: None

Genome editing

- Facilitates knockout or knock-in of mutations by introducing a double-strand break at a desired site in the genome
- Dramatically increases the efficiency of mutagenesis
- Can be used *in vitro* and *in vivo*

Genome editing

- The cell has two methods to repair double-strand breaks

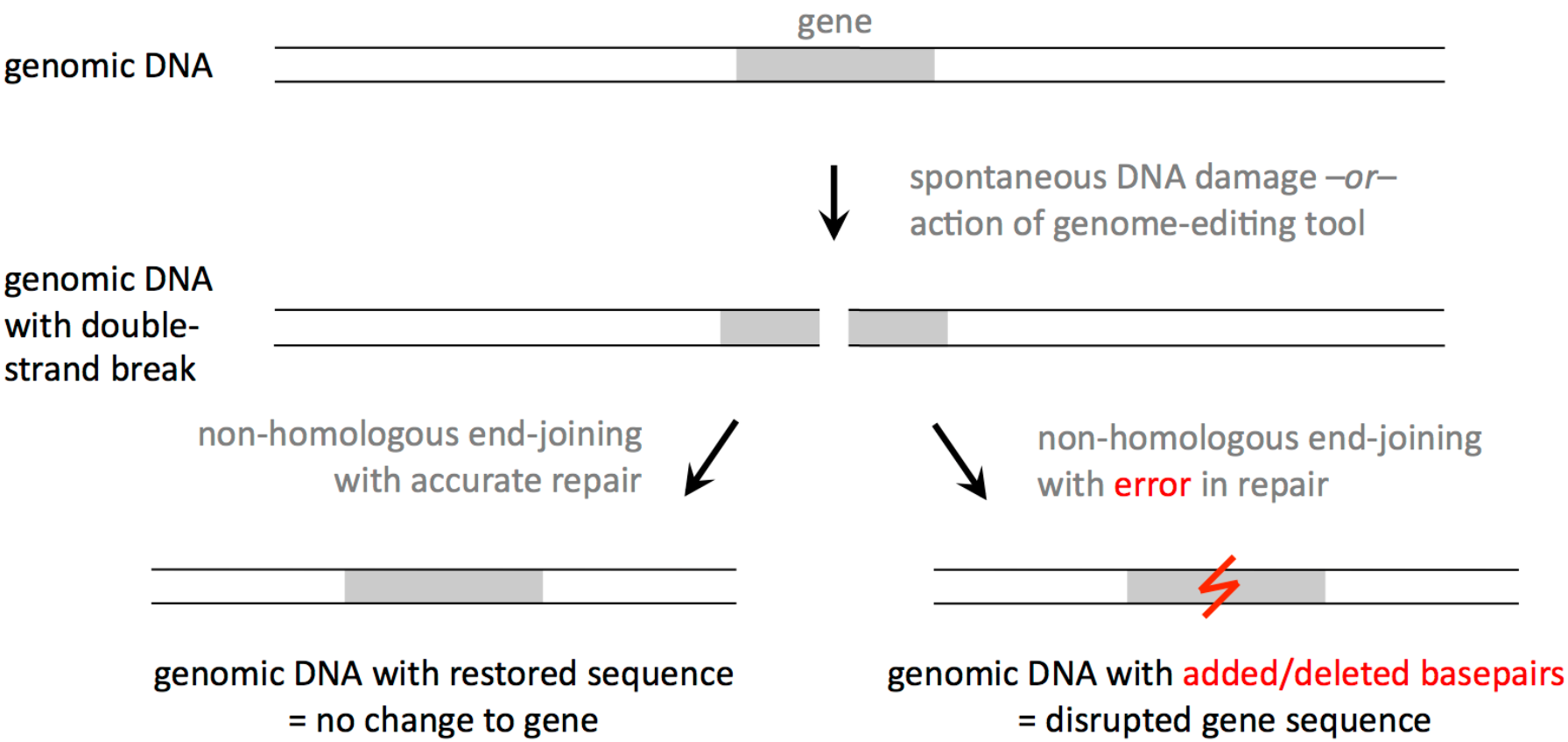
- Non-homologous end-joining (NHEJ) – rejoins two free ends, error-prone → indel/frameshift mutations

- Homology-directed repair (HDR) – uses sister chromatid/chromosome as a template to replace the area of the break via homologous recombination

Genome editing

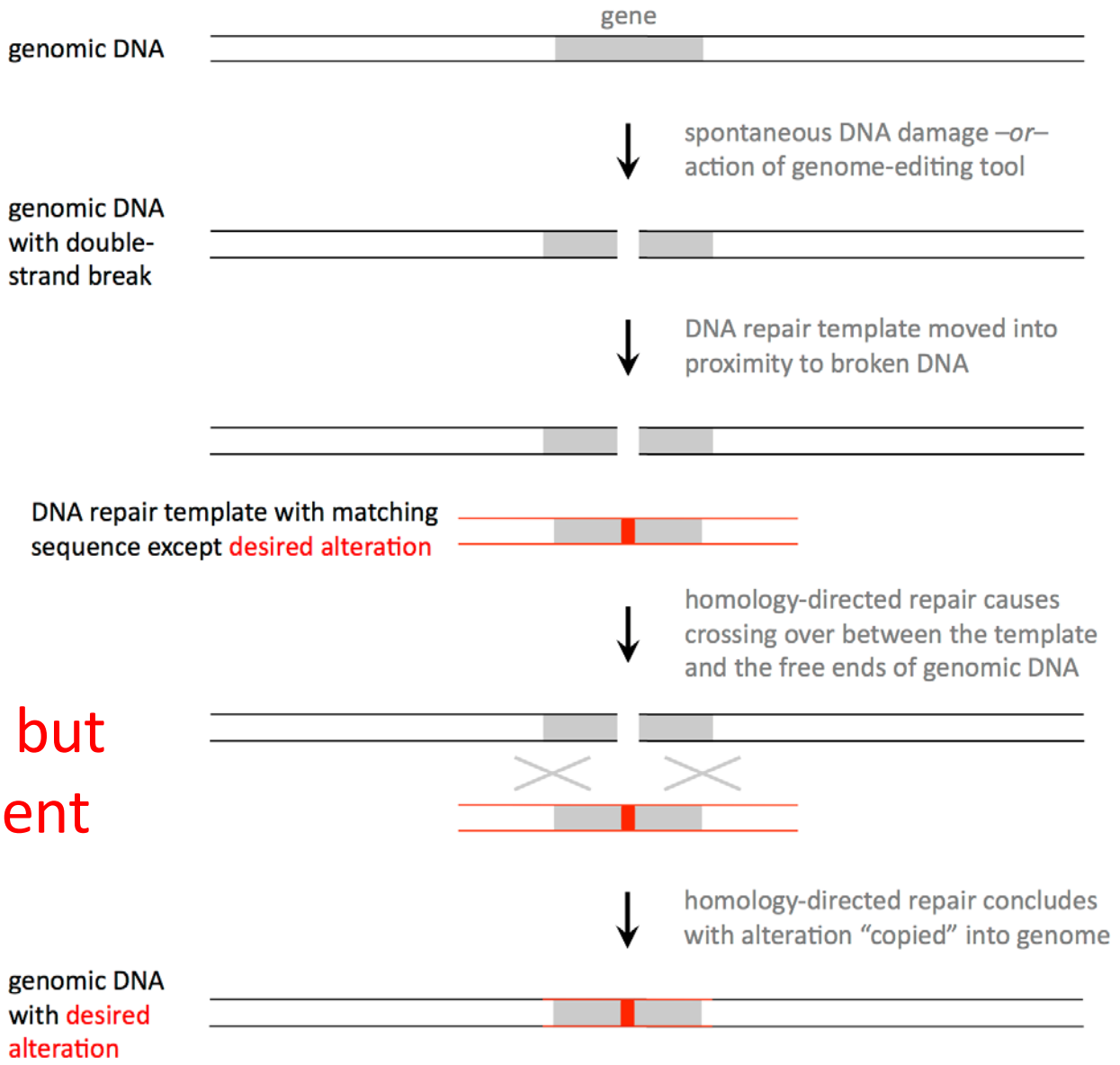
- Can fool the cell into using a custom-made piece of DNA as a repair template
- If the custom-made DNA harbors a mutation, can exploit HDR to knock in the mutation into the genome (or to correct a disease-causing mutation)
- HDR only works in proliferating cells, less efficient than NHEJ

Non-homologous end-joining (NHEJ)



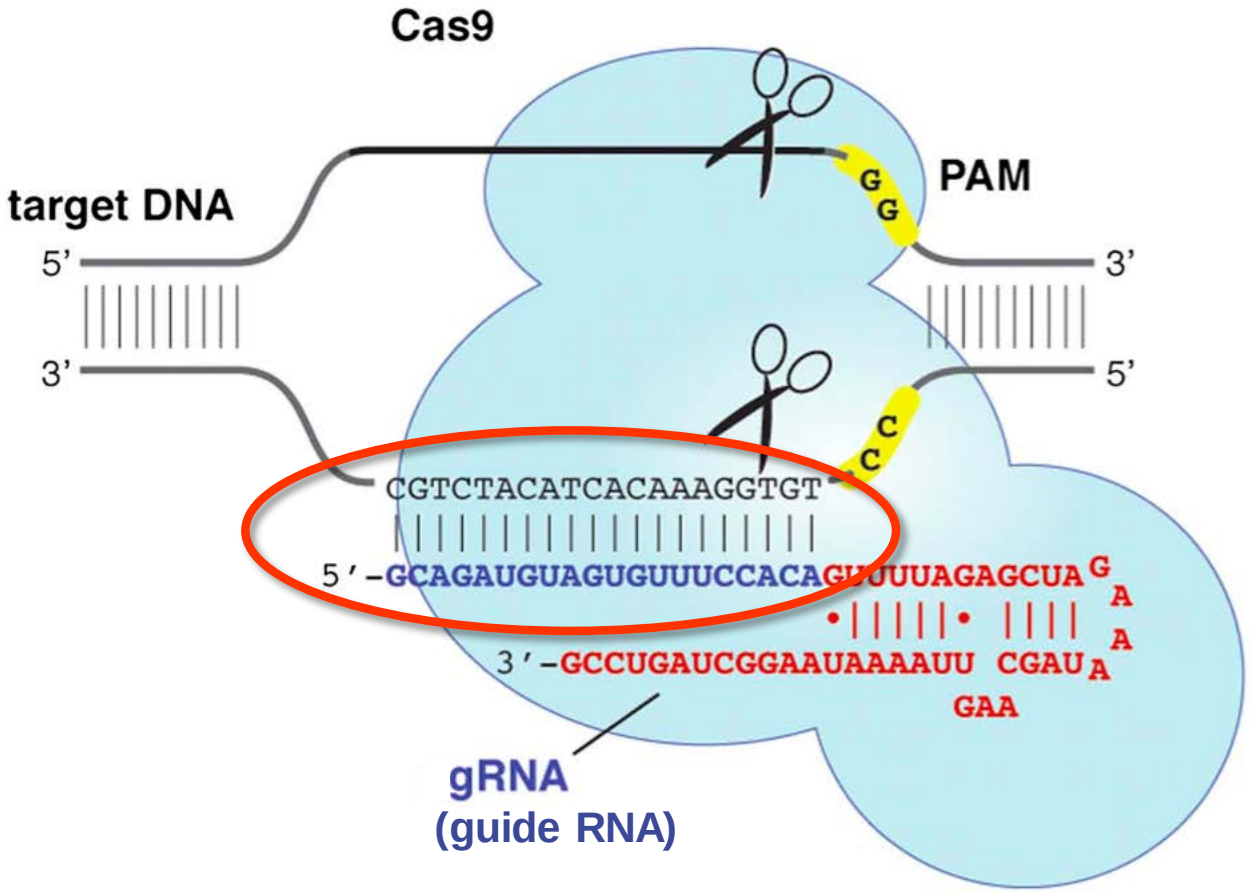
crude but efficient

Homology-directed repair (HDR)



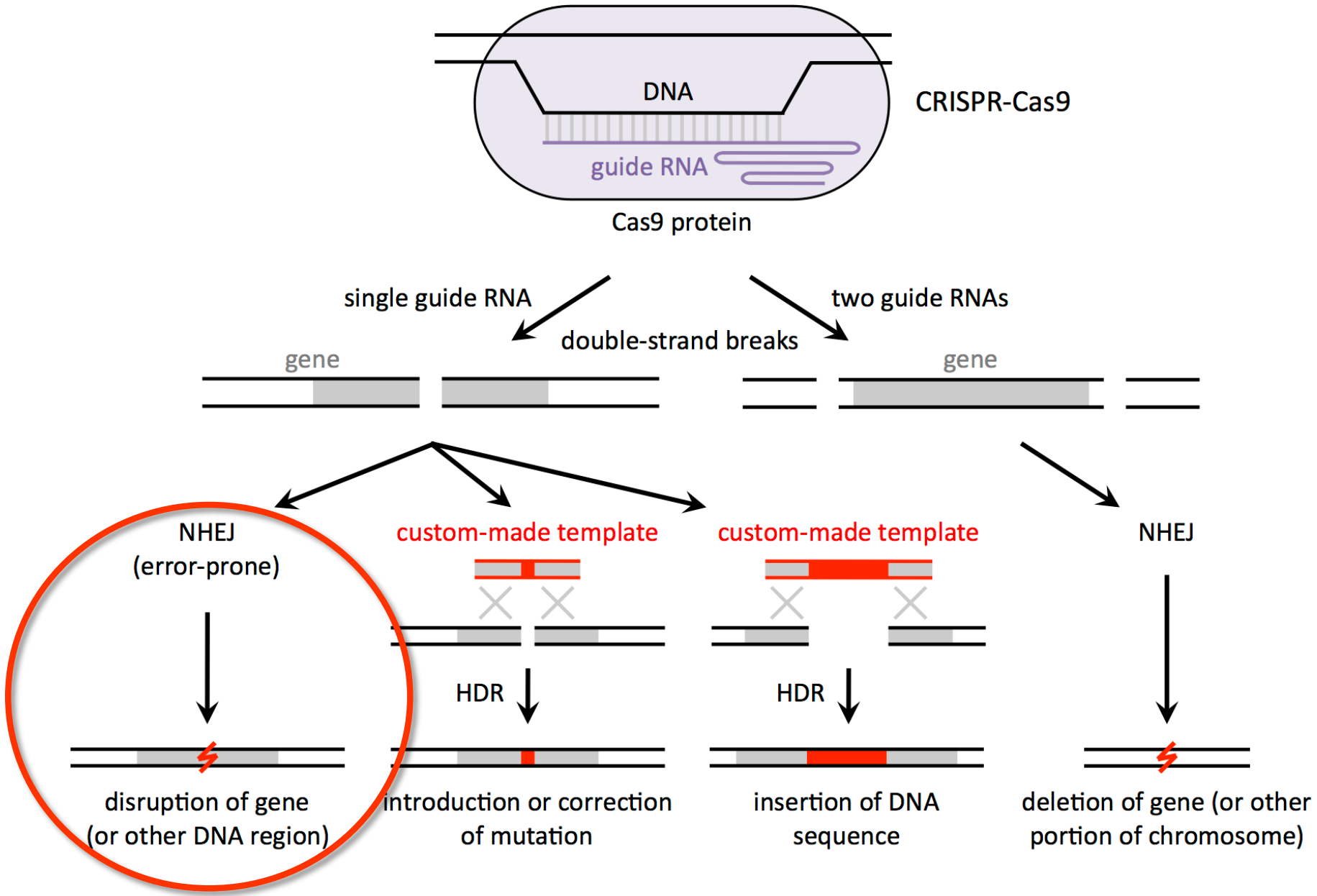
precise but inefficient

CRISPR-Cas9 in mammalian cells

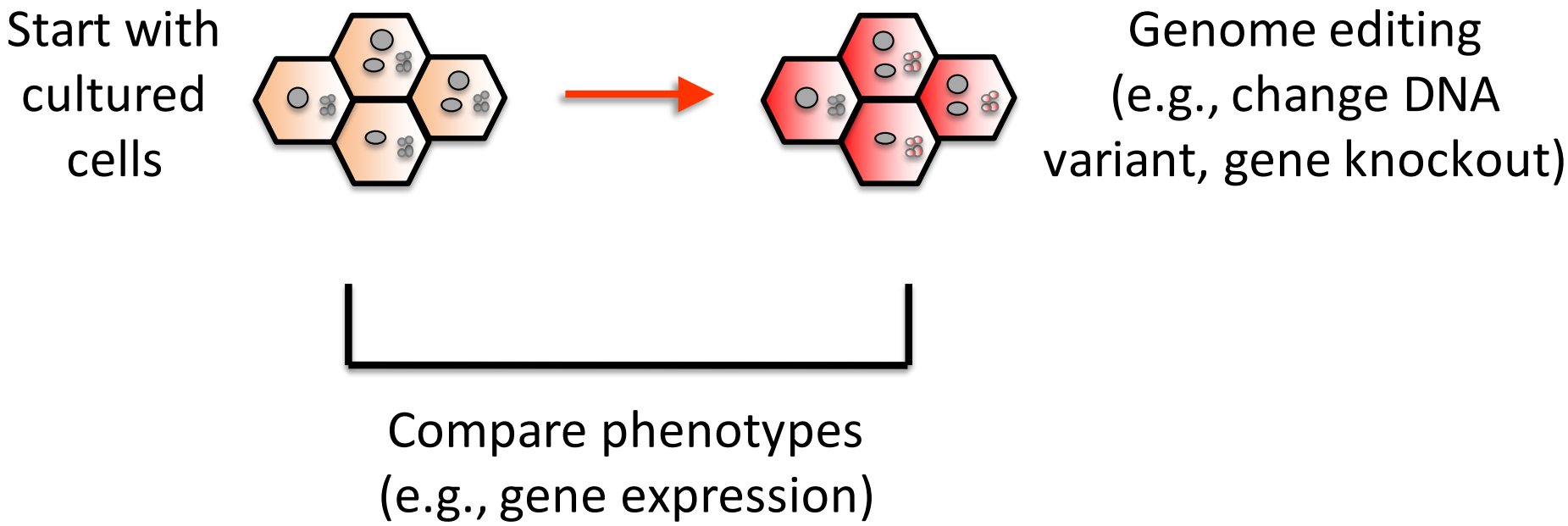


Jinek et al. *eLife* 2013; 2:e00471
Mali et al. *Science* 2013; 339:823-6
Cong et al. *Science* 2013; 339:819-23
Cho et al. *Nat Biotechnol* 2013; 31:230-2

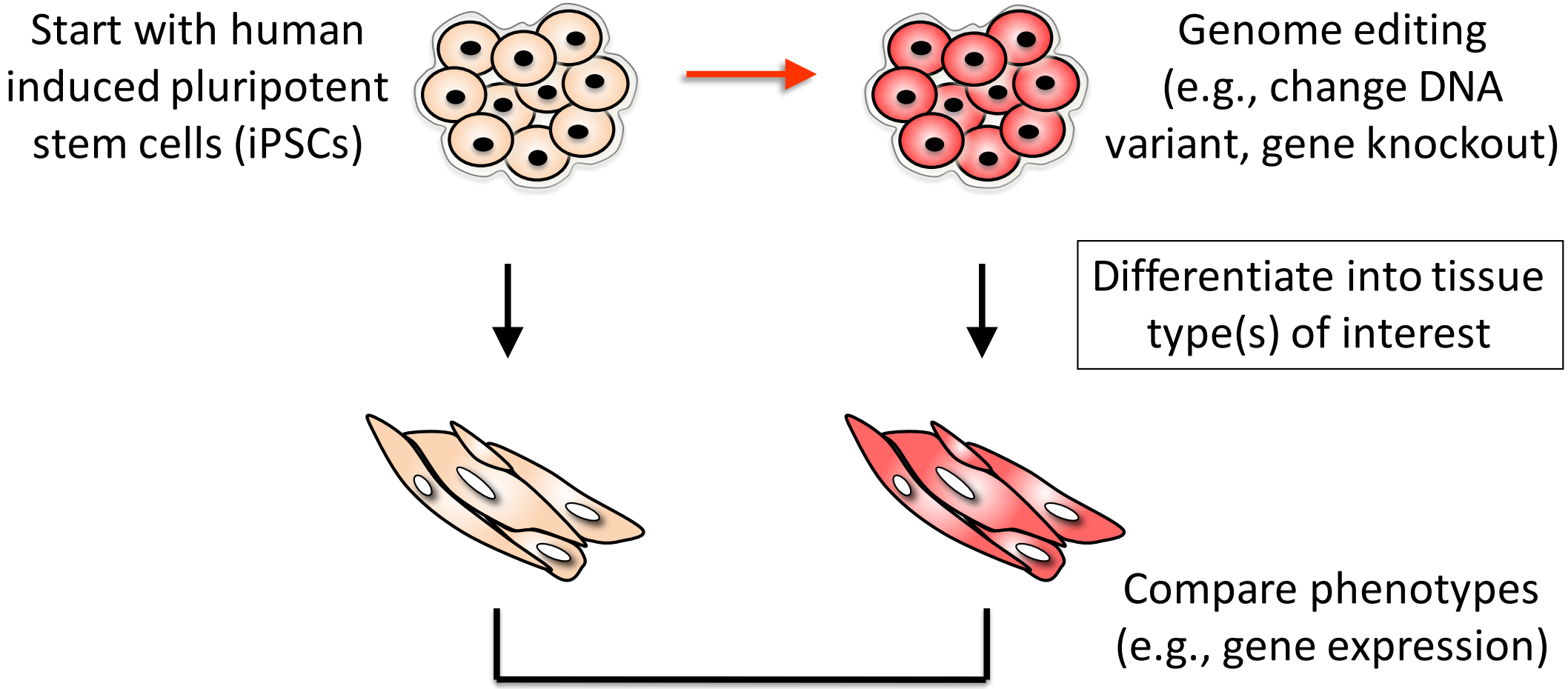
The CRISPR-Cas9 system for genome editing



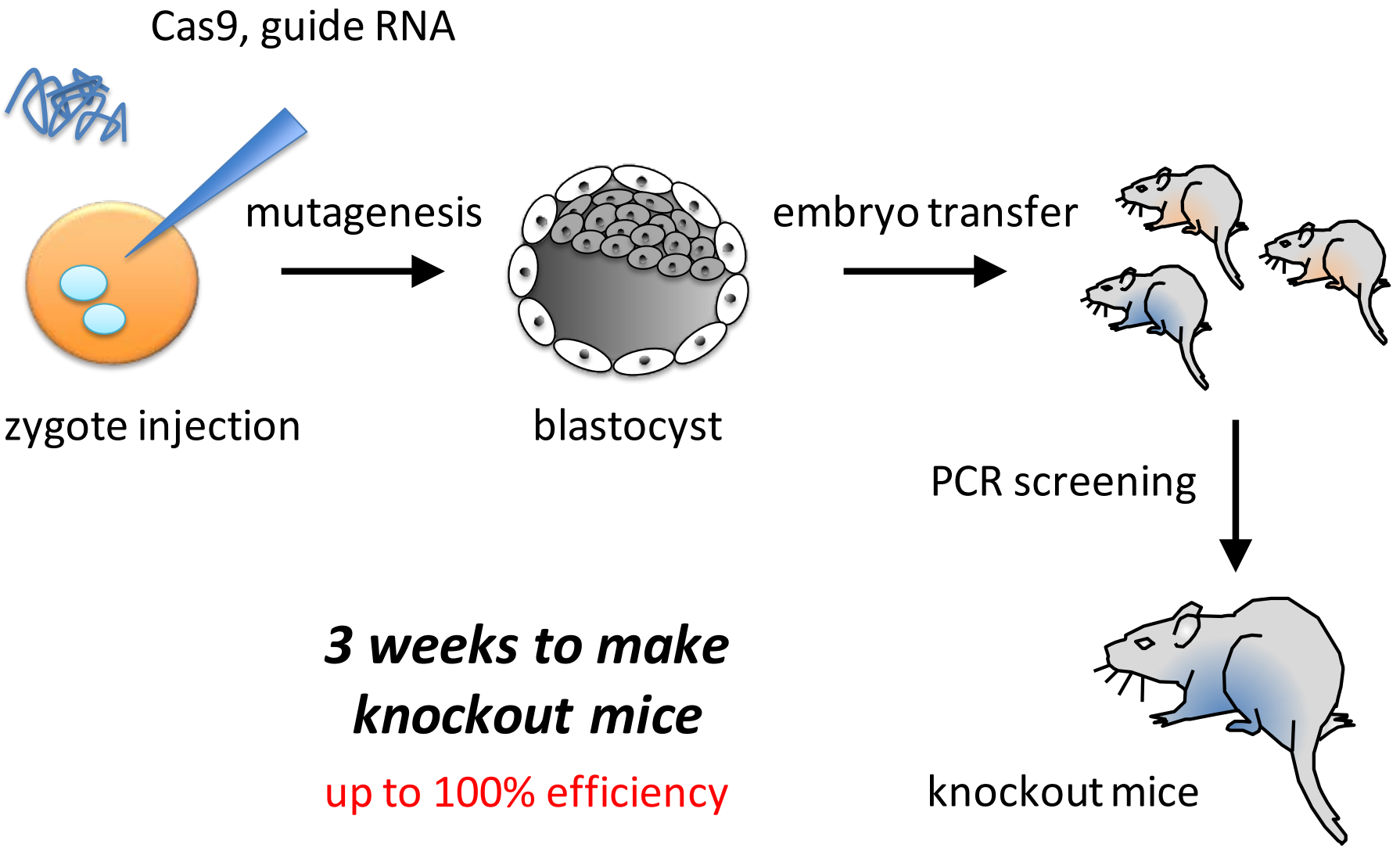
1. Generating altered cells with CRISPR-Cas9



2. Generating altered iPSC-derived cells with CRISPR-Cas9



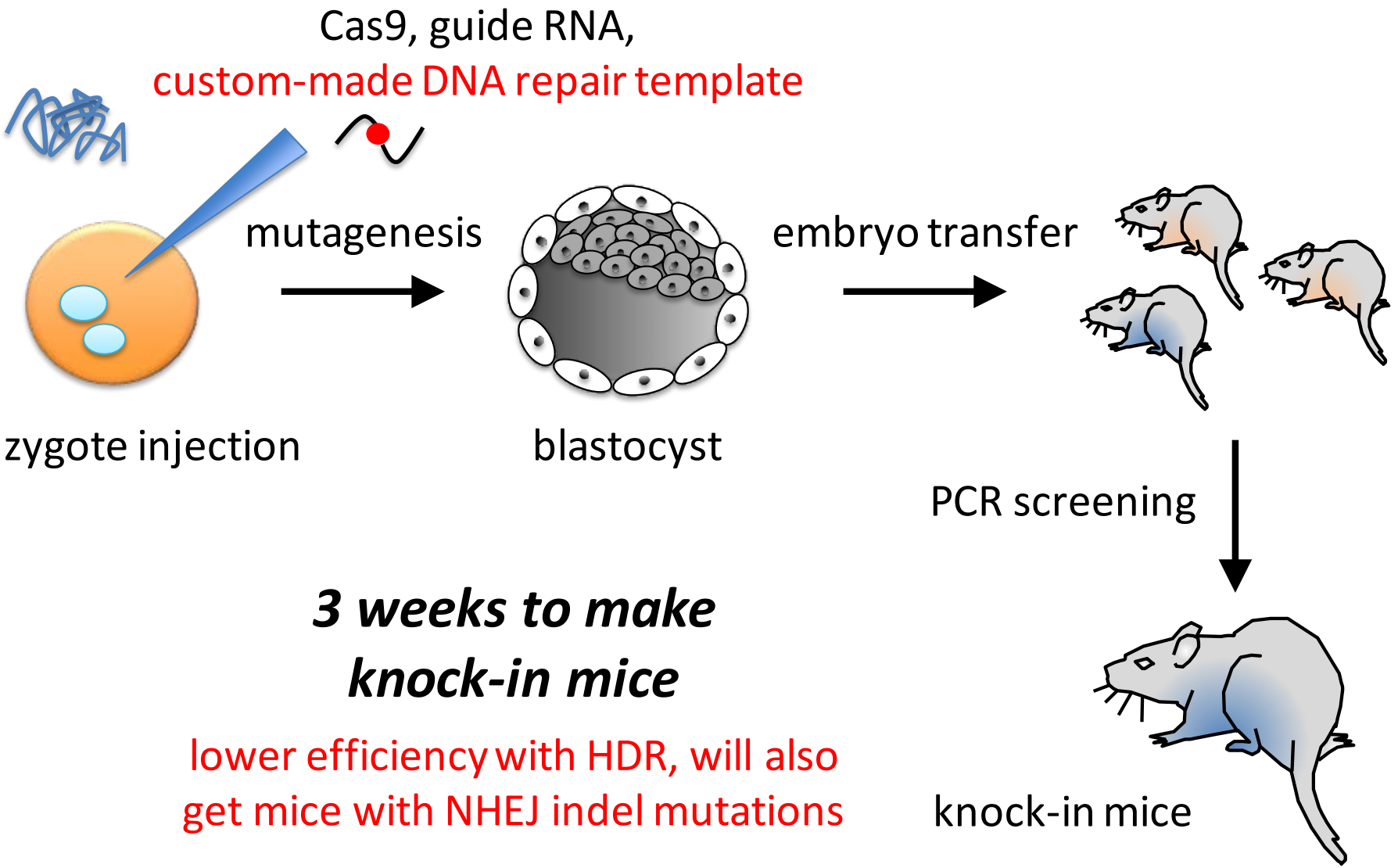
3. Generating knockout animals with CRISPR-Cas9 (NHEJ)



3 weeks to make knockout mice

up to 100% efficiency

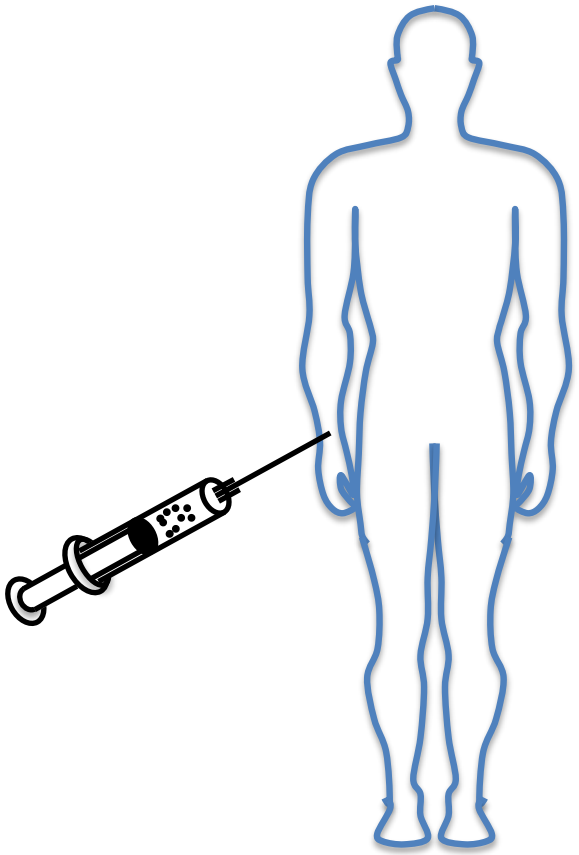
4. Generating knock-in animals with CRISPR-Cas9 (HDR)



3 weeks to make knock-in mice

lower efficiency with HDR, will also get mice with NHEJ indel mutations

5. Clinical uses of genome editing



- Strategy 1: Disrupt disease-causing genes (NHEJ)
- Strategy 2: Repair disease mutations (HDR)
- Strategy 3: Insert genes that attenuate/cure disease (HDR)

PCSK9 and coronary heart disease (CHD)

Individuals with total loss-of-function mutations in *PCSK9*:

SINGLE mutation → LDL-C ↓ 30-40%; CHD risk ↓ 80-90%

TWO mutations → LDL-C ↓ ~80%; CHD risk eliminated?

No apparent adverse health consequences

3% in populations have loss-of-function *PCSK9* mutations

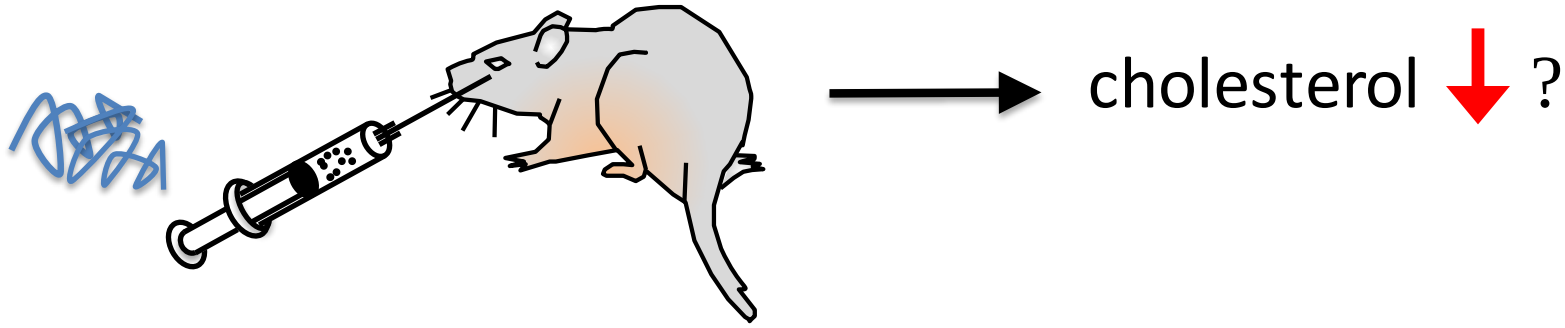
Cohen et al. *Nat Genet* 2005; 37:161-5

Cohen et al. *N Engl J Med* 2006; 356:1264-72

Zhao et al. *Am J Hum Genet* 2006; 79:514-23

Hooper et al. *Atherosclerosis* 2007; 193:445-8

Targeting mouse *Pcsk9* with somatic genome editing



CRISPR-Cas9 targeting *Pcsk9* in the mouse liver using virus

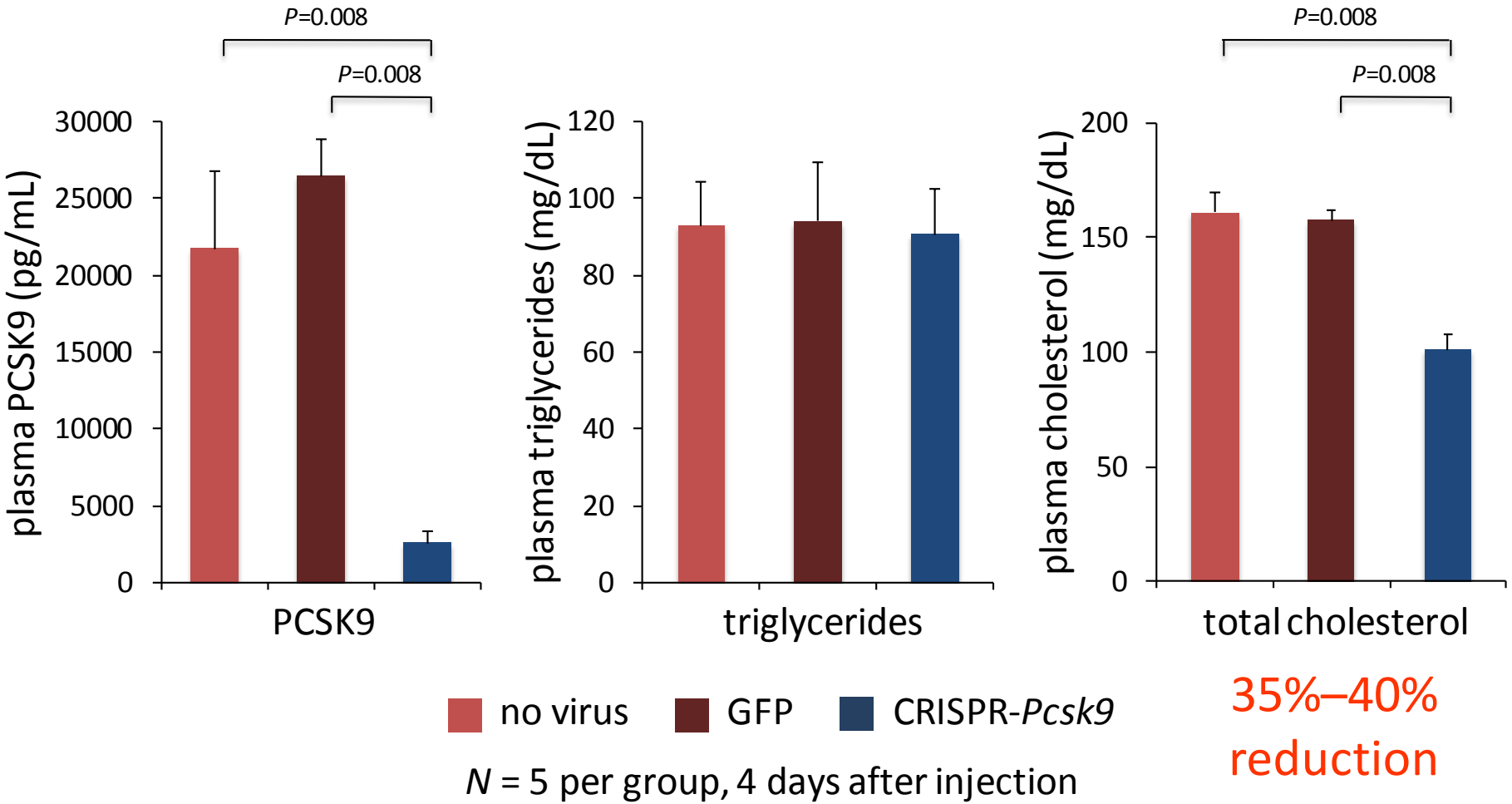
Molecular Medicine

Permanent Alteration of PCSK9 With In Vivo CRISPR-Cas9 Genome Editing

Qiurong Ding, Alanna Strong, Kevin M. Patel, Sze-Ling Ng, Bridget S. Gosis, Stephanie N. Regan, Chad A. Cowan, Daniel J. Rader, Kiran Musunuru

Ding et al. *Circ Res* 2014; 115:488-92

Targeting mouse *Pcsk9* with somatic genome editing



Ding et al. *Circ Res* 2014; 115:488-92

In vivo genome editing for therapy

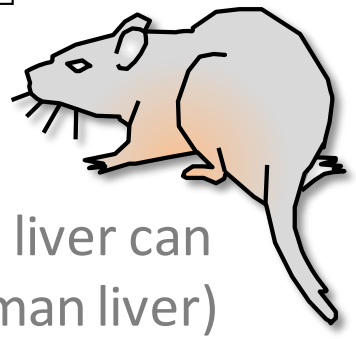
Traditional therapies	Genome-editing therapy
• Repeated dosing	• One-time therapy
• Short-term effect	• Permanent effect

Big concern is safety – what is the extent of off-target mutagenesis elsewhere in the genome? Risk of cancer?

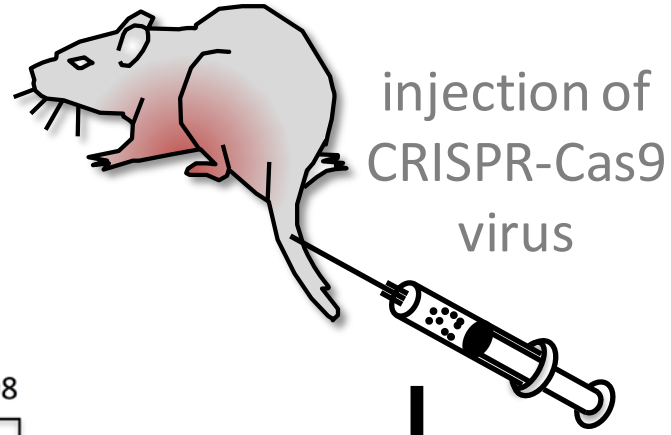
Targeting human *PCSK9* in liver-humanized mice

"Humanized" mouse model

transplantation of primary human hepatocytes

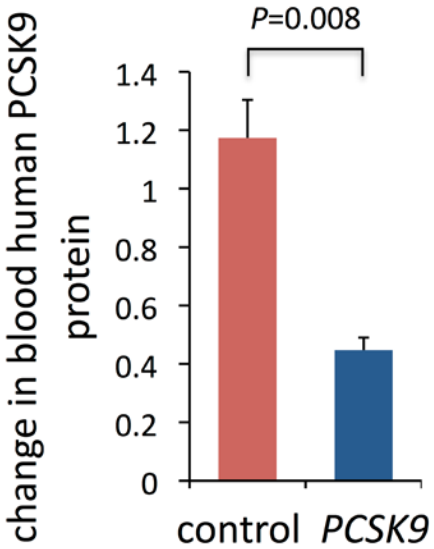


FRG KO mice (mouse liver can be replaced with human liver)



injection of CRISPR-Cas9 virus

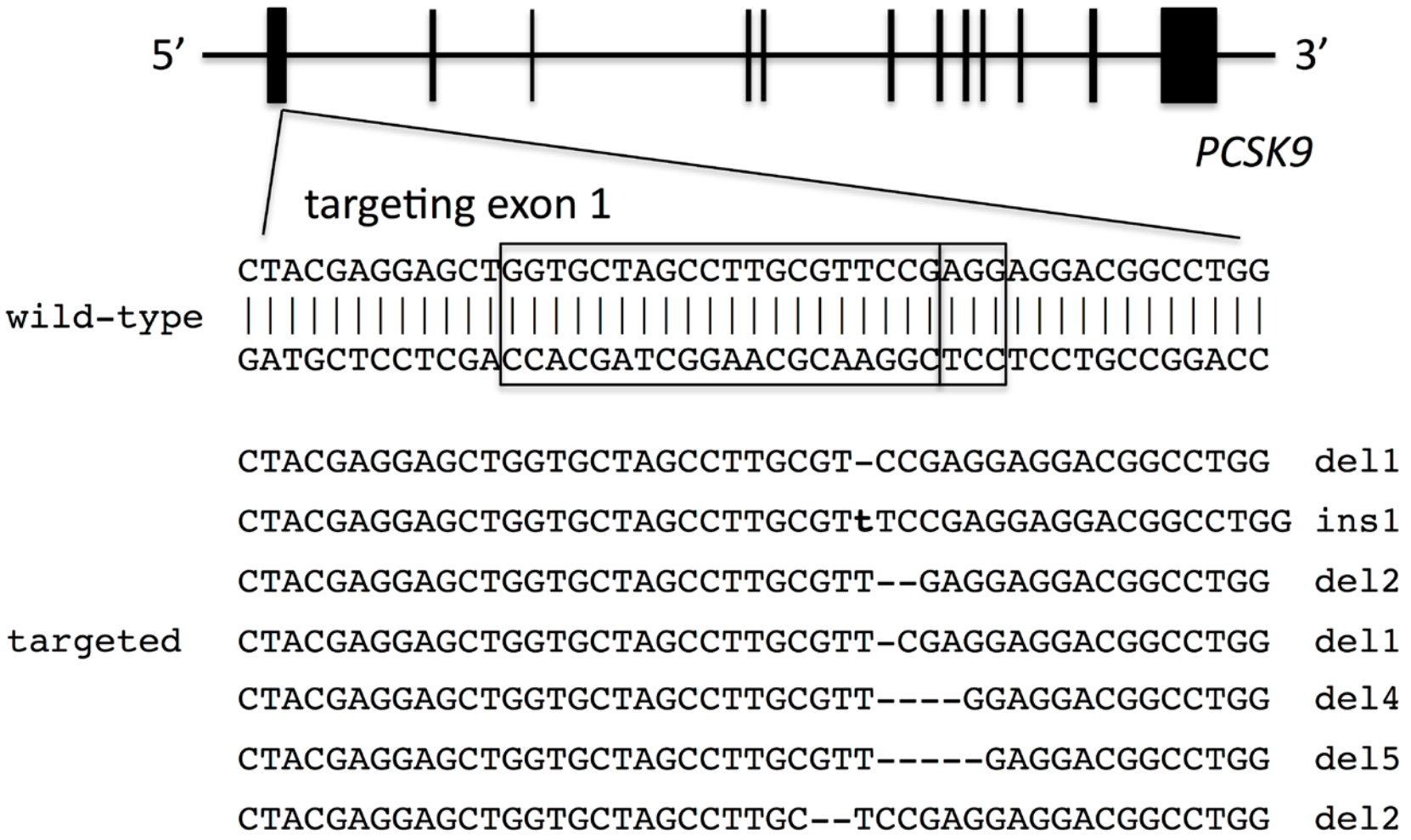
can gauge efficacy and safety in authentic human cells, with human genomes, in a living animal



changes in **human** characteristics

Wang et al., *Arterioscler Thromb Vasc Biol* 2016; 36:783-6

Targeting human *PCSK9* – on-target mutagenesis



Wang et al., *Arterioscler Thromb Vasc Biol* 2016; 36:783-6

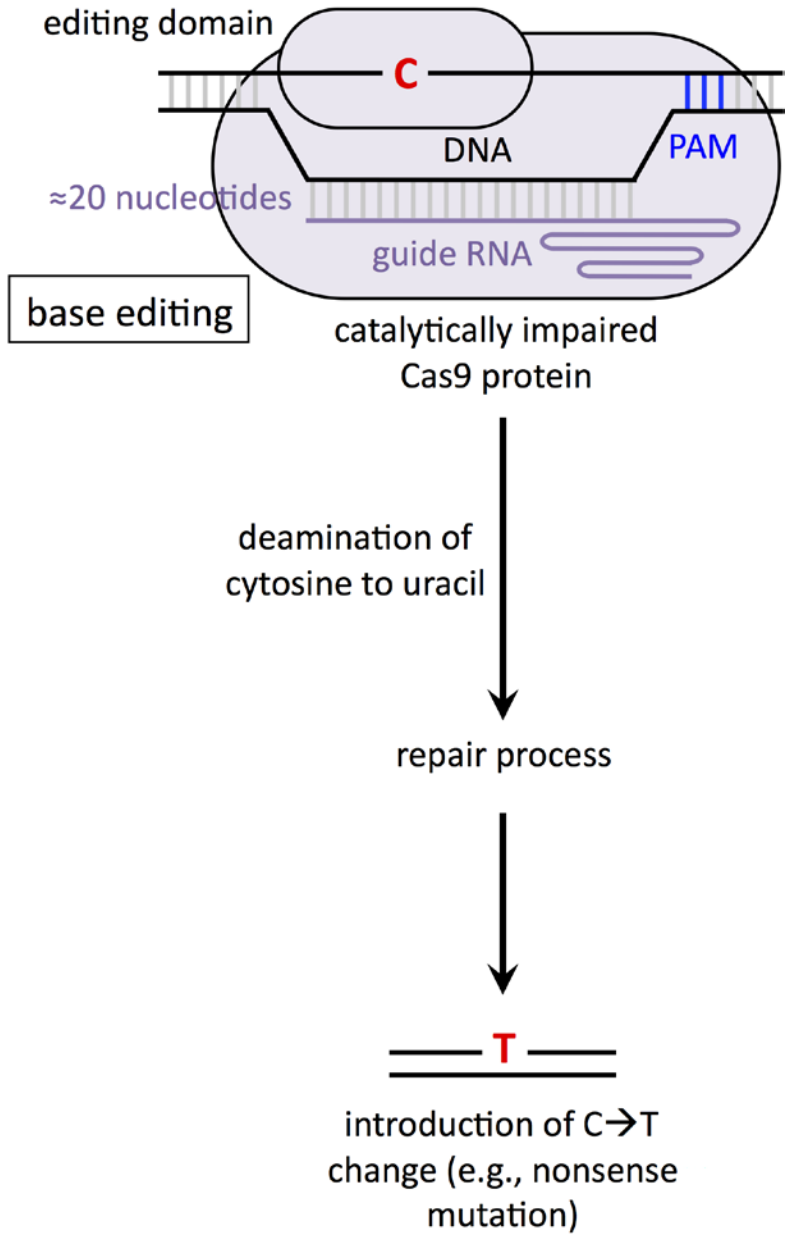
Targeting human *PCSK9* – off-target mutagenesis

site	location	sequence	CRISPR-*PCSK9* (%indels)	control (%indels)
PCSK9	chr1	GGTGCTAGCCTTGCGTTCCGAGG	47.4%, 41.7%	
OT1	chr17	AGTGCTGCCCGTGCGTTCCGAGG	0.01%, 0.01%	0.01%
OT2	chr13	AGGGCTAGCCTGGCGTTCCCCAG	0.07%, 0.04%	0.07%
OT3	chr15	GTTGCTGGCATTGCCTTCCGCAG	0.02%, 0.01%	0.01%
OT4	chr10	GCTGCAAGCTTTGCTTTCCGAAG	0.02%, 0.03%	0.02%
OT5	chr8	GAGGCTAACCTTGAGTTCCGAGG	0.06%, 0.01%	0.01%
OT6	chr12	AGGGCTAGCCTCGCATTCCGGAG	0.02%, 0.02%	0.02%
OT7	chr13	ATTGCTAGCCTTGCTTTCCAGAG	0.01%, 0.02%	0.01%
OT8	chr5	GGTGC-AGCCTTGCTTTCCGAGG	0.03%, 0.03%	0.03%

No evidence of off-target (OT) mutagenesis

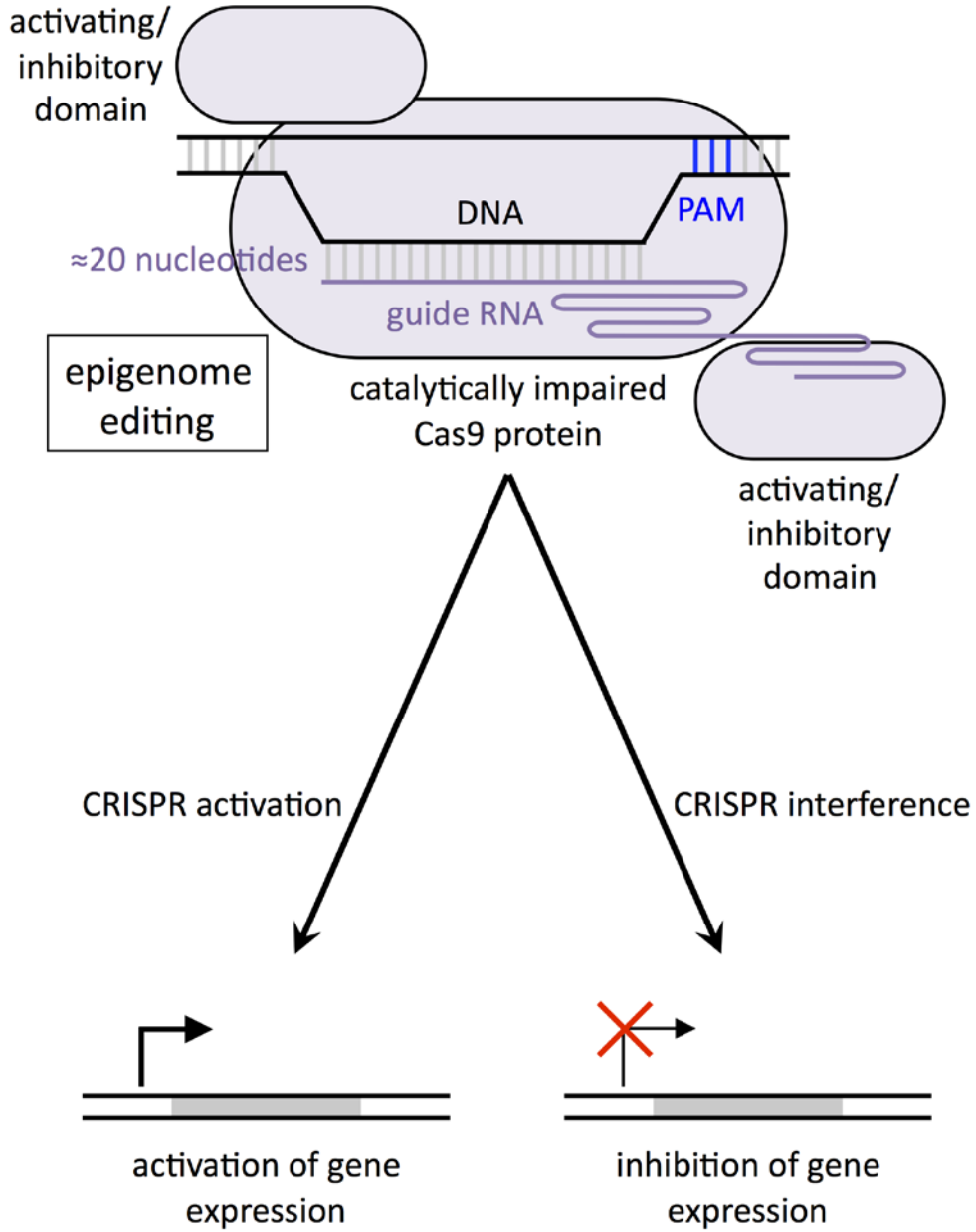
Wang et al., *Arterioscler Thromb Vasc Biol* 2016; 36:783-6

Base editing and epigenome editing

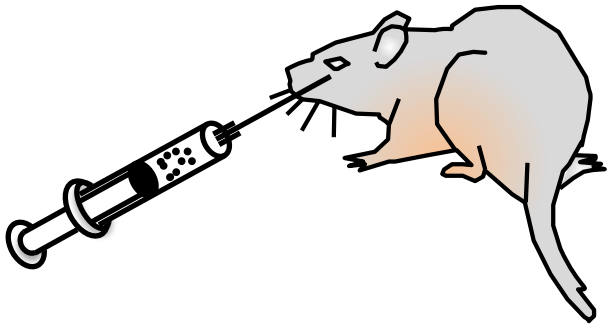


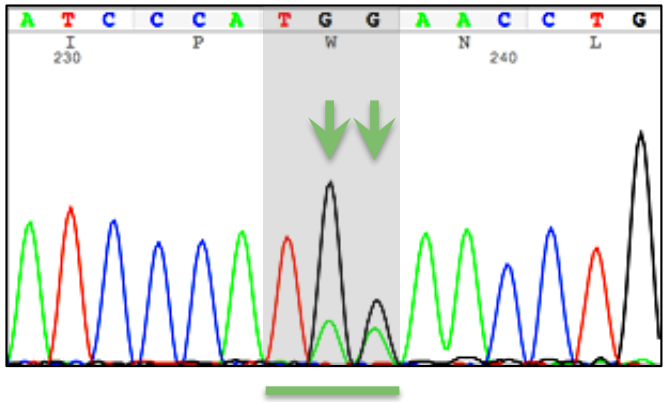
Safer?

Reversible?

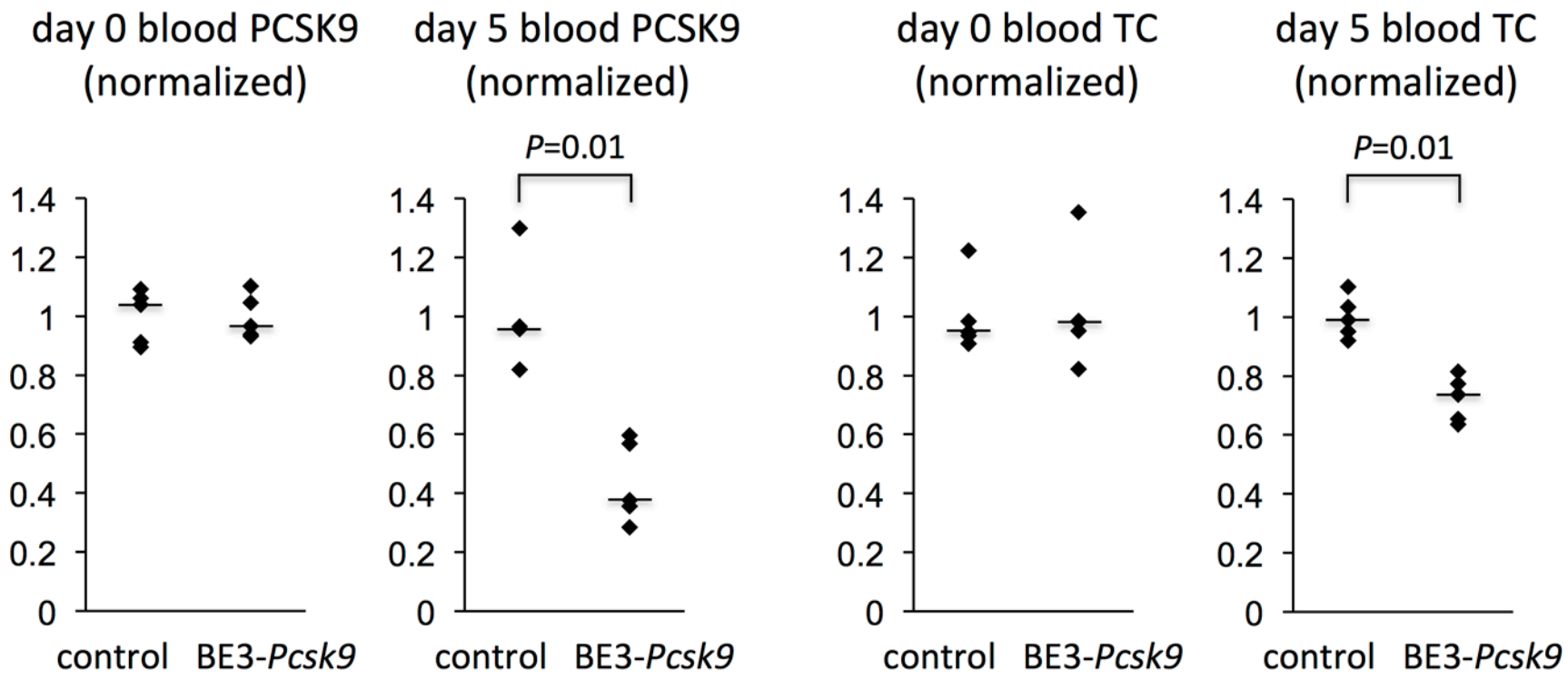


In vivo base editing of murine *Pcsk9*





Pcsk9 W159X
(T**GG**→T**AA**)
nonsense mutation

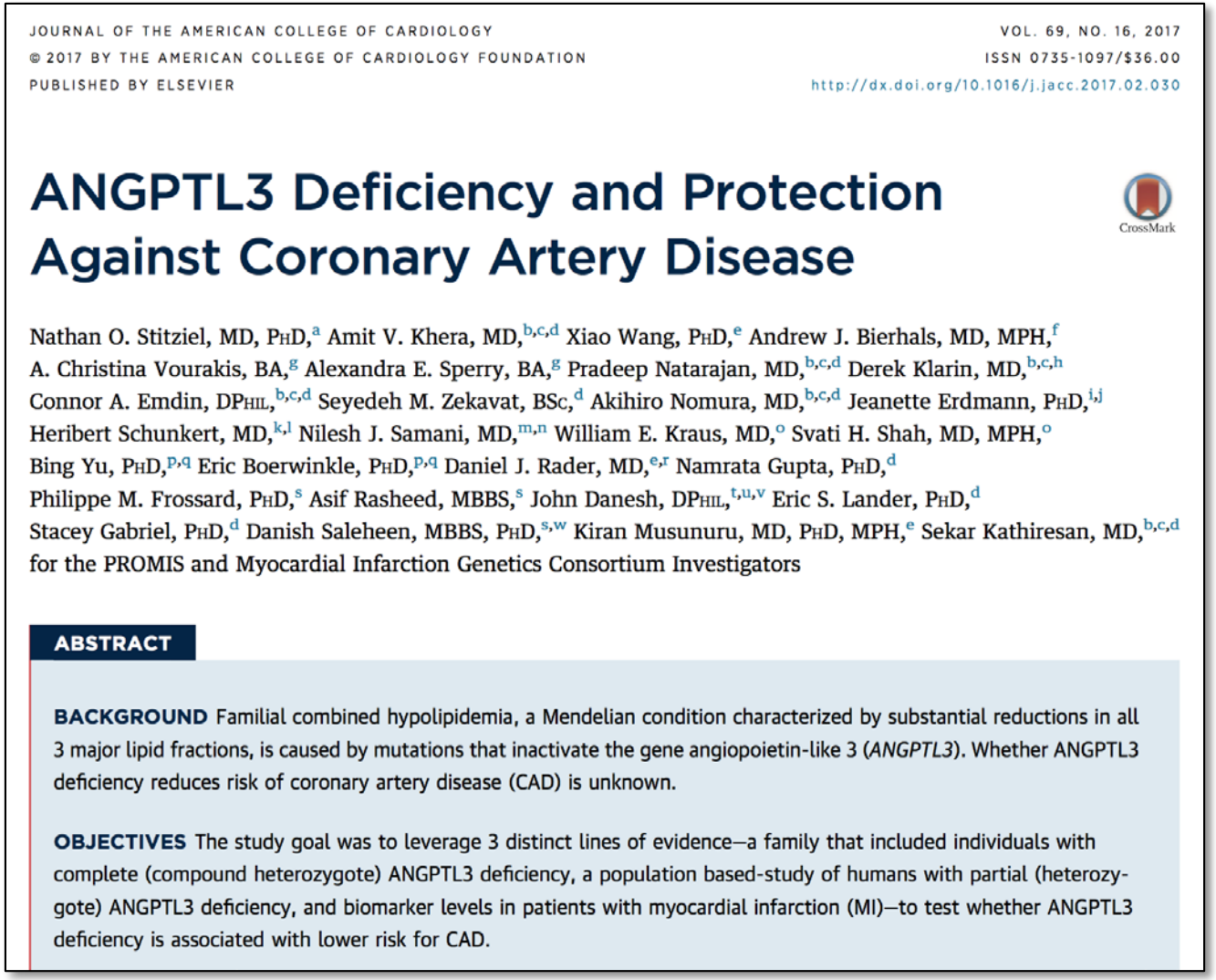


~60% reduction
of blood PCSK9

~30% reduction
of cholesterol

Chadwick et al., *Arterioscler Thromb Vasc Biol* 2017; 37:1741-7

ANGPTL3 as a therapeutic target is similar to *PCSK9*



JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2017 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER

VOL. 69, NO. 16, 2017
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2017.02.030

ANGPTL3 Deficiency and Protection Against Coronary Artery Disease

Nathan O. Stitziel, MD, PhD,[a] Amit V. Khera, MD,[b,c,d] Xiao Wang, PhD,[e] Andrew J. Bierhals, MD, MPH,[f] A. Christina Vourakis, BA,[g] Alexandra E. Sperry, BA,[g] Pradeep Natarajan, MD,[b,c,d] Derek Klarin, MD,[b,c,h] Connor A. Emdin, DPhil,[b,c,d] Seyedeh M. Zekavat, BSc,[d] Akihiro Nomura, MD,[b,c,d] Jeanette Erdmann, PhD,[i,j] Heribert Schunkert, MD,[k,l] Nilesh J. Samani, MD,[m,n] William E. Kraus, MD,[o] Svati H. Shah, MD, MPH,[o] Bing Yu, PhD,[p,q] Eric Boerwinkle, PhD,[p,q] Daniel J. Rader, MD,[e,r] Namrata Gupta, PhD,[d] Philippe M. Frossard, PhD,[s] Asif Rasheed, MBBS,[s] John Danesh, DPhil,[t,u,v] Eric S. Lander, PhD,[d] Stacey Gabriel, PhD,[d] Danish Saleheen, MBBS, PhD,[s,w] Kiran Musunuru, MD, PhD, MPH,[e] Sekar Kathiresan, MD,[b,c,d] for the PROMIS and Myocardial Infarction Genetics Consortium Investigators

ABSTRACT

BACKGROUND Familial combined hypolipidemia, a Mendelian condition characterized by substantial reductions in all 3 major lipid fractions, is caused by mutations that inactivate the gene angiopoietin-like 3 (*ANGPTL3*). Whether ANGPTL3 deficiency reduces risk of coronary artery disease (CAD) is unknown.

OBJECTIVES The study goal was to leverage 3 distinct lines of evidence—a family that included individuals with complete (compound heterozygote) ANGPTL3 deficiency, a population based-study of humans with partial (heterozygote) ANGPTL3 deficiency, and biomarker levels in patients with myocardial infarction (MI)—to test whether ANGPTL3 deficiency is associated with lower risk for CAD.

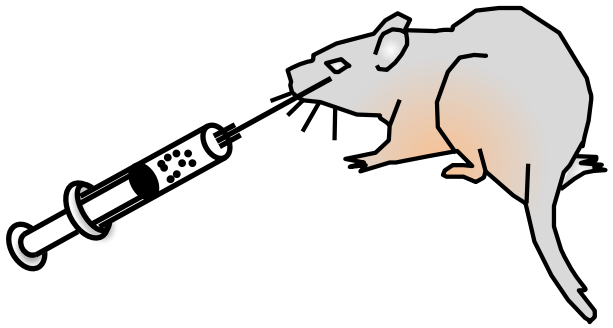
Individuals with one loss-of-function mutation in *ANGPLT3*:

LDL-C, TG ↓ 15-30%
CHD risk ↓ 35-40%

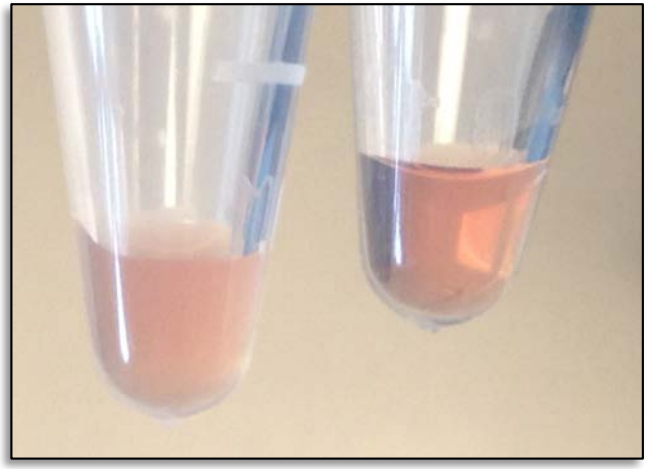
Individuals with two loss-of-function mutation in *ANGPLT3*: totally healthy

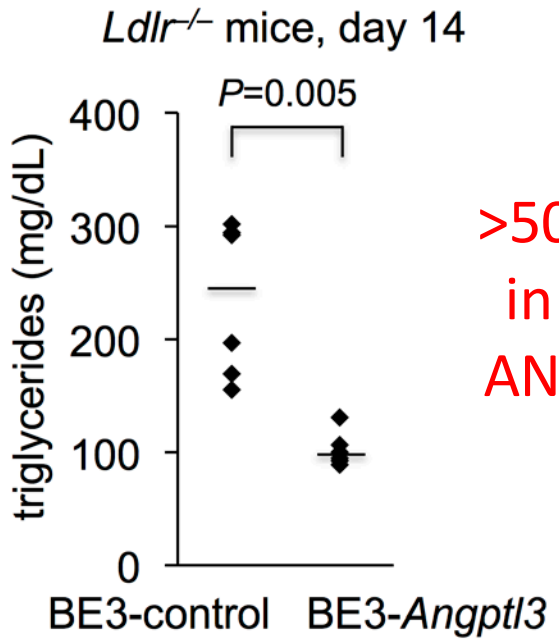
Musunuru et al. *N Engl J Med* 2010; 363:2220-7
Stitziel et al. *J Am Coll Cardiol* 2017; 69:2054-63
Dewey et al. *N Engl J Med* 2017; 377:211-21

Base editing of *Angptl3* in mouse model of familial hypercholesterolemia (FH)

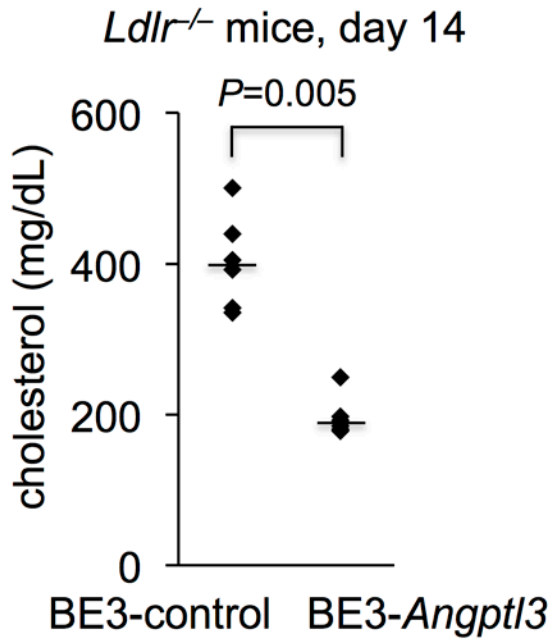


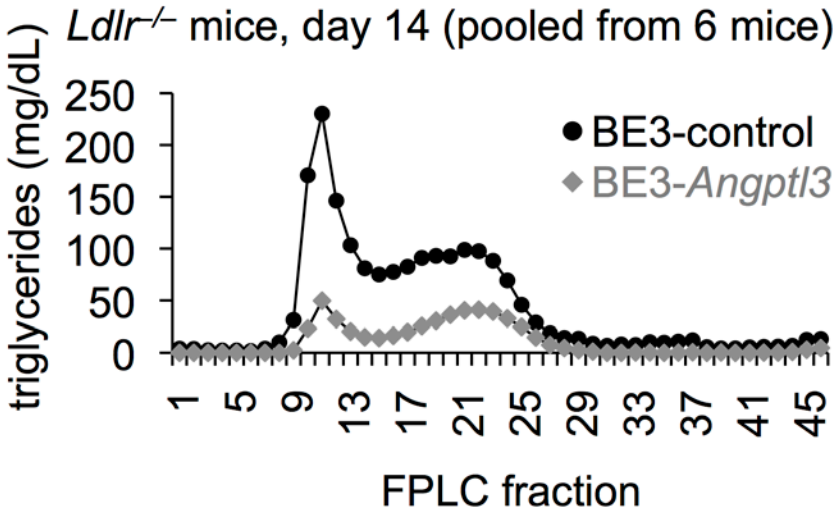
BE3 targeting *Angptl3* in the mouse liver using virus:
Q135X (CAA→TAA)

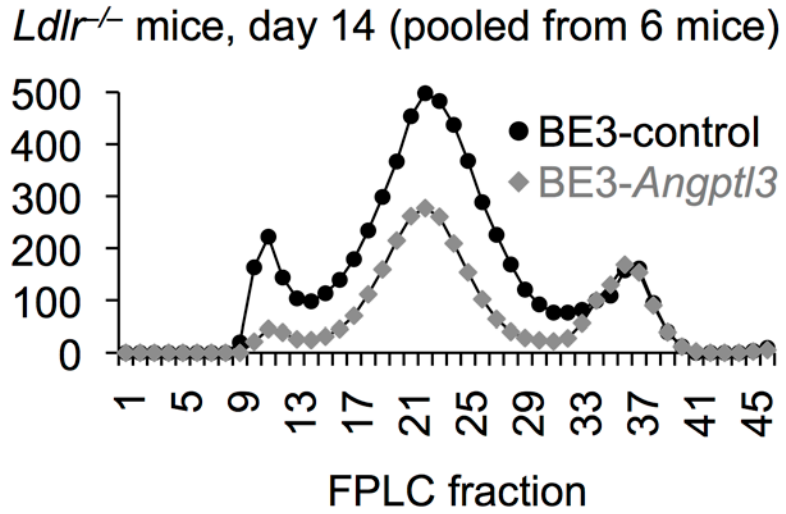




>50% reductions in triglycerides AND cholesterol







PCSK9/*ANGPTL3* and coronary heart disease (CHD)

- Degree of CHD risk reduction probably depends on length of protection (few years vs. lifelong)
- Who to treat?
  - adult with strong risk factor profile?
  - all adults?
  - child with strong family history or FH?
  - *in utero* with strong family history or FH?
  - in embryo with strong family history or FH (with implications for future generations)?

Generating altered human embryos with CRISPR-Cas9

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.

Ma et al., *Nature* 2017; 548:413-9

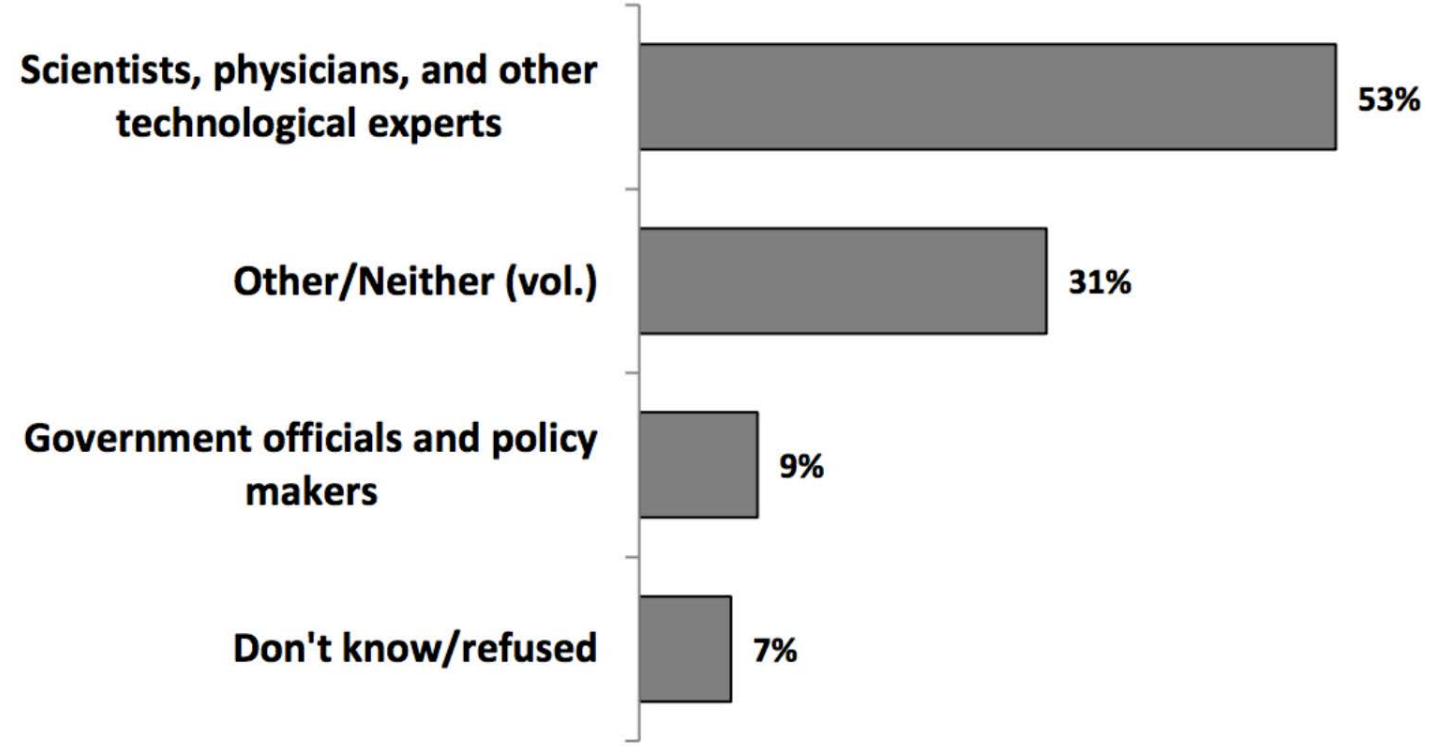
Potential clinical uses of germline genome editing

- Treating/pre-empting severe genetic disorders
- Addressing genetic causes of infertility (e.g., block in gamete development)
- Reducing risk of common/complex diseases
- “Enhancement”

Who decides?

FIGURE 3: Who Should Decide Whether or Not to Allow Changing the Genes of Unborn Babies?

For decisions on whether or not to allow changing the genes of unborn babies to improve their healthy, physical traits, or intelligence, do you think we should leave it up to...



https://www.statnews.com/2016/02/11/stat-harvard-poll-gene-editing/

A poll of 300 scientists and physicians at AHA meeting

Perspective

What Do We Really Think About Human Germline Genome Editing, and What Does It Mean for Medicine?

Kiran Musunuru, MD, PhD, MPH; William R. Lagor, PhD; Joseph M. Miano, PhD

Genome editing has captured widespread attention because of its potential therapeutic applications. Early studies with human embryos have established the feasibility of human germline genome editing but raise complex social, ethical, and legal questions. In light of the potential impact of genome editing on the practice of cardiovascular medicine, we surveyed ≈300 attendees at a recent American Heart Association conference to elicit their opinions on somatic and germline genome editing. The results were revealing and highlight the need to broadly engage the public and solicit the opinions of various constituencies before proceeding with clinical germline genome editing.

(mutations present in some cells in the embryo but not in other cells) was largely eliminated. Furthermore, the relevance to cardiovascular medicine was unambiguous because the sperm used for the study originated from a man with severe hypertrophic cardiomyopathy, with the corrected mutation being in the cardiac myosin-binding protein C (*MYBPC3*) gene. In light of this study, it is clear that human GGE is now feasible and might be achieved for some genes without off-target effects. In addition to remaining safety concerns, the path to clinical use will now have to contend with important social, ethical, and legal issues. The future is on us, whether we like it or not.

Recognizing the relevance and potential impact of these issues on the future practice of cardiovascular medicine,

Musunuru et al., *Circ Cardiovasc Genet* 2017; 10:e001910

Survey results

If you had the opportunity to receive a one-shot somatic genome-editing therapy that would permanently reduce your risk of CHD, would you do so (assuming the therapy is 100% safe)?

- Yes 69%
- No 19%
- Don’t know 12%

Survey results

Do you think it would be acceptable for parents to use human germline genome editing to have a healthy biological child when there is no other means to do so?

- Yes 68%
- No 21%
- Don’t know 11%

Survey results

Do you think it would be acceptable for parents to use human germline genome editing to reduce the risk of their child having a serious medical condition (e.g., premature CHD or Alzheimer disease)?

- Yes 45%
- No 40%
- Don’t know 15%

Survey results

Do you think it would be acceptable for parents to use human germline genome editing to increase the odds of their child having a desired trait (e.g., athletic ability)?

- Yes 2%
- No 95%
- Don’t know 3%

AMERICAN
COLLEGE *of*
CARDIOLOGY