Technical Challenges to Achieving Safe and Effective Human Germline/Heritable based on Data from Somatic Cell Editing

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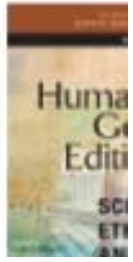
Strict Criteria Proposed by 2017 Report

Criteria for heritable germline editing

The committee recommends that clinical trials using heritable genome editing should be permitted only within a robust and effective regulatory framework that encompasses:

- **Absence of resonable alternatives**
- **Restriction to preventing a serious disease or condition**
- **Restriction to editing genes that have been convincingly demonstrated to cause or to strongly predispose to the disease or condition**
- **Restriction to converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects**
- **Availability of credible pre-clinical and/or clinical data on risks and potential health benefits of the procedures**
- **Ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants**
- **Comprehensive plans for long-term, multigenerational follow-up while still respecting personal autonomy**
- **Maximum transparency consistent with patient privacy**
- **Continued reassessment of both health and societal benefits and risks, with broad on-going participation and input by the public**
- **Reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition**



Proposed Technical Criteria for Heritable Genome Editing

- **Absence of alternatives (e.g IVF with PGD)**
  - ***Since efficiency is poor, need research to make it more efficient***
  - ***Using editing to increase the potential number of implantable embryos is not a compelling rationale***
    1. ***Additional manipulation of embryo likely will make it less likely to successfully generate a viable pregnancy***
    2. ***Expose at least 50% of embryos to un-necessary engineering/manipulation***
  - ***State supported, a la the UK health system, with a defined list of diseases***
    1. ***Prevent suffering***
    2. ***Side effect of being economically cost effective***

- **Restriction to serious disease or condition and to genes with clearly proven causality**

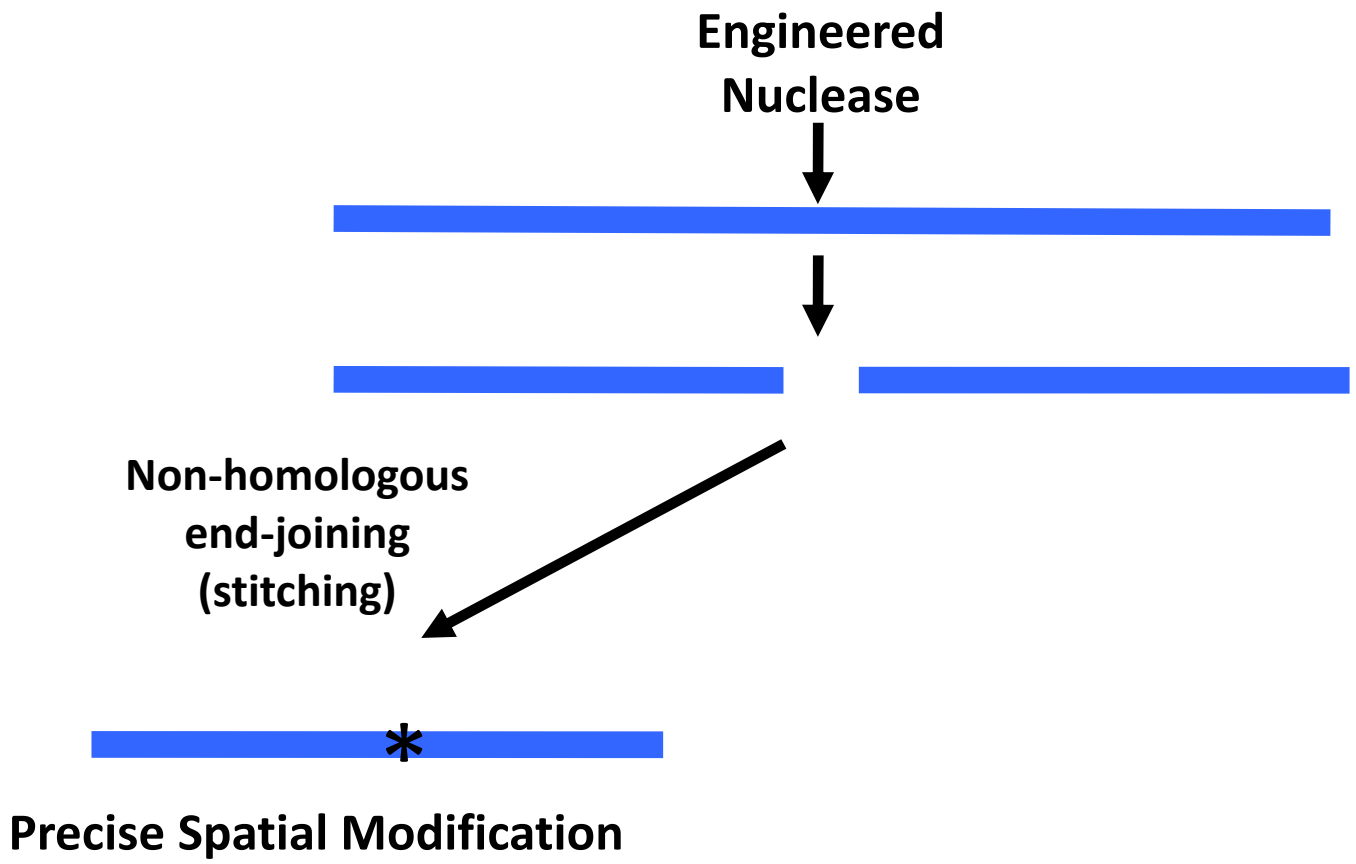
- **Restriction to editing *to* sequences that are common and known to be healthy in the human population**
  - ***Revert pathologic sequences to normal sequences***
  - ***Don't modify healthy sequences to bespoke sequences (humility)***
  - ***Just because there are a few people in the world who have a certain gene sequence, does NOT mean that it would be broadly healthy (the problem of what is missing)***

- **Reliable oversight mechanism to prevent extension to uses other than treating serious diseases *(not needed if bullet point 3 is adopted)***

Issues Related to these Technical Criteria

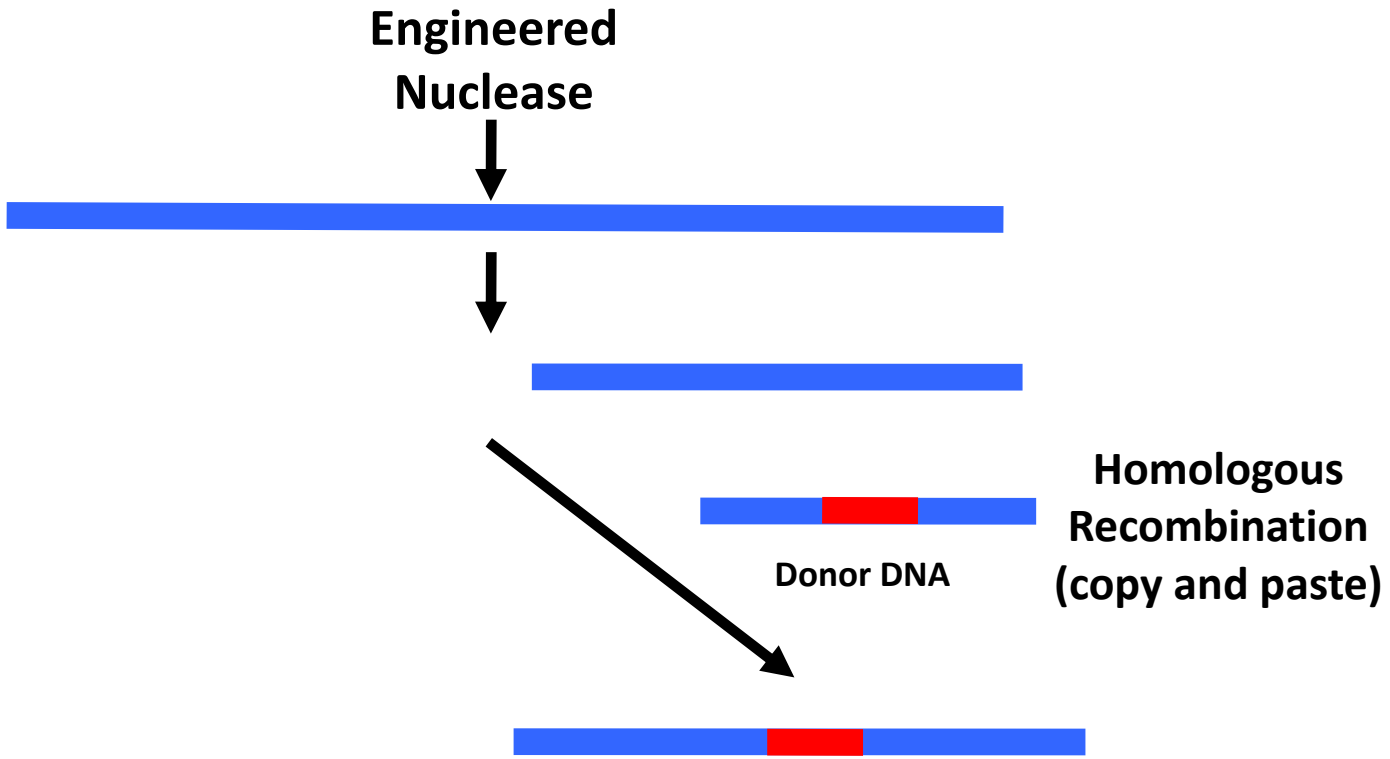
1. Specificity
2. Chimerism
3. Generating only common and healthy sequences
4. Potential solutions by not editing zygotes

Heritable NHEJ based editing should be prohibited because it generates sequences that are neither common nor known to be healthy in the human population



Method to Break Things

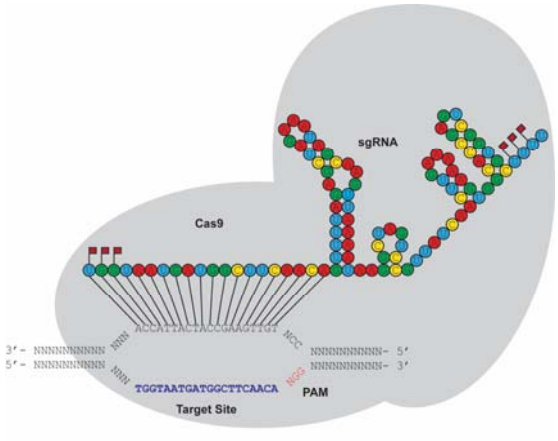
Only HDR/HR Based Editing Can Reliably Generate Sequences that are Known to be Common and Healthy in the Human Population

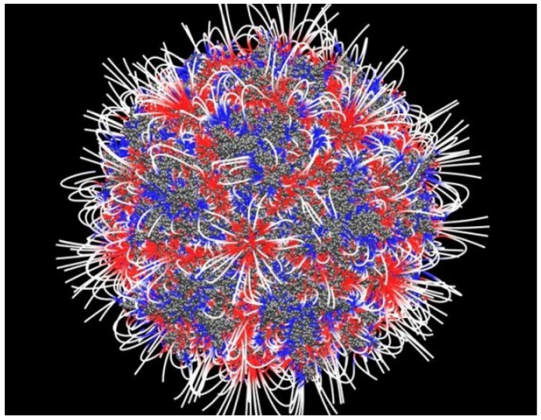


Precise Spatial AND Nucleotide Modification of Genome

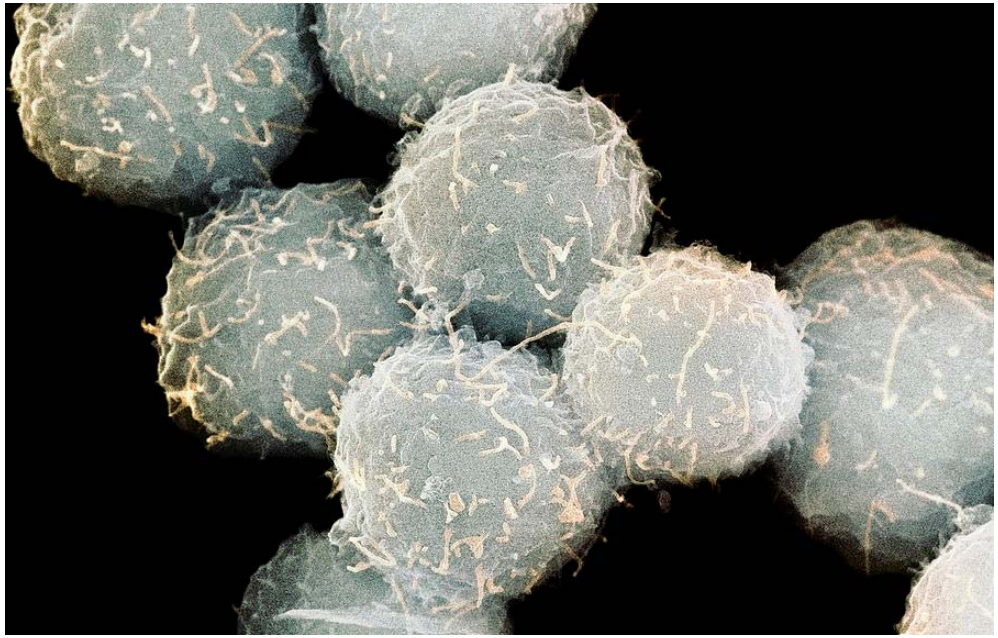
Method to Make Single Nucleotide Changes

Gene Correction using Cas9/gRNA RNP and a non-integrating virus (AAV6)





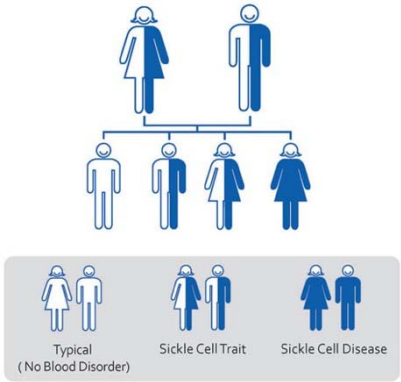
Recombinant Adeno-Associated Virus (AAV6) (non-integrating)



Bak, Dever et al Nature Protocols (2018)

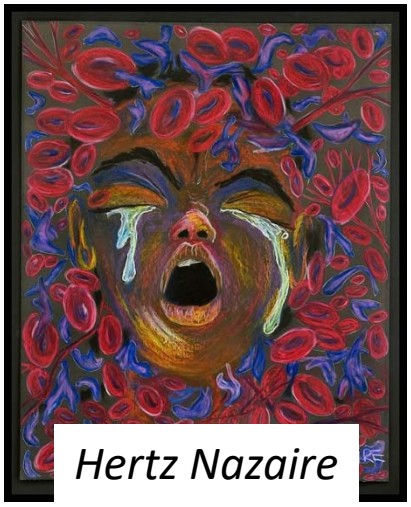
Sickle Cell Disease is Caused by a Single Nucleotide Variant in the *HBB* Gene

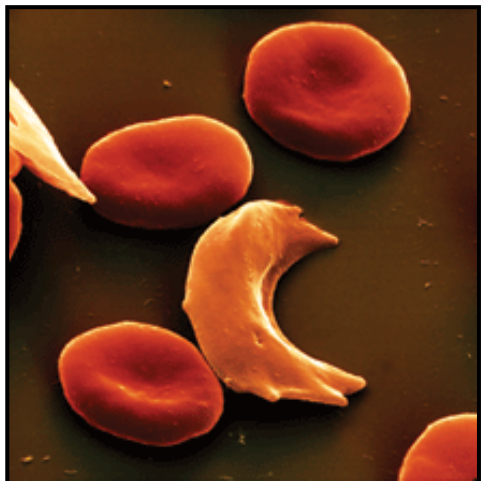
	Hgb A	Hgb S Sickle (E6V)
Partial DNA Sequence of Beta Globin Gene:	CCT GAG GAG / GGA CTC CTC	CCT GTG GAG / GGA CAC CTC
Partial RNA Sequence:	CCU GAG GAG	CCU GUG GAG
Partial Amino Acid Sequence for Beta Globin:	Pro — Glu — Glu	Pro — Val — Glu



Hemoglobin Molecule:

Red Blood Cell:



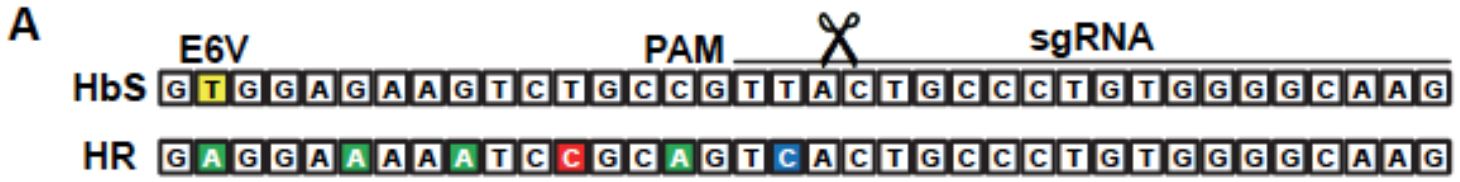


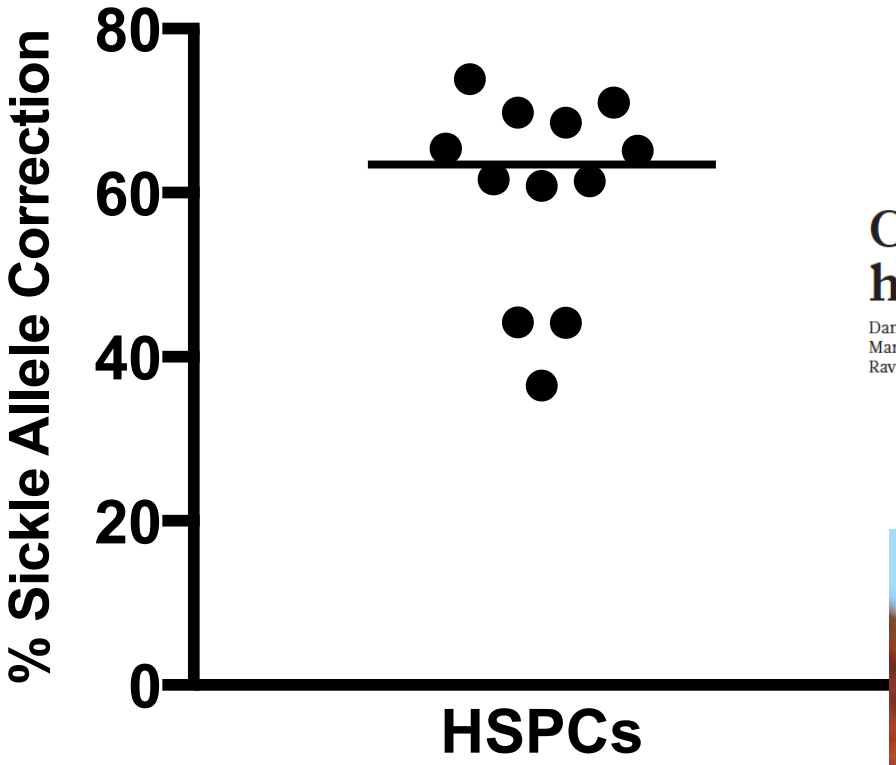
Median Lifespan

United States: mid-40s (though taking medicine for pain >3 times/week) (neurocognitive damage starts occurring in first years of life)

Africa: 5-8 years old

Editing Sickle Variant in CD34+ Somatic Hematopoietic Stem and Progenitor from Six Sickle Cell Disease Patients



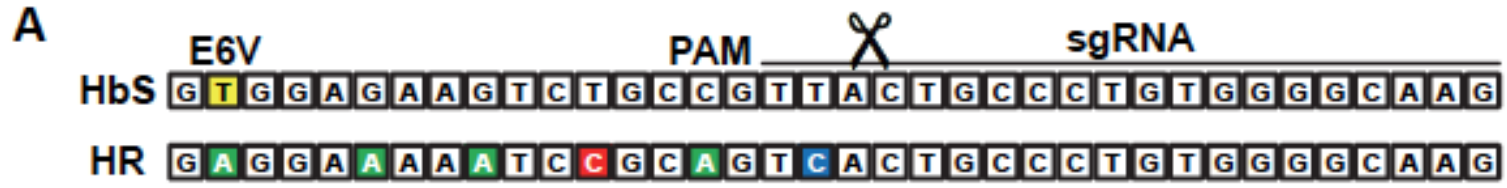


CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells

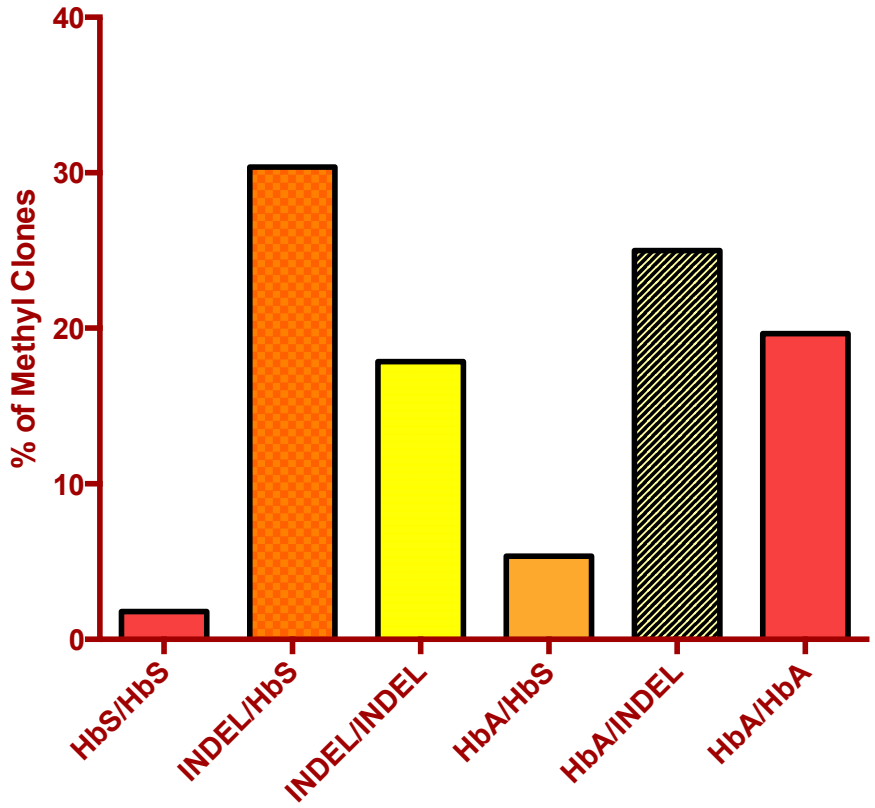
Daniel P. Dever[1]*, Rasmus O. Bak[1]*, Andreas Reinisch[2], Joab Camarena[1], Gabriel Washington[1], Carmencita E. Nicolas[1], Mara Pavel-Dinu[1], Nivi Saxena[1], Alec B. Wilkens[1], Sruthi Mantri[1], Nobuko Uchida[3]†, Ayal Hendel[1], Anupama Narla[4], Ravindra Majeti[2], Kenneth I. Weinberg[1] & Matthew H. Porteus[1]

Nature 2016

1. Silent changes are not present in human population
2. Even so, 70% of cells have at least one allele with a non-natural sequence (INDEL)
3. Highly chimeric population



S/S →



What about specificity?

Qualitative Biochemical Understanding of Nuclease Specificity

$$p(\text{Editing}) \approx c_1[\text{conc}] * c_2(\text{Time}) * c_3(\text{Kd}) \text{ (on/off)} * c_4(\text{Kcat}) * c_5(p(\text{repair fidelity}))$$

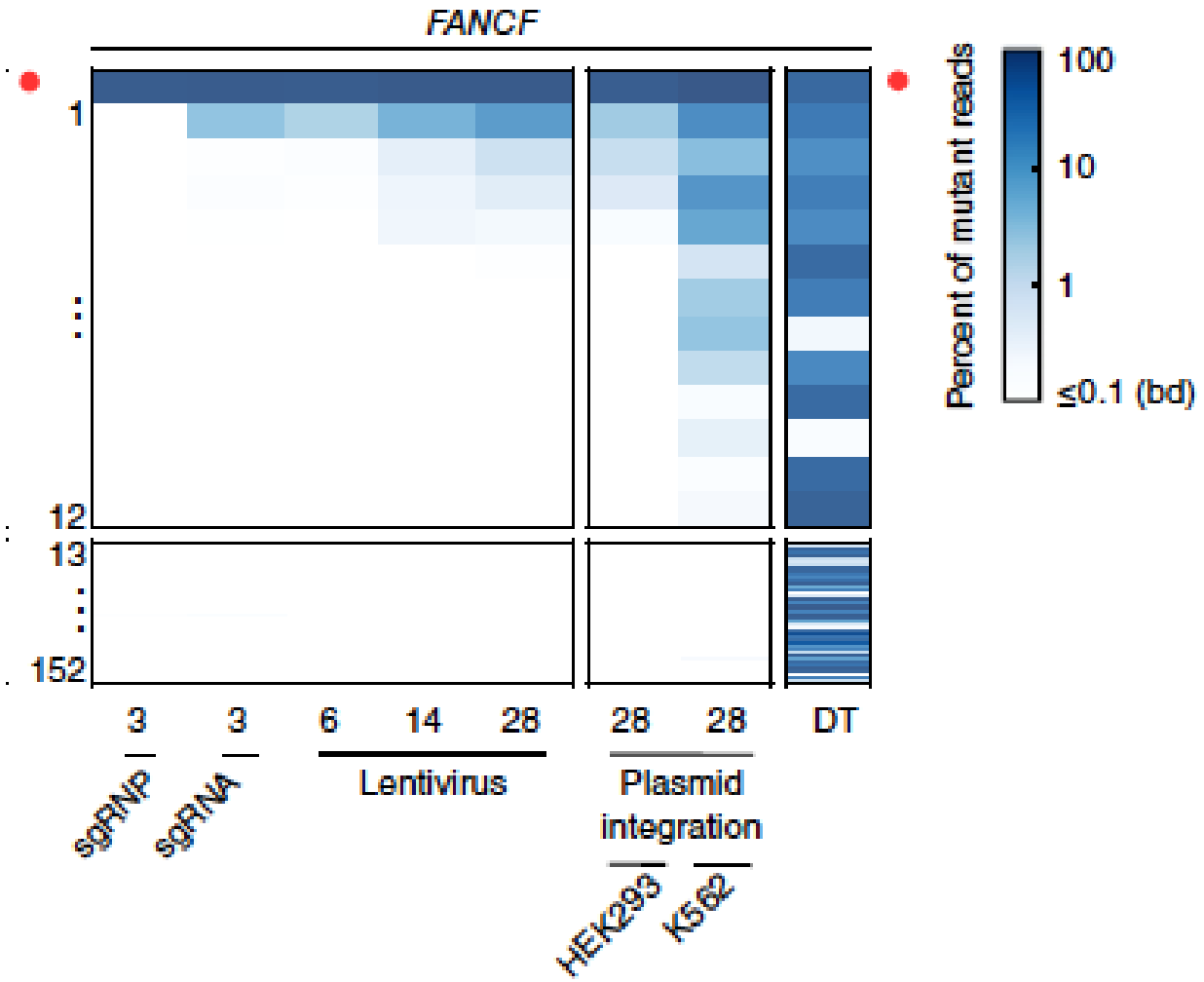
$$\text{Kd} \approx (\text{guideRNA binding energy})(\text{Cas9-DNA binding energy})$$

Improvement in specificity by using RNP Delivery

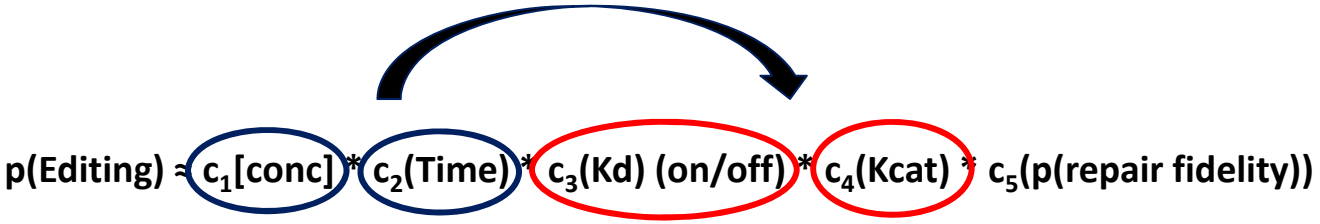
Mapping the genomic landscape of CRISPR–Cas9 cleavage

Peter Cameron[1,4], Chris K Fuller[1,4], Paul D Donohoue[1], Brittnee N Jones[1,3], Matthew S Thompson[1], Matthew M Carter[1], Scott Gradia[1], Bastien Vidal[1], Elizabeth Garner[1], Euan M Slorach[1], Elaine Lau[1], Lynda M Banh[1], Alexandra M Lied[1], Leslie S Edwards[1], Alexander H Settle[1], Daniel Capurso[1], Victor Llaca[2], Stéphane Deschamps[2], Mark Cigan[2,3], Joshua K Young[2] & Andrew P May[1,3]

Nature Methods (2017)



Qualitative Biochemical Understanding of Nuclease Specificity



Kd ≈ (guideRNA binding energy)(Cas9-DNA binding energy)

ARTICLES
https://doi.org/10.1038/s41591-018-0137-0

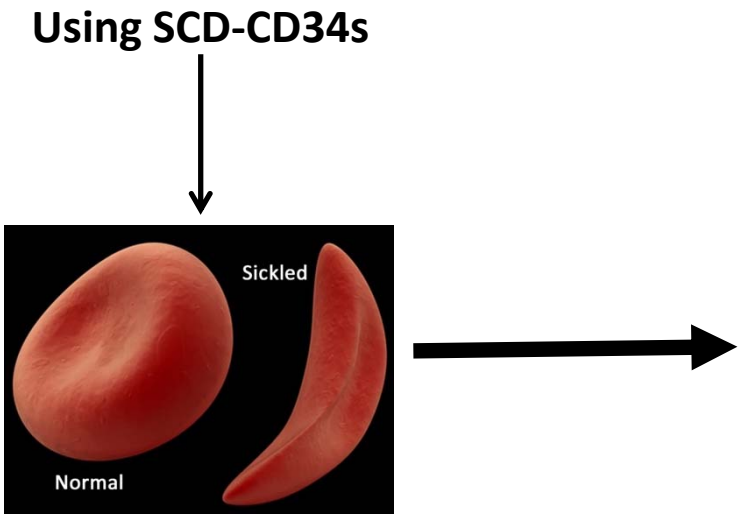
nature medicine

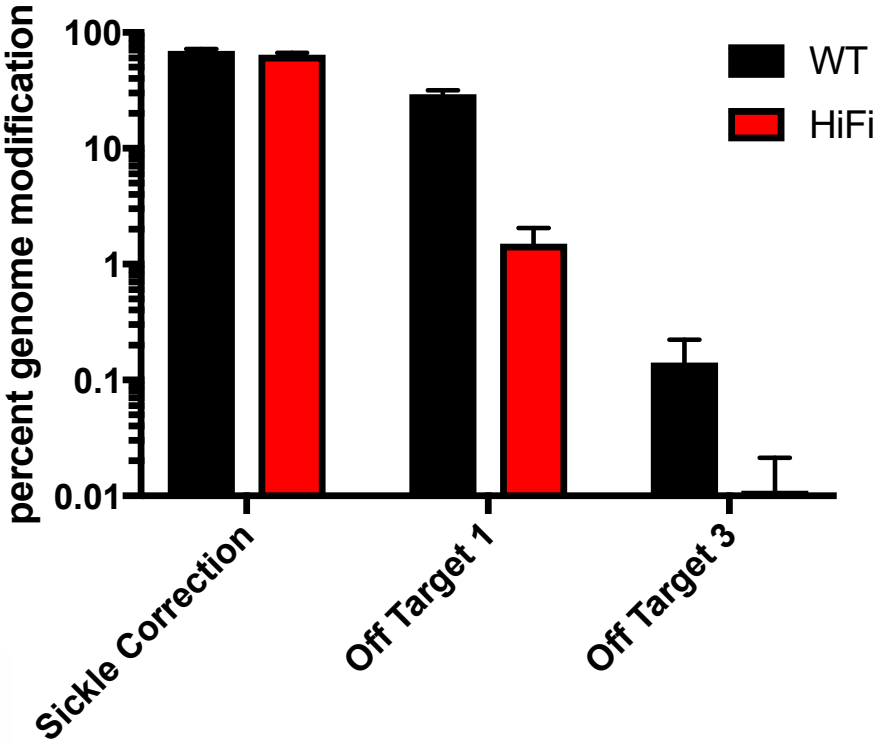
A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells

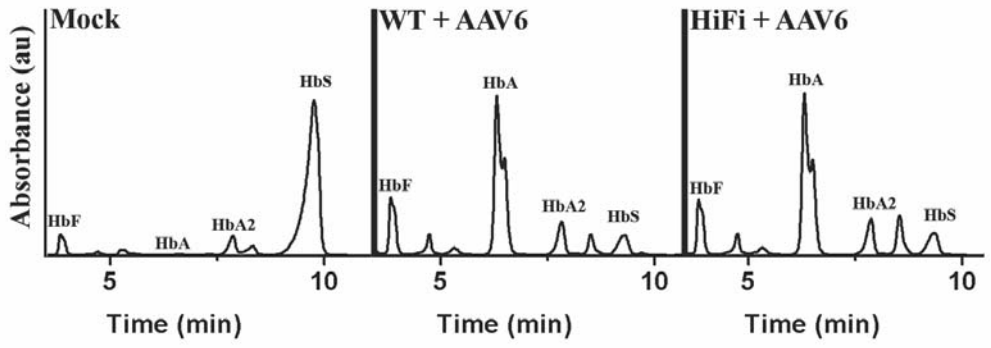
Christopher A. Vakulskas[1,7], Daniel P. Dever[2,7], Garrett R. Rettig[1], Rolf Turk[1], Ashley M. Jacobi[1], Michael A. Collingwood[1], Nicole M. Bode[1], Matthew S. McNeill[1], Shuqi Yan[1], Joab Camarena[2], Ciaran M. Lee[3], So Hyun Park[3], Volker Wiebking[2], Rasmus O. Bak[4,5], Natalia Gomez-Ospina[2], Mara Pavel-Dinu[2], Wenchao Sun[6], Gang Bao[3], Matthew H. Porteus[2]* and Mark A. Behlke[1]*

NATURE MEDICINE | VOL 24 | AUGUST 2018 | 1216–1224 | www.nature.com/naturemedicine

IDT HiFi SpCas9 (R691A) Mediates High Level Sickle Gene Correction while Reducing Off-Target INDELs by > 1-log in Sickle Cell Patient Derived CD34+ HSPCs







Vakulskas, Dever, Camarena, Lee, Bao, Behlke (Nature Medicine (2018))

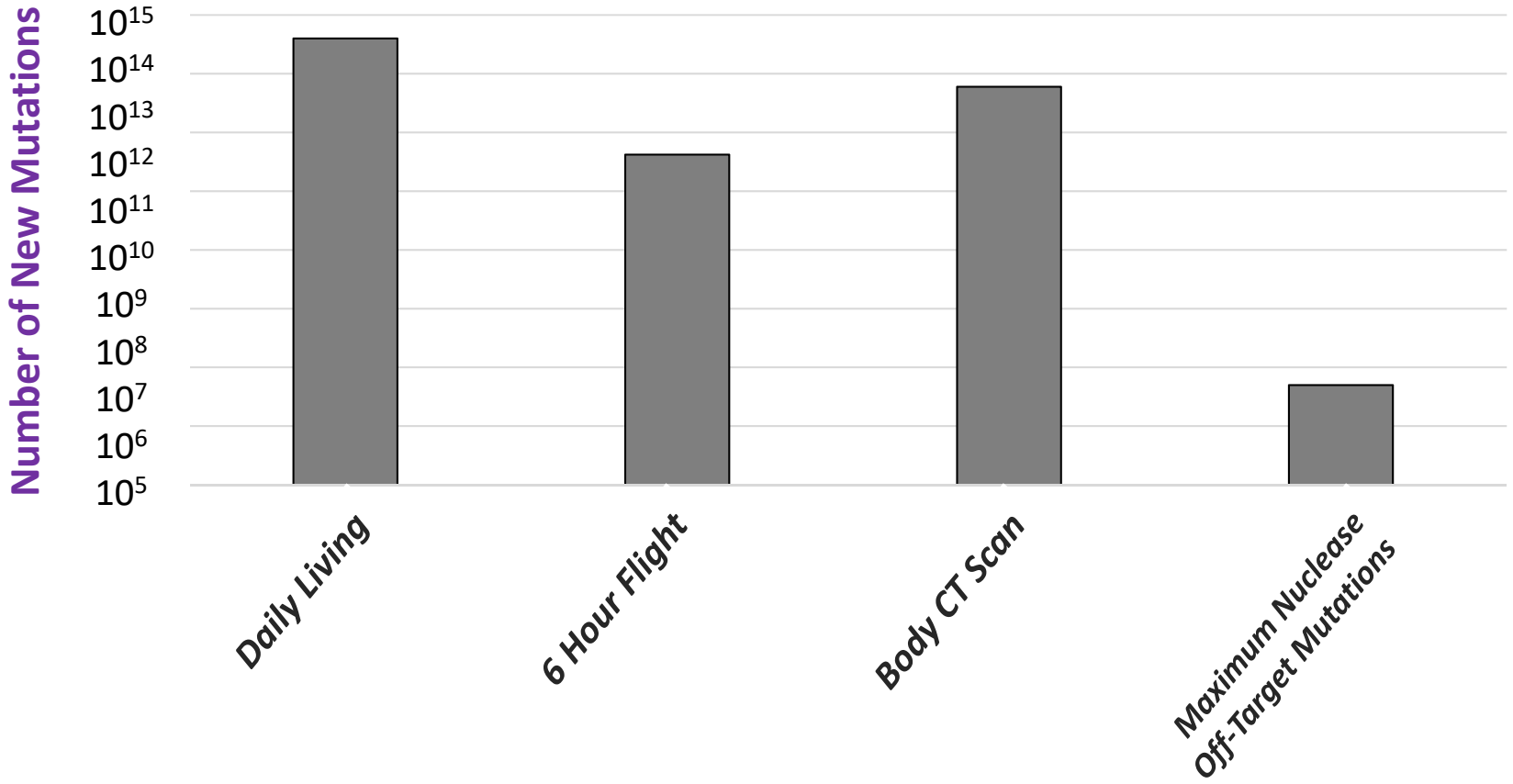
Specificity of RNP genome editing is more specific than cellular life in terms of new mutations generated (by >2000-4000 fold)

RNP Editing with HiFi Cas9: <1 mutation in 1% of cells

Life: 20-40 mutations in 100% of cells

Putting Potential Nuclease Toxicity in Context: Natural and Acquired Genetic Variation

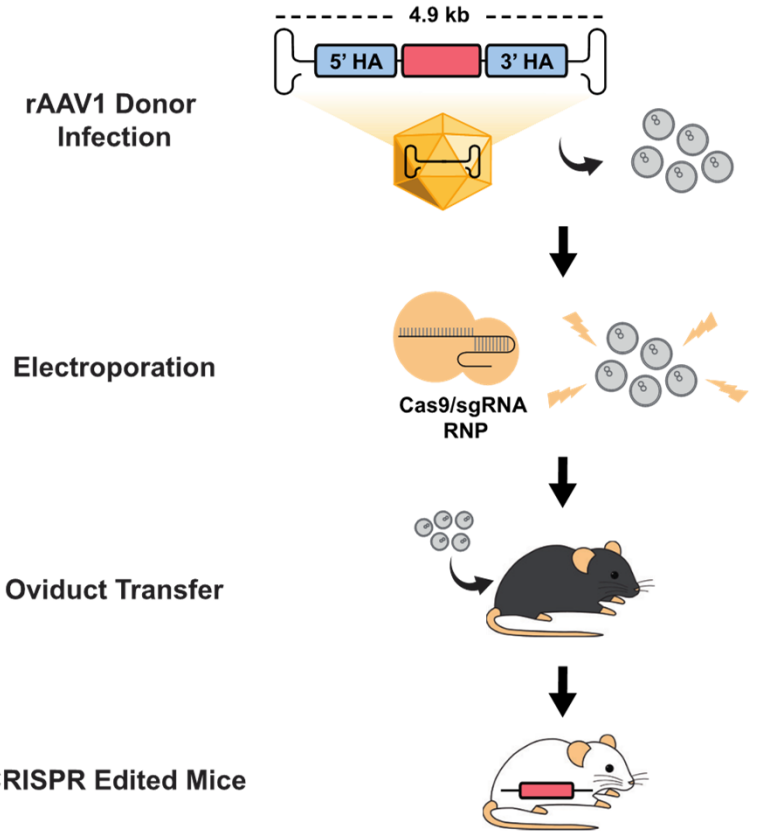
- **Tremendous genetic diversity among humans to begin with:**
  Baseline Variation Per Person: 2.4 million SNVs, 500-600K In/Dels (355 Exonic, 91 Frameshift), ~3000 structural variants (i.e. Dewey et al 2014 JAMA)
- **Tremendous ongoing genetic diversity within each person**
  ~20 new mutations per every cell division

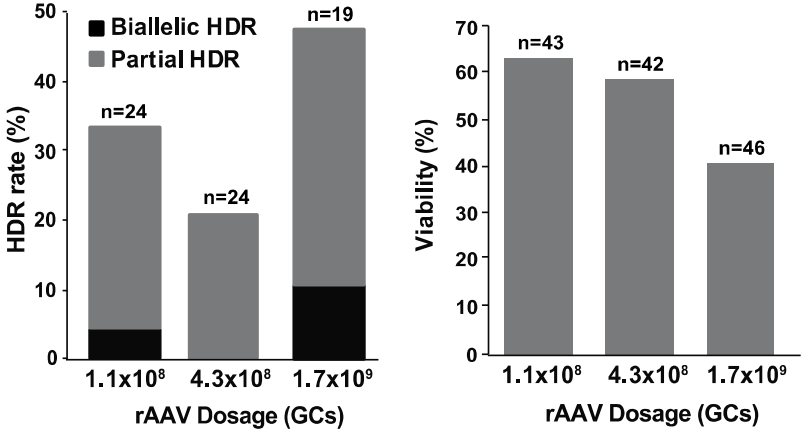


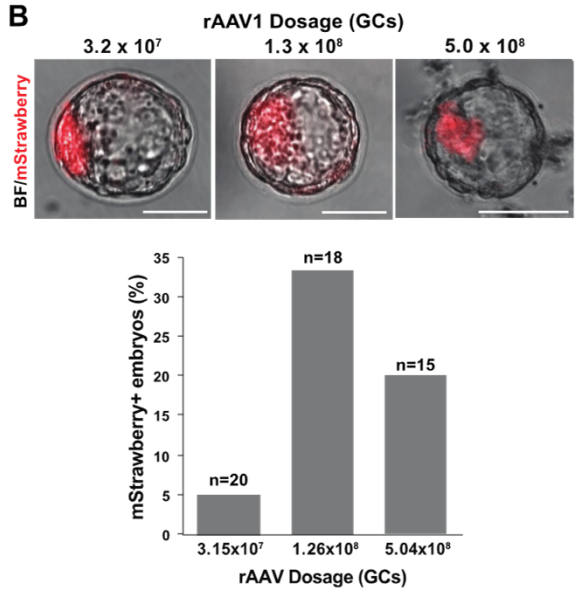
Assumes high fidelity of DSB Repair: 90% (9 out of 10 breaks are repaired precisely)

RNP/AAV6 System is Highly Efficient in Mouse Zygotes

CRISPR-READI: Efficient Generation of Knockin Mice by CRISPR RNP Electroporation and AAV Donor Infection

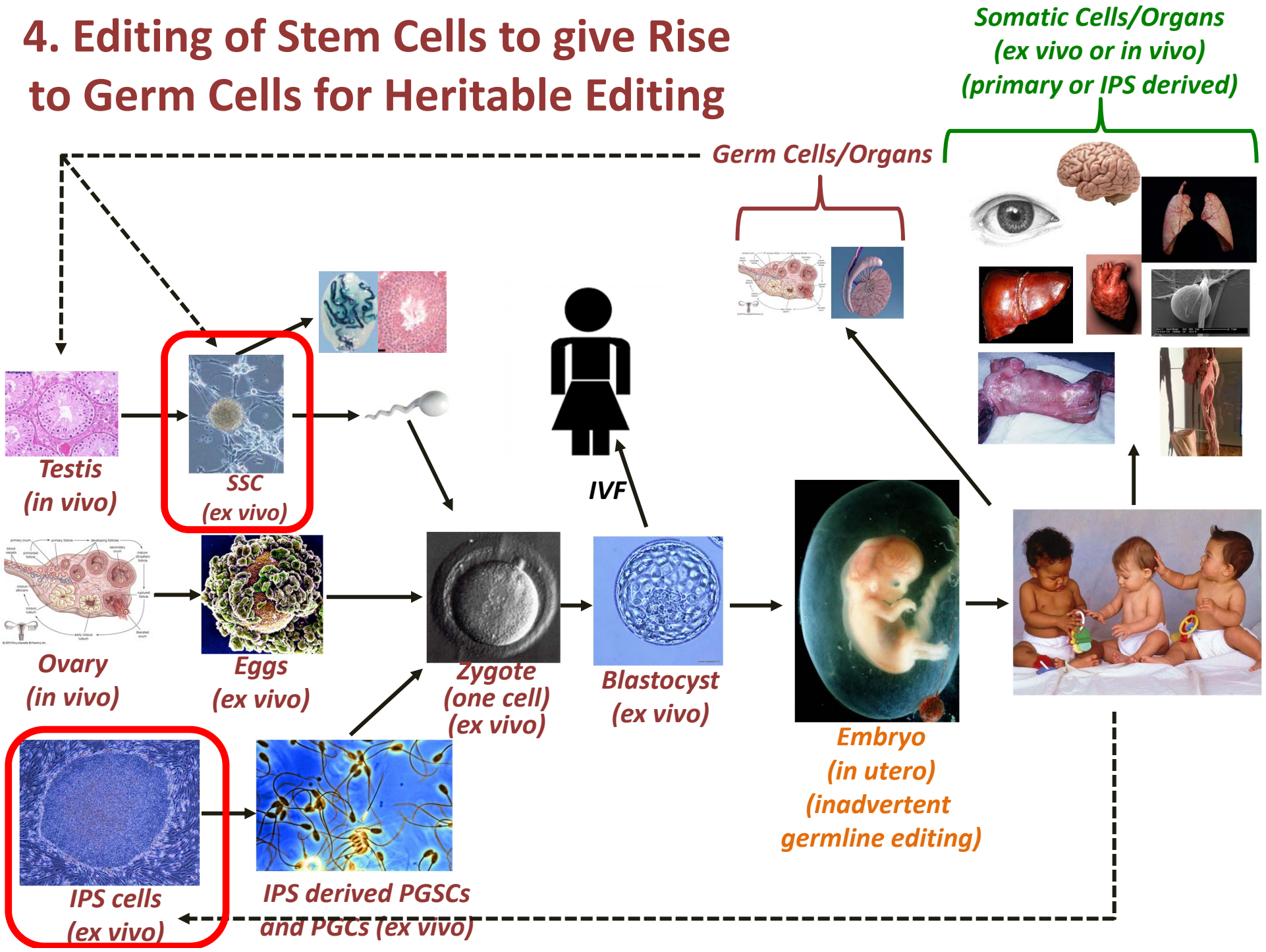




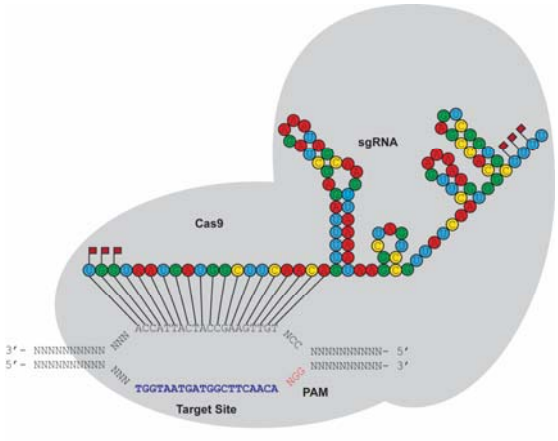


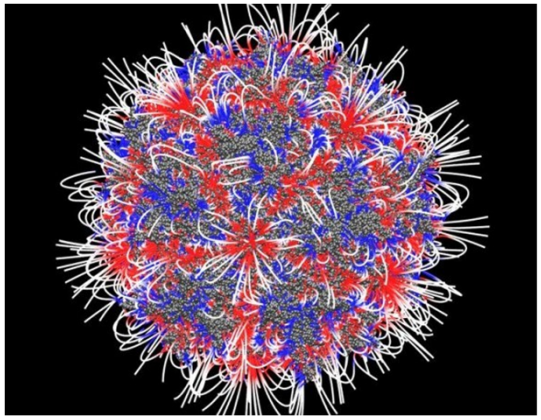
Chen et al., 2019, Cell Reports *27*, 3780–3789
June 25, 2019 © 2019
https://doi.org/10.1016/j.celrep.2019.05.103

4. Editing of Stem Cells to give Rise to Germ Cells for Heritable Editing

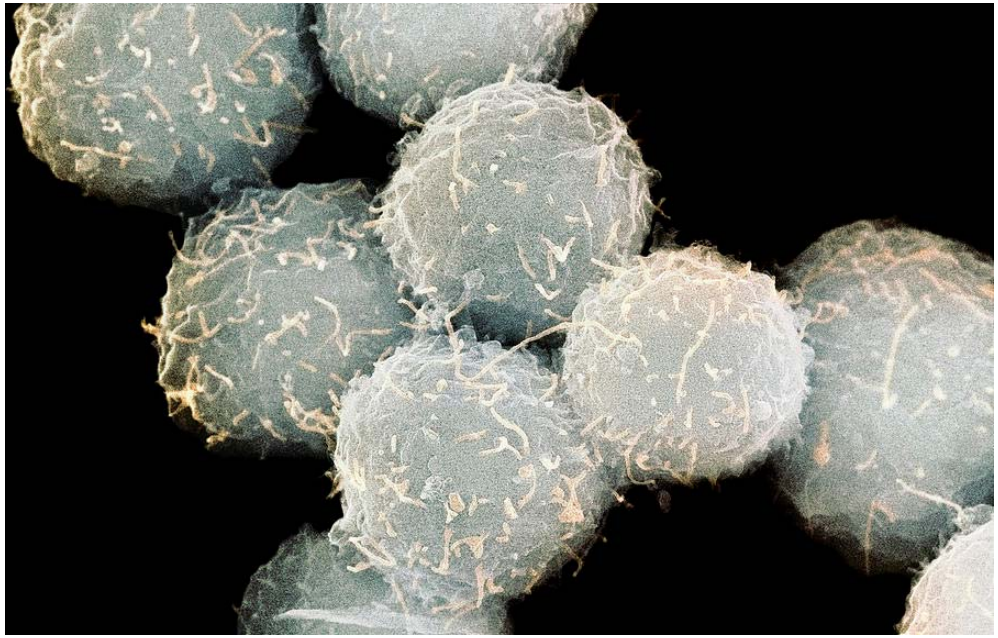


Targeted Gene Integration using RNP/AAV6 System in Human Pluripotent Cells





Recombinant Adeno-Associated Virus (AAV6) (non-integrating)



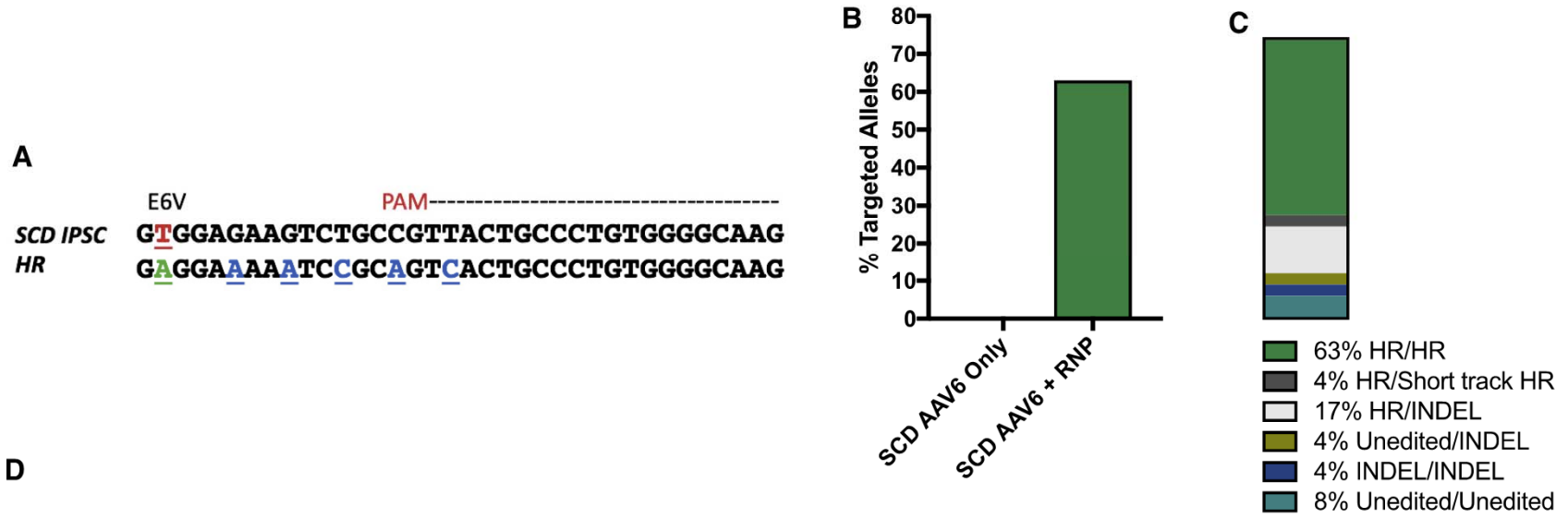
Resource

Cell Stem Cell

Highly Efficient and Marker-free Genome Editing of Human Pluripotent Stem Cells by CRISPR-Cas9 RNP and AAV6 Donor-Mediated Homologous Recombination

Martin, Ikeda, Cromer et al Cell Stem Cell (2019)

Efficient Targeted Integration and Single Nucleotide Correction (>40%)



Editing of Mouse Spermatogonial Stem Cells (SSCs)

OPEN ACCESS Freely available online

PLOS ONE

Genome Editing in Mouse Spermatogonial Stem/Progenitor Cells Using Engineered Nucleases

PLoS One (2014)

Danielle A. Fanslow[1], Stacey E. Wirt[2], Jenny C. Barker[3], Jon P. Connelly[3], Matthew H. Porteus[2]*, Christina Tenenhaus Dann[1]*

Cell Research (2015) 25:67-79.

www.nature.com/cr

ORIGINAL ARTICLE

Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells

Yuxuan Wu[1, 2, *], Hai Zhou[1, 2, 3, *], Xiaoying Fan[4, *], Ying Zhang[2, 5, *], Man Zhang[1, 2, *], Yinghua Wang[1, 2], Zhenfei Xie[1, 2], Meizhu Bai[1, 2, 6], Qi Yin[1, 2], Dan Liang[1, 2], Wei Tang[7], Jiaoyang Liao[1, 2], Chikai Zhou[1, 2], Wujuan Liu[1, 2], Ping Zhu[4], Hongshan Guo[4], Hong Pan[1, 2], Chunlian Wu[3], Huijuan Shi[8], Ligang Wu[2, 5], Fuchou Tang[4], Jinsong Li[1, 2, 6]

Human Reproduction Update, Vol.22, No.5 pp. 561–573, 2016
Advanced Access publication on May 30, 2016 doi:10.1093/humupd/dmw017

human reproduction update

Spermatogonial stem cell autotransplantation and germline genomic editing: a future cure for spermatogenic failure and prevention of transmission of genomic diseases

Callista L. Mulder†, Yi Zheng†, Sabrina Z. Jan, Robert B. Struijk, Sjoerd Repping, Geert Hamer*, and Ans M.M. van Pelt

Center for Reproductive Medicine, Amsterdam Research Institute Reproduction and Development, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands

Summary/Personal Conclusions

1. **There are only a few examples in which heritable/germline editing prevents a child being born with a serious disease that could not be prevented by IVF with PGD.**
   - **Using zygote editing to create "more" such zygotes is not a justification to cross what remains an important line when considering the global human population with diverse, robust, meaningful, and differing belief systems.**
2. **Restricting edits to sequences that are known to be common and healthy in the human population protects from hubris and from leakage to applications beyond the prevention of disease.**
   - **Does not fundamentally alter human evolution/genetics**
   - **Could generate a list of acceptable sequences that permissible to edit to**
3. **Quantitative, public disclosures/international review about efficacy, specificity, and chimerism should be required before any attempt at creating a pregnancy.**
4. **Editing to sequences that are common and healthy in the human population in a non-chimeric way without creating sequences that are novel remains technically challenging.**
5. **Advances in stem cell biology, germ cell biology, and somatic cell editing, however, may provide solutions to the technical challenges of #4 and regulations should be put into place anticipating that the technical challenges will be solved.**
6. **Societal, ethical, and governance issues remain just as, if not more, important than the technical issues.**