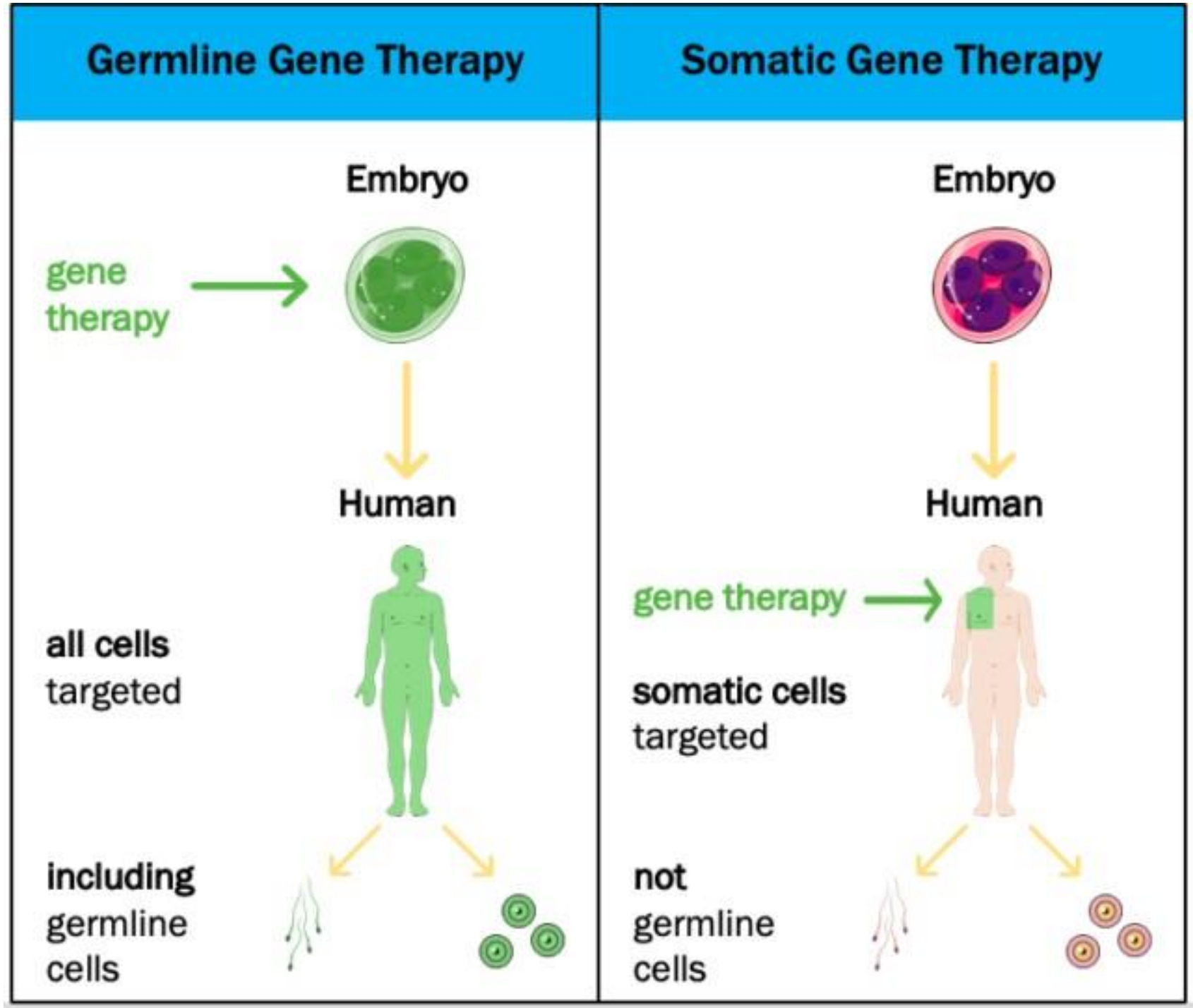
Germline gene editing

- Germline editing is the process by which the genome of an individual is edited in such a way that the change is heritable.
- This is achieved through genetic alterations within the germ cells, or the reproductive cells, such as the egg and sperm.

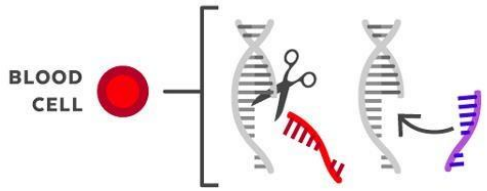
- Human germline engineering is a type of genetic modification that directly manipulates the genome using molecular engineering techniques.

- Aside from germline engineering, genetic modification can be applied in another way, somatic genetic modification.
- Somatic gene modification consists of altering somatic cells, which are all cells in the body that are not involved in reproduction.
- While somatic gene therapy does change the genome of the targeted cells, these cells are not within the germline, so the alterations are not heritable and cannot be passed on to the next generation.

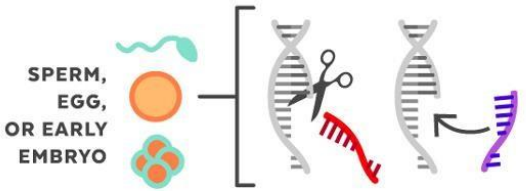

Germline Gene Therapy
Embryo
gene therapy
Human
all cells targeted
including germline cells
Somatic Gene Therapy
Embryo
Human
gene therapy
somatic cells targeted
not germline cells

SOMATIC GENE EDITING VS. GERMLINE GENE EDITING

EDIT



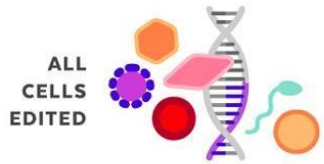
Somatic therapies target genes in specific types of cells (blood cells, for example).



Germline modifications are made so early in development that any change is copied into all of the new cells.

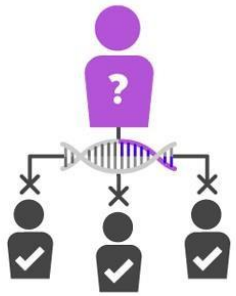
COPY

The edited gene is contained only in the target cell type. No other types of cells are affected.

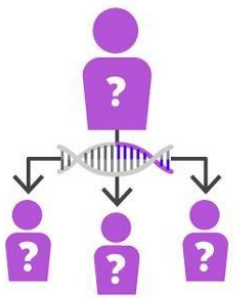


The edited gene is copied in every cell, including sperm or eggs.

RISKS



Any changes, including potential off-target effects, are limited to the treated individual.



If the person has children, the edited gene is passed on to future generations.

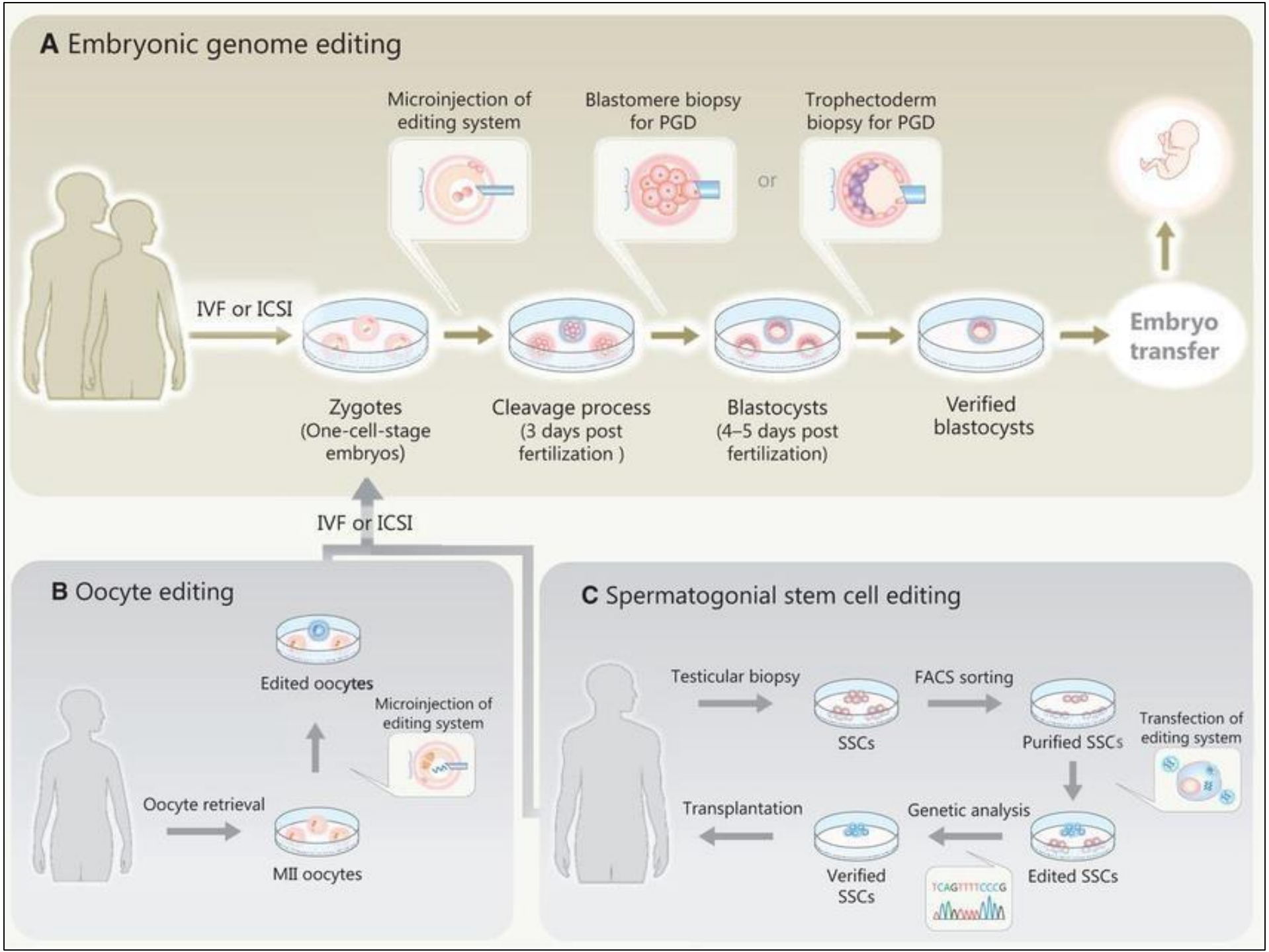
NEXT GENERATION

The edited gene is not passed down to future generations.

CONSENSUS

Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.

Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.


A Embryonic genome editing
Microinjection of editing system
Blastomere biopsy for PGD
Trophectoderm biopsy for PGD
or
IVF or ICSI
Embryo transfer
Zygotes (One-cell-stage embryos)
Cleavage process (3 days post fertilization)
Blastocysts (4–5 days post fertilization)
Verified blastocysts
IVF or ICSI
B Oocyte editing
Edited oocytes
Microinjection of editing system
Oocyte retrieval
MII oocytes
C Spermatogonial stem cell editing
Testicular biopsy
SSCs
FACS sorting
Purified SSCs
Transfection of editing system
Transplantation
Verified SSCs
Genetic analysis
Edited SSCs
TCAGTTTTCCCG

- The Lulu and Nana controversy refers to the two Chinese twin girls born in November 2018, who had been genetically modified as embryos by the Chinese scientist He Jiankui.
- The twins are believed to be the first genetically modified babies.
- The girls' parents had participated in a clinical project run by He, which involved IVF, PGD and genome editing procedures in an attempt to edit the gene CCR5. CCR5 encodes a protein used by HIV to enter host cells, so by introducing a specific mutation into the gene CCR5 Δ32 He claimed that the process would confer innate resistance to HIV.

- The project run by He recruited couples wanting children where the man was HIV-positive and the woman uninfected.
- During the project, He performed IVF with sperm and eggs from the couples and then introduced the CCR5 Δ32 mutation into the genomes of the embryos using CRISPR/Cas9.
- He then used PGD on the edited embryos during which he sequenced biopsied cells to identify whether the mutation had been successfully introduced. He reported some mosaicism in the embryos, whereby the mutation had integrated into some cells but not all, suggesting the offspring would not be entirely protected against HIV.
- He claimed that during the PGD and throughout the pregnancy, foetal DNA was sequenced to check for off-target errors introduced by the CRISPR/Cas9 technology, however the NIH released a statement in which they announced "the possibility of damaging off-target effects has not been satisfactorily explored".
- The girls were born in early November 2018, and were reported by He to be healthy.

He Jiankui speaking at the Second International Summit on Human Genome Editing, November 2018

- People inherit two copies of CCR5, one from each parent.
- He chose the gene as a target because he knew that about 1% of Northern European populations are born with both copies missing 32 base pairs, resulting in a truncated protein that doesn’t reach the cell surface.
- These people, known as CCR5Δ32 homozygotes, appear healthy and are highly resistant to HIV infection.

- Researchers showed in 2016 that knocking out one or both CCR5s in mice enhances their memory and cognition.
- A subsequent study that crippled CCR5 in mice found that, compared with control animals, the mutants recovered from strokes more quickly and had improved motor and cognitive functions following traumatic brain injury.
- The later study, in the 21 February issue of Cell, also included an analysis of 68 stroke patients who had one copy of CCR5 with the HIV resistance mutation; it concluded they had improved recovery, too.

Two female monkeys named Mingming and Ningning are the first primates to have their genes precisely edited by scientists.

NrOb1, which is involved in keeping embryonic stem cells flexible and for determining sex; **Ppar-gamma**, which helps regulate metabolism; and **Rag1**, an immune system gene.

SUMMARY

Monkeys serve as important model species for studying human diseases and developing therapeutic strategies, yet the application of monkeys in biomedical researches has been significantly hindered by the difficulties in producing animals genetically modified at the desired target sites. Here, we first applied the CRISPR/Cas9 system, a versatile tool for editing the genes of different organisms, to target monkey genomes. By coinjection of Cas9 mRNA and sgRNAs into one-cell-stage embryos, we successfully achieve precise gene targeting in cynomolgus monkeys. We also show that this system enables simultaneous disruption of two target genes (*Ppar-γ* and *Rag1*) in one step, and no off-target mutagenesis was detected by comprehensive analysis. Thus, coinjection of one-cell-stage embryos with Cas9 mRNA and sgRNAs is an efficient and reliable approach for gene-modified cynomolgus monkey generation.

Human Germline Genome Editing

Kelly E. Ormond,[1,19,*] Douglas P. Mortlock,[2,19] Derek T. Scholes,[3,19] Yvonne Bombard,[4] Lawrence C. Brody,[5] W. Andrew Faucett,[6,7] Nanibaa' A. Garrison,[8,9] Laura Hercher,[7,10] Rosario Isasi,[11] Anna Middleton,[12,13] Kiran Musunuru,[14] Daniel Shriner,[15,16] Alice Virani,[17,18] and Caroline E. Young[3]

With CRISPR/Cas9 and other genome-editing technologies, successful somatic and germline genome editing are becoming feasible. To respond, an American Society of Human Genetics (ASHG) workgroup developed this position statement, which was approved by the ASHG Board in March 2017. The workgroup included representatives from the UK Association of Genetic Nurses and Counsellors, Canadian Association of Genetic Counsellors, International Genetic Epidemiology Society, and US National Society of Genetic Counselors. These groups, as well as the American Society for Reproductive Medicine, Asia Pacific Society of Human Genetics, British Society for Genetic Medicine, Human Genetics Society of Australasia, Professional Society of Genetic Counselors in Asia, and Southern African Society for Human Genetics, endorsed the final statement. The statement includes the following positions. (1) At this time, given the nature and number of unanswered scientific, ethical, and policy questions, it is inappropriate to perform germline gene editing that culminates in human pregnancy. (2) Currently, there is no reason to prohibit in vitro germline genome editing on human embryos and gametes, with appropriate oversight and consent from donors, to facilitate research on the possible future clinical applications of gene editing. There should be no prohibition on making public funds available to support this research. (3) Future clinical application of human germline genome editing should not proceed unless, at a minimum, there is (a) a compelling medical rationale, (b) an evidence base that supports its clinical use, (c) an ethical justification, and (d) a transparent public process to solicit and incorporate stakeholder input.