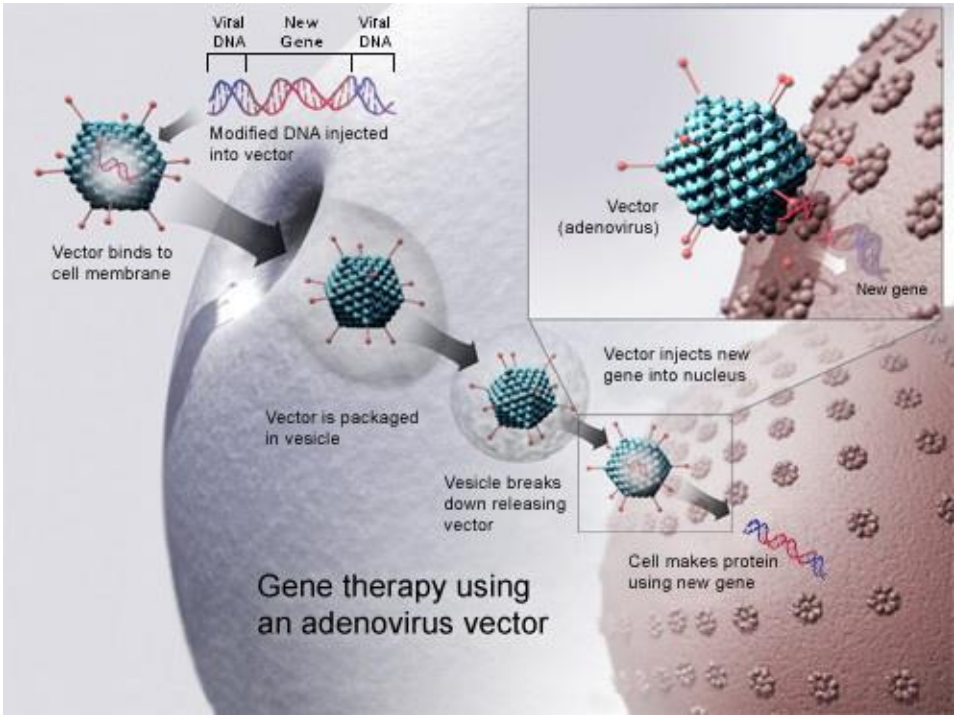
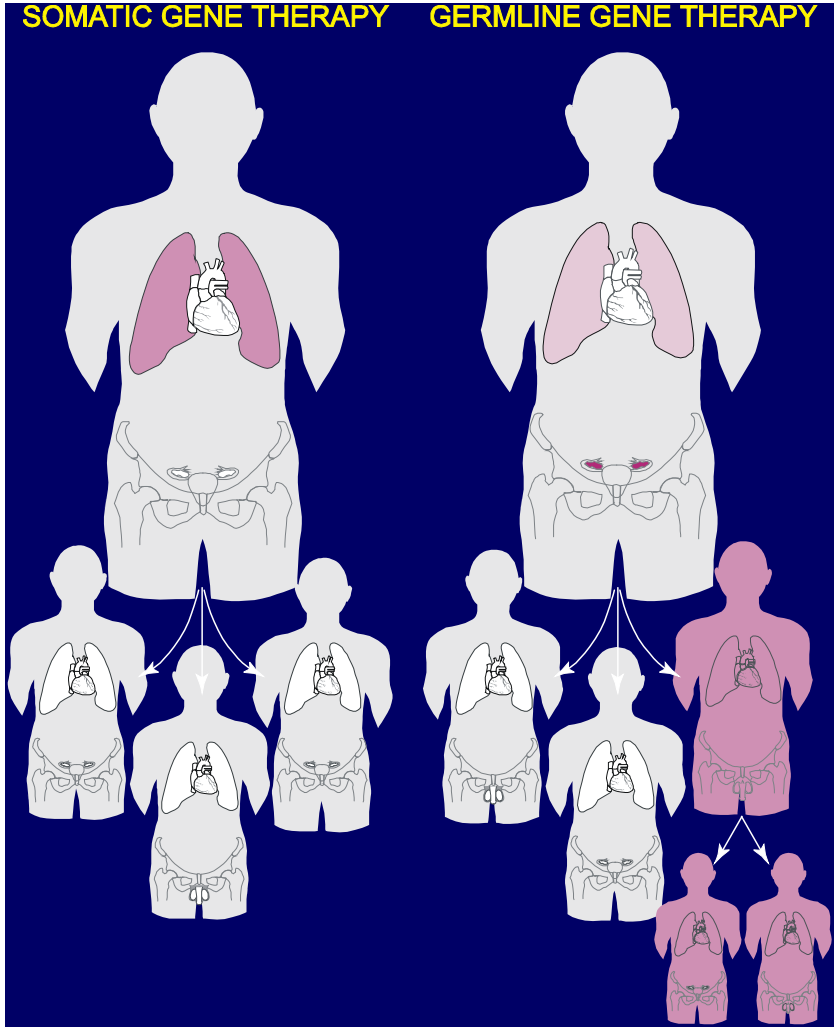
Genová terapie: na modelu léčby cystické fibrózy

Prof. MUDr. Milan Macek, DrSc.
Ústav biologie a lékařské genetiky
2. lékařské fakulty Univerzity Karlovy, Praha

Obecné principy



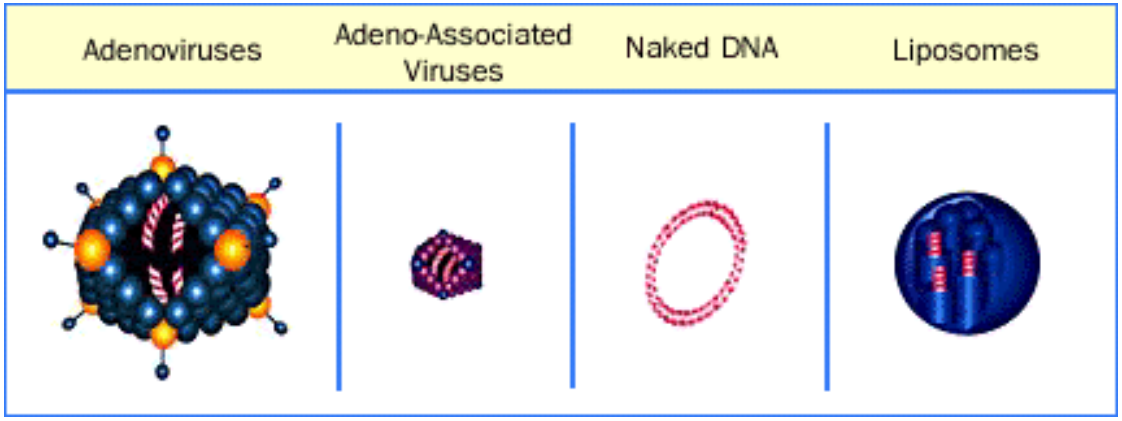


TD Gelehrter, FS Collins, D Ginsburg.
Principles of Medical Genetics. 1997.

Obecné problémy

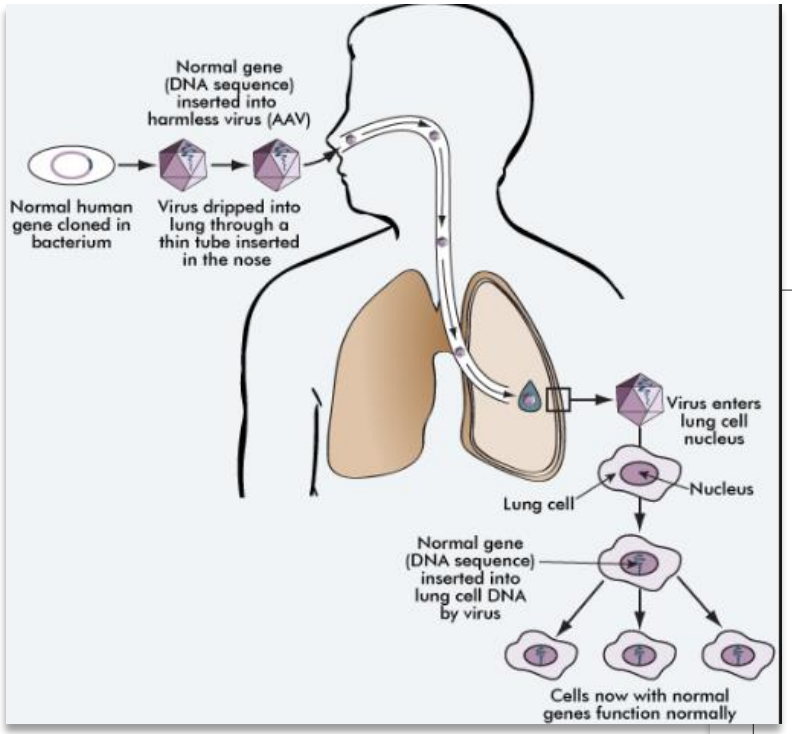
Jak dodat DNA ?
Dosažení žádoucí exprese?
Udržení žádoucí exprese?
Tkáňová specifičnost
in vivo regulace?

GT1 vektory



- Retroviry
  - Lentiviry
- Adenoviry
- Adeno-associované viry (AAV)
- Ostatní virové vektory
  - Vaccinia– Hepatitis virus
  - Herpes virus – Polio virus
  - Papilloma virus– Sindbis and other RNA viruses
- Nevirové metody
  - Ligand-DNA conjugates – Adenovirus- ligand-DNA
  - Lipofection – Direct DNA injection
  - CaPO4 precipitation – Ribozymes
  - chimeric oligo/gene correction

Genová terapie 1.0 versus Genová terapie 2.0



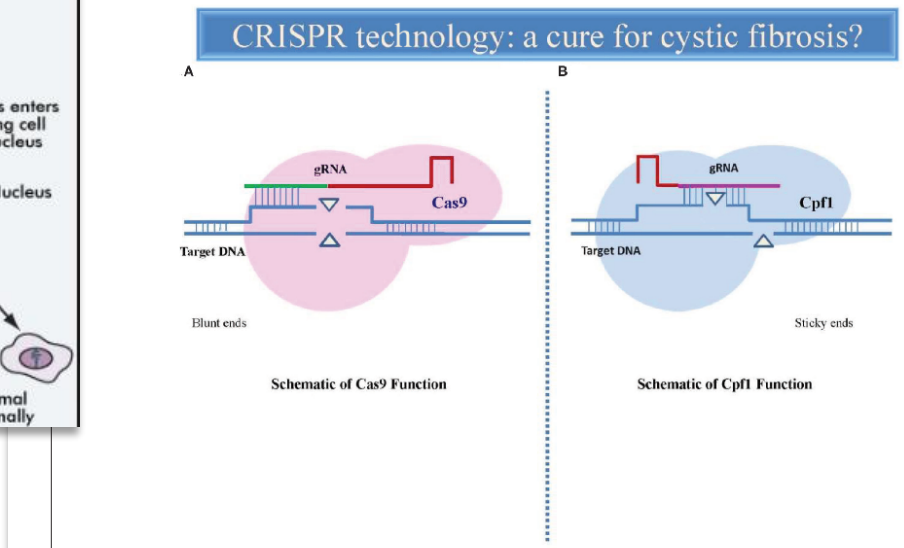
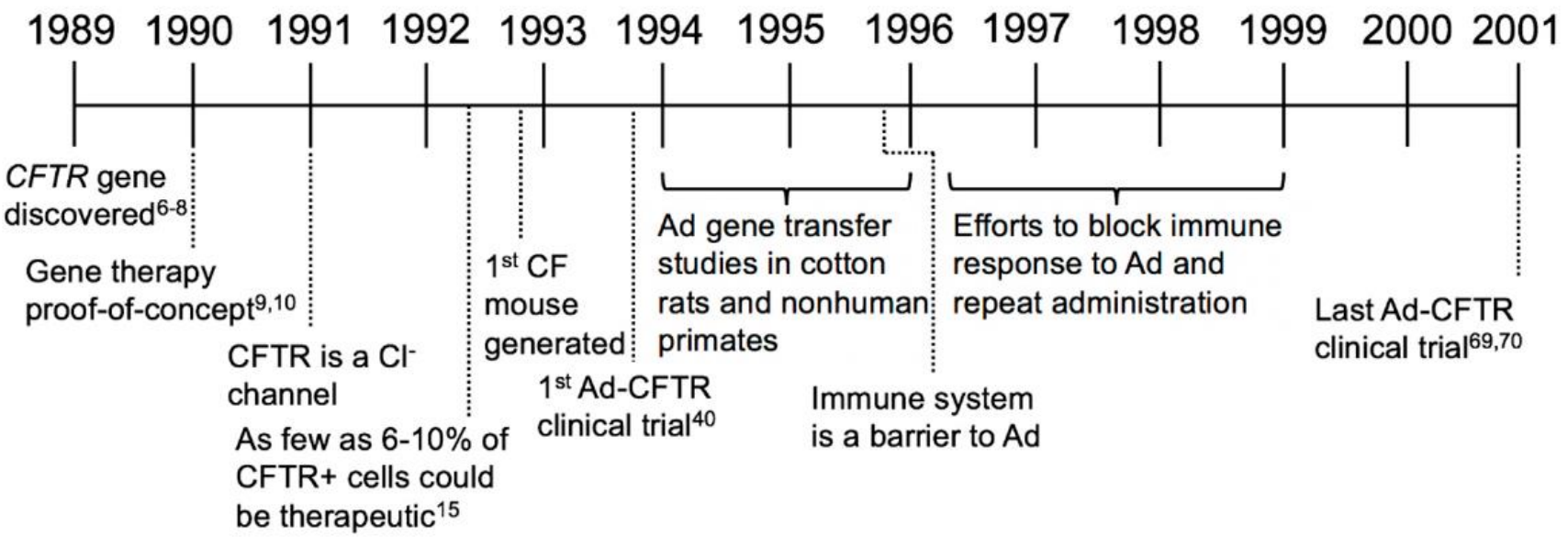
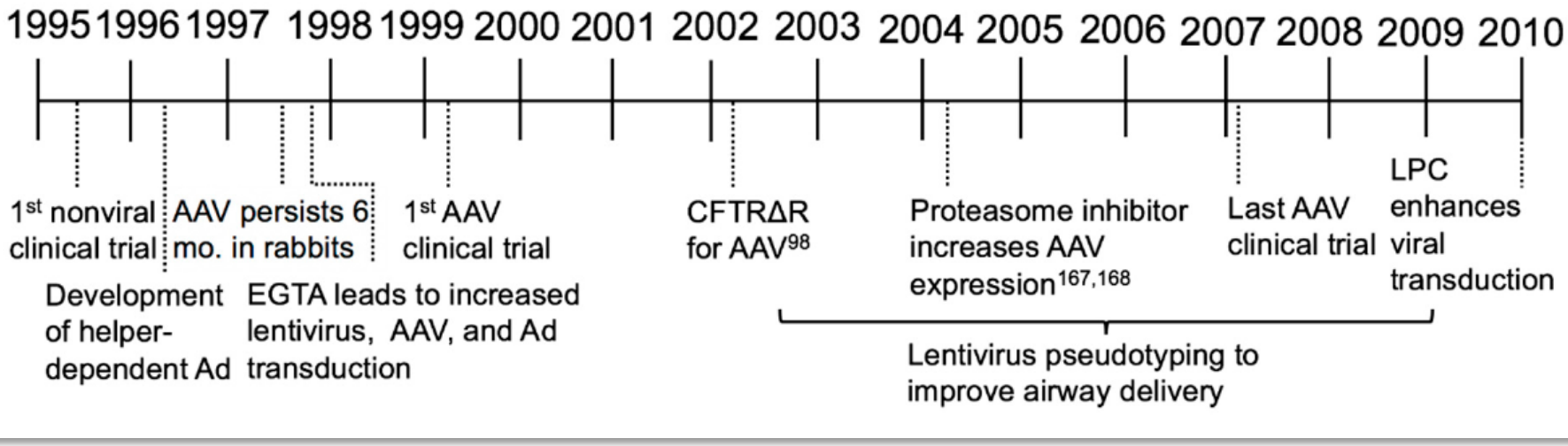


FIGURE 1 | Comparative representation of CRISPR-Cas9 and CRISPR-Cpf1-mediated genome editing. The gRNA directs endonuclease Cas9 **(A)** to the target DNA sequence (blue) where it induces a double-strand break, leading to a sequence deletion. Cas9 uses a structural region of gRNA as a handle (red) and a variable targeting region (green) which identifies the target sequence to match and cleave. Cas9 can be specifically directed to the any target site of genome simply by modifying the sequence of the gRNA. Cpf1 endonuclease **(B)** contains a shorter and single identified nuclease domain (CRISPR-RNA), in contrast to the two nuclease domains present in Cas9. Cpf1-crRNA efficiently cleaves target DNA without the requirement for any additional RNA species. Cpf1 generates a staggered cut, in contrast to the blunt ends generated by Cas9. In both cases, the DSBs are subsequently repaired by two major cellular mechanisms, non-homologous end joining (NHEJ) and homology-directed repair (HDR).

doi: 10.3389/fphar.2018.00396

Vývoj genové terapie u CF (1989-2010)



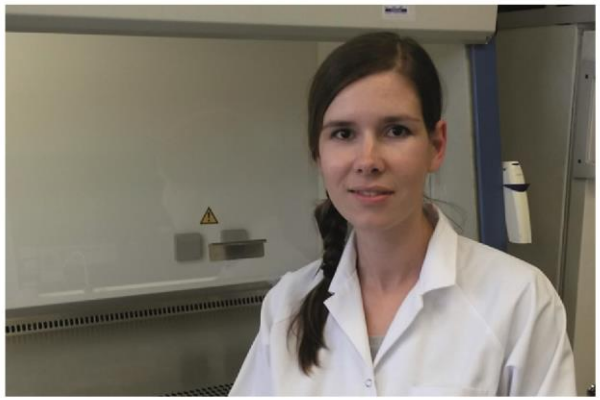


doi:10.3390/genes9110538

Vývoj genové terapie u CF (2008-2021)

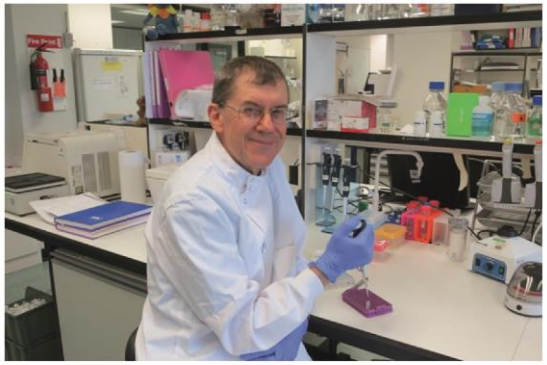
Source: Eric Alton

Eric Alton, professor of gene therapy and respiratory medicine at Imperial College London, is coordinator of the UK Cystic Fibrosis Gene Therapy Consortium



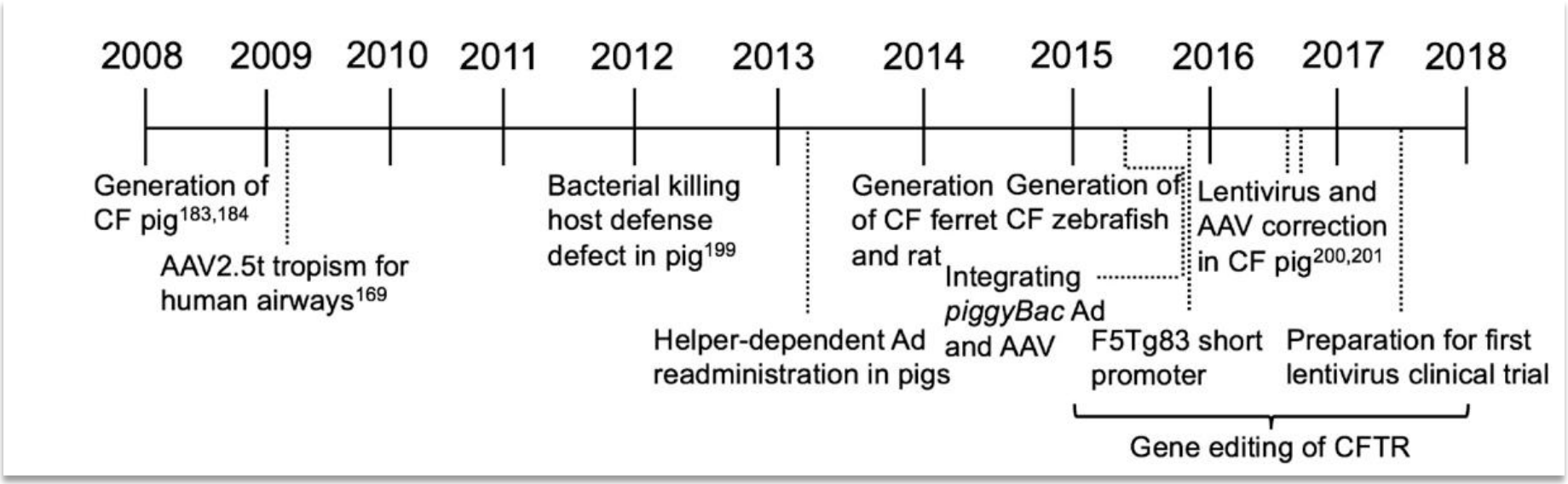
Source: Marianne Carlon

Marianne Carlon, a gene therapy researcher at Catholic University in Leuven, Belgium, says liposomes are not very efficient at getting their genetic cargo into the host nucleus



Source: Chris Boyd

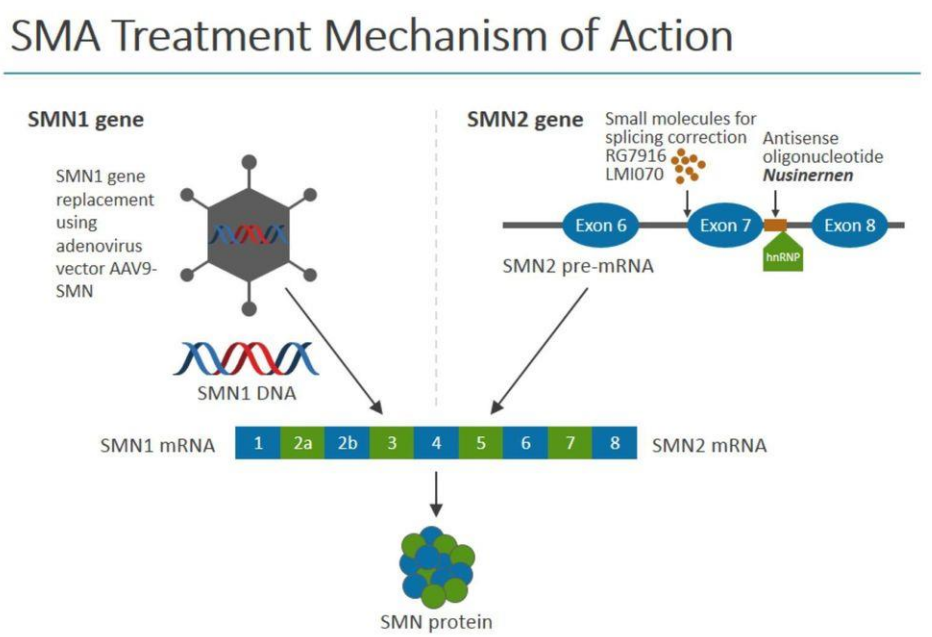
Chris Boyd, a medical geneticist at the University of Edinburgh and a member of the UK Cystic Fibrosis Gene Therapy Consortium, says the cells lining the airways perceive gene therapy delivery systems as alien invaders, and do their best to keep them out



http://www.cfgenetherapy.org.uk/

doi:10.3390/genes9110538

Spinální svalová atrofie (2020)

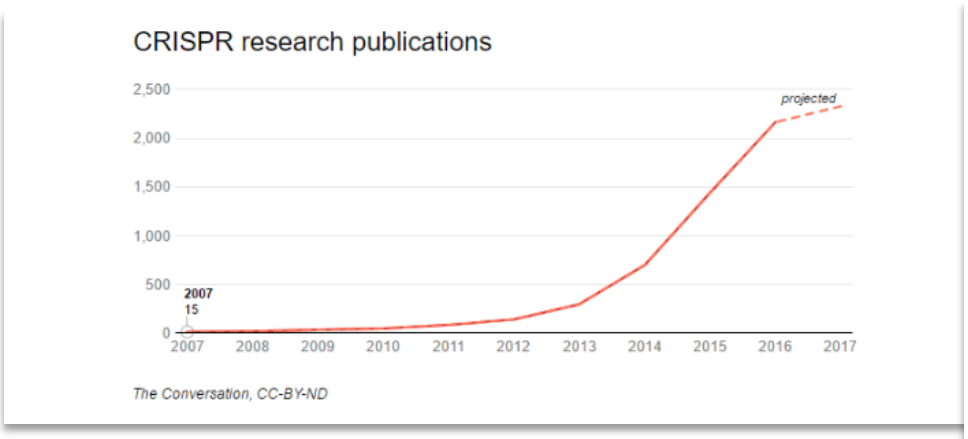


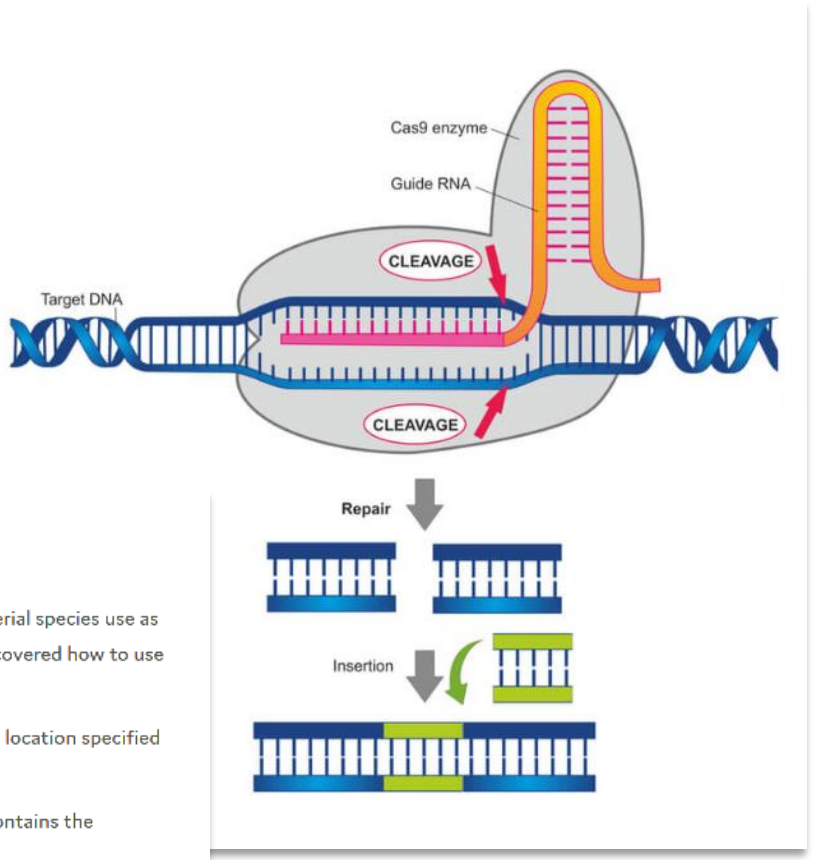
Farrar MA, et al. *Ann Neurol*. 2017;81:355-368.

Plus a minusy virových vektorů

Vector	Advantages	Disadvantages
Retrovirus	High efficiency transduction of appropriate target cells. Long-term expression- integration into chromosomal DNA).	Potential for insertional mutagenesis. Requires dividing cells. Limited size of DNA insert.
Adenovirus	High transduction efficiency. Broad range of target cells. Does not require cell division. Low risk of insertional mutagenesis.	Transient expression. Immunogenicity. Direct cytopathic effects of virus.
Adeno-associated virus (AAV)	Does not require cell division. ? Site specific integration.	Potential for insertional mutagenesis if integration not site-specific. Limited size of DNA insert.
Non-viral vectors	No infectious risk. Completely synthetic. No limitation on insert size.	Low efficiency. Limited target cell range. Transient expression.

GeneTerapy 2.0: CRISPR-Cas9





CRISPR Lexicon

- **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats of genetic information that some bacterial species use as part of an antiviral system. A group of scientists, including our co-founder Dr. Emmanuelle Charpentier, discovered how to use this system as a gene-editing tool (Jinek, *et al.* Science 2012)
- **Cas9:** a CRISPR-associated (Cas) endonuclease, or enzyme, that acts as "molecular scissors" to cut DNA at a location specified by a guide RNA
- **Deoxyribonucleic acid (DNA):** the molecule that most organisms use to store genetic information, which contains the "instructions for life"
- **Ribonucleic acid (RNA):** a molecule related to DNA that living things use for a number of purposes, including transporting and reading the DNA "instructions"
- **Guide RNA (gRNA):** a type of RNA molecule that binds to Cas9 and specifies, based on the sequence of the gRNA, the location at which Cas9 will cut DNA

http://www.crisprtx.com/gene-editing/crispr-cas9

Genová editace u CF- základní principy

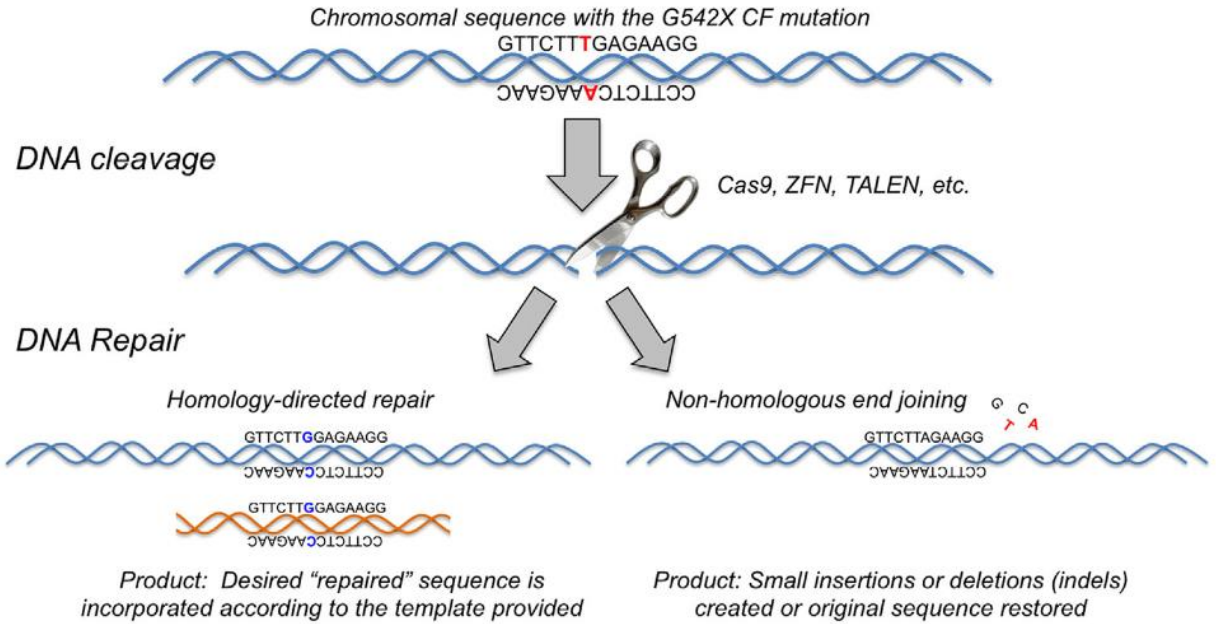
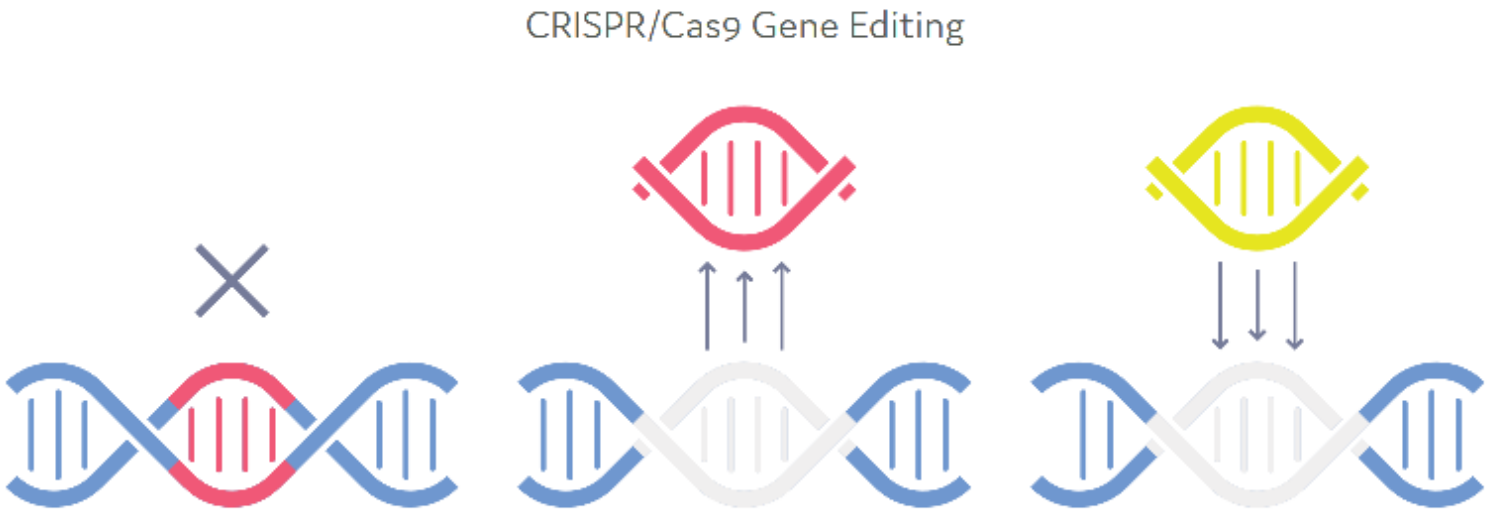


FIGURE 1 The gene editing process. Gene editing takes advantage of a cell's ability to repair DNA damage. A DNA break can be induced at a position of choice, such as a disease causing mutation like *G542X*, by any number of methods (CRISPR/Cas9, ZFNs, TALENs), and the broken ends may be reconnected by a process called nonhomologous end-joining (at right) that often results in a small number of nucleotides being lost or inserted during the repair. If a template is available (in orange), DNA polymerase can use the homology between the template and the breakage site during repair to incorporate the template's sequences, in this case an arrangement that would repair the *G542X* stop codon mutation

DOI: 10.1002/ppul.23791

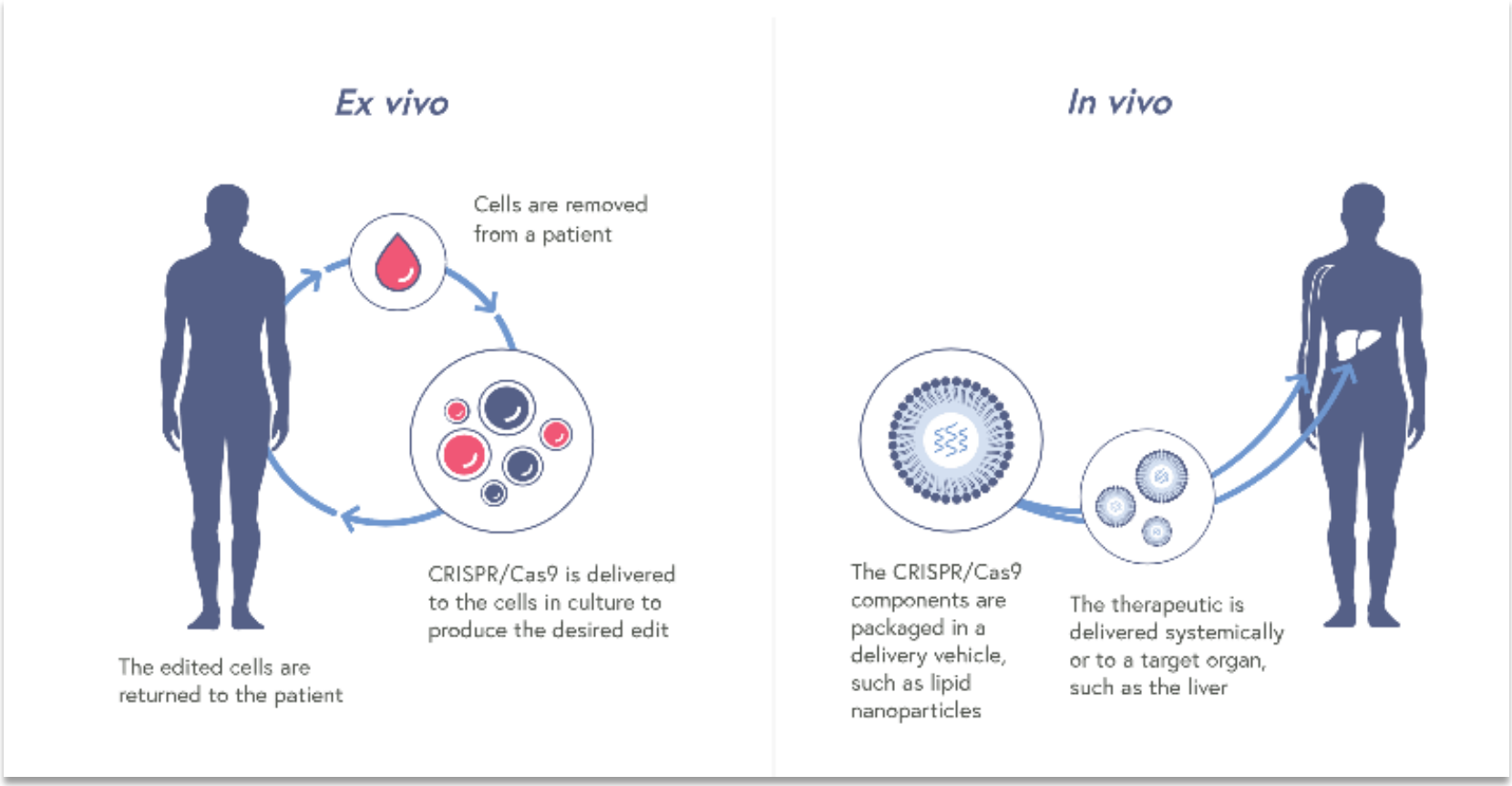
CRISPR Therapeutics 1.

Three main categories of genetic edits can be performed with CRISPR/Cas9:



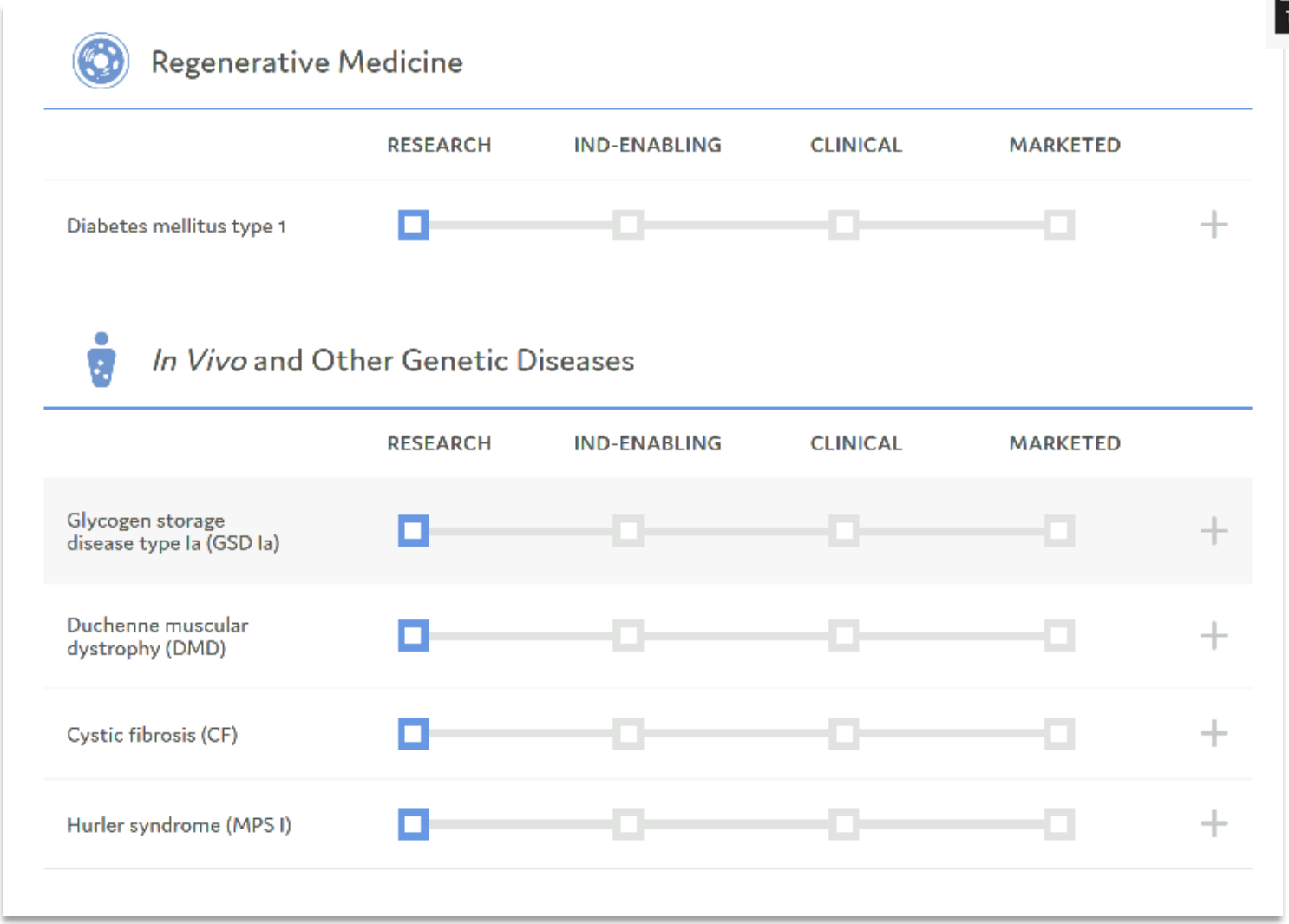
http://www.crisprtx.com/gene-editing/crispr-cas9

CRISPR Therapeutics 2.



http://www.crisprtx.com/gene-editing/crispr-cas9

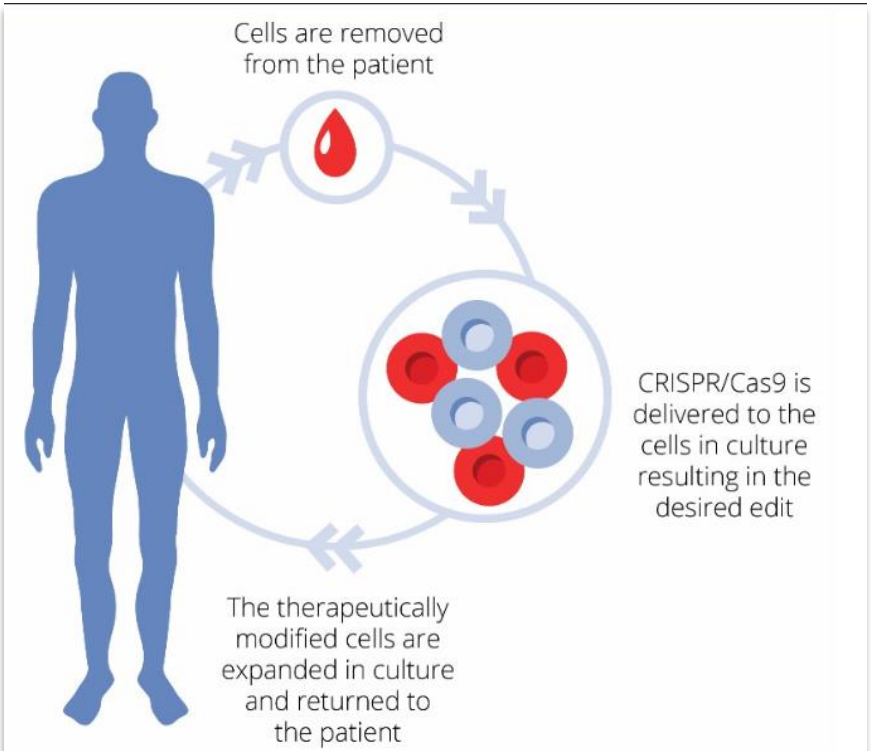
CRISPR Therapeutics 4.



http://www.crisprtx.com/gene-editing/crispr-cas9

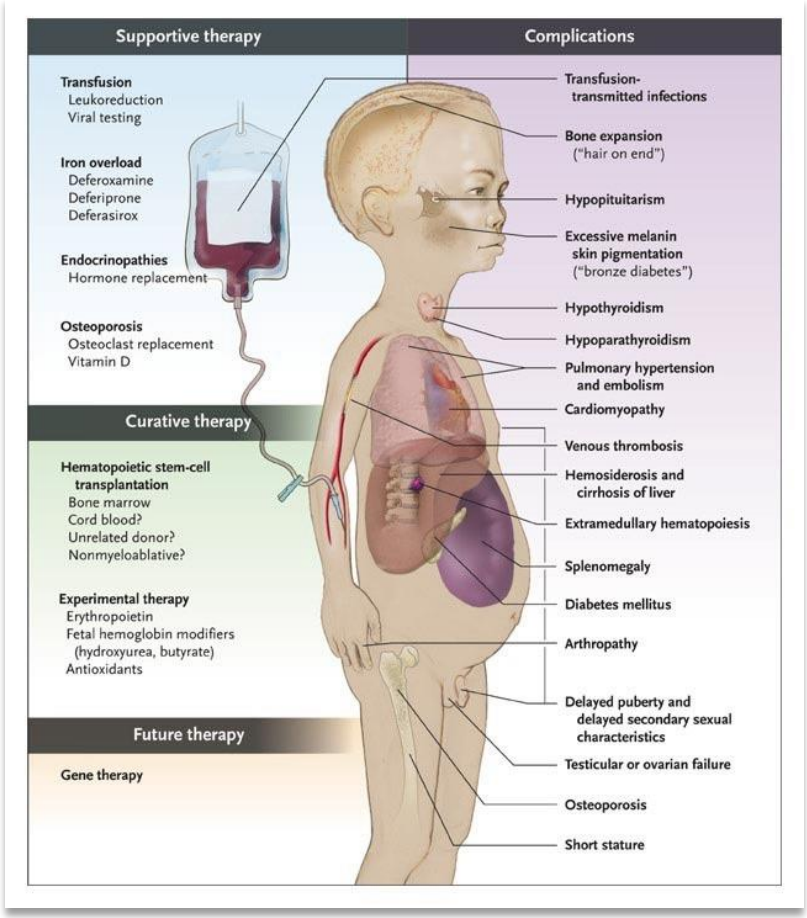
Thalasemie – ex vivo terapie (2018)

CRISPR THERAPEUTICS



CRISPR Therapeutics and Vertex presented preclinical data supporting the efficacy of the therapy last weekend at ASH 2017. The researchers showed that they were able to consistently edit over **80%** of human hematopoietic stem cells using CTX001 and that their infusion into mice models resulted in an increased production of fetal hemoglobin. The team is now ready to test if these results are consistent in humans.

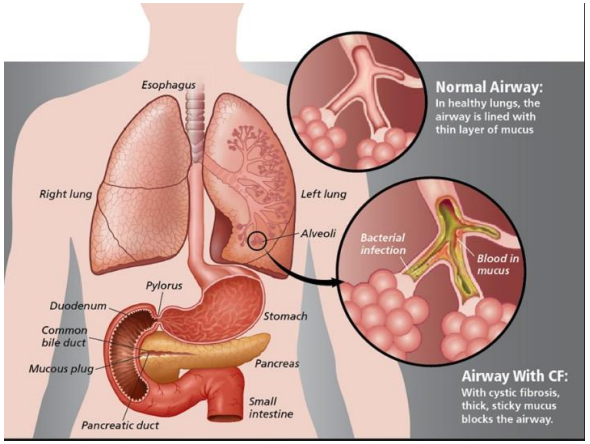
If the EMA approves the application, CRISPR Therapeutics will be the first to run a clinical trial in humans with CRISPR/Cas9 genome editing technology in Europe. The gene editing company, based in Basel, Switzerland, and co-founded by CRISPR/Cas9 co-inventor **Emmanuelle Charpentier**, is one of the most advanced players in the development of human therapeutics

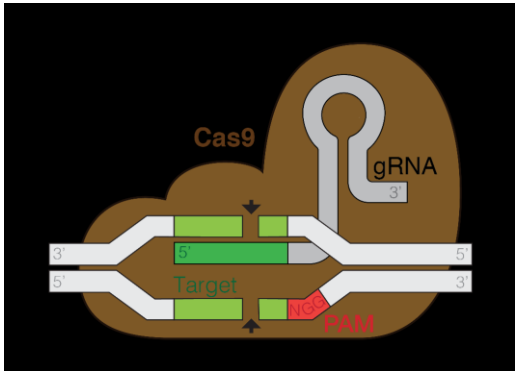


https://labiotech.eu/medical/crispr-therapeutics-clinical-trials/

Hlavní problémy CRISPR-Cas9 (nejenom u CF)

Jak dodat komponenty in vitro ?
Jak dodat komponenty in vivo ?
Co přesně aplikovat ?
Kdy editovat ?
Jaké buňky editovat ?
Jako dlouho editace vydrží?
Imunologická odpověď ?
Off target editace a jak ji zjistíme?





DOI: 10.1002/ppul.23791

„One – size – fits“ strategie CRISPR-Cas9 u CF

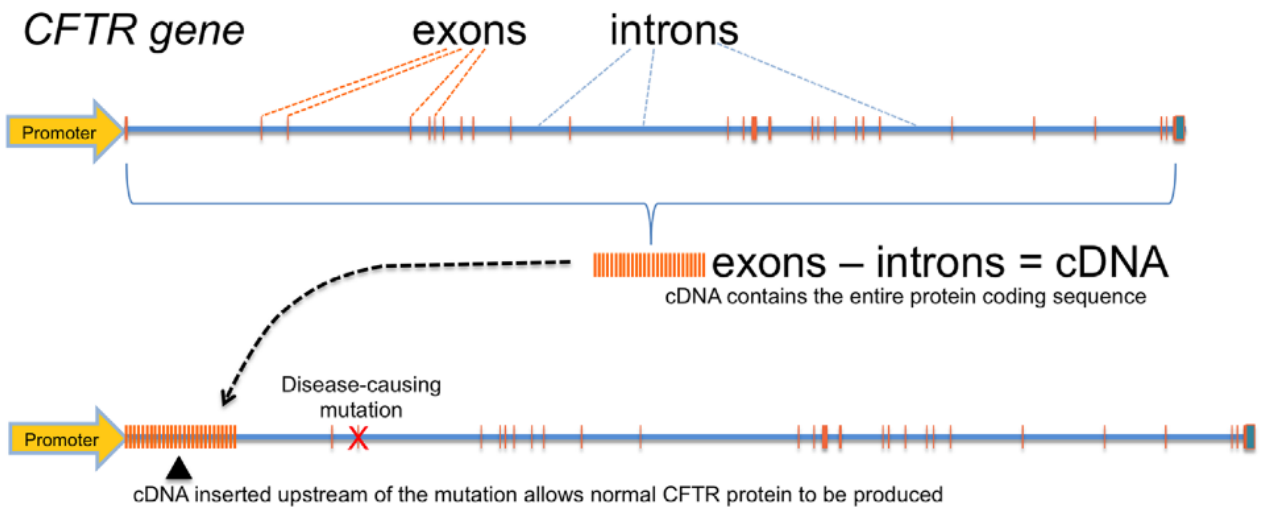
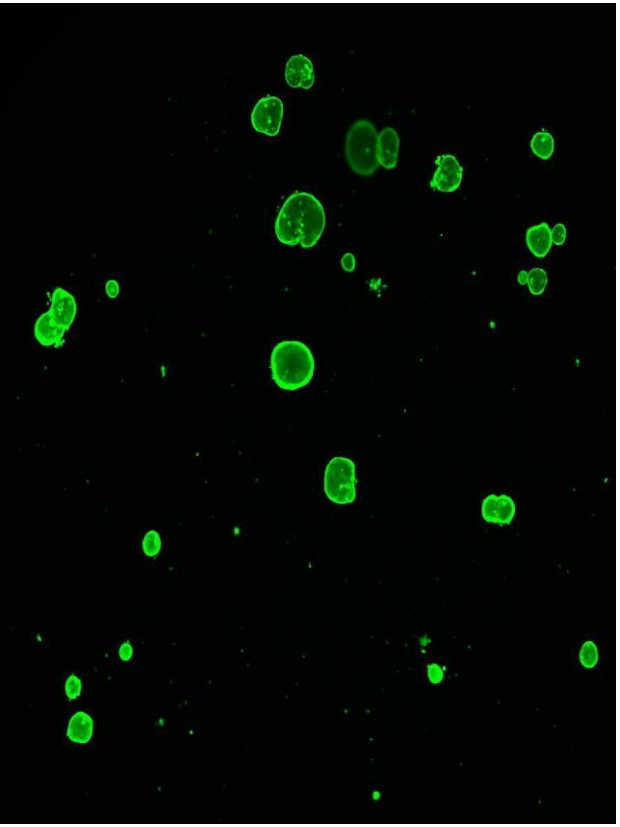


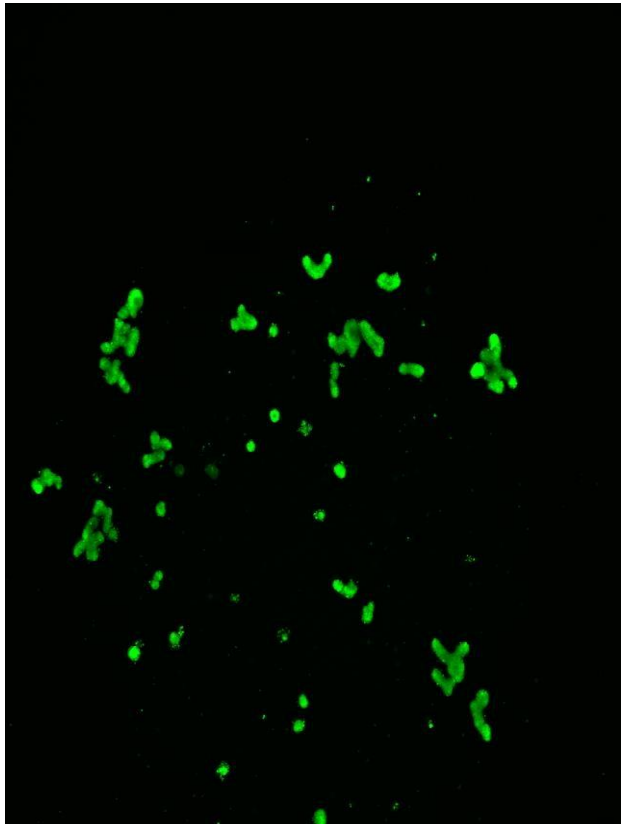
FIGURE 2 One strategy to circumvent all mutations. A DNA break provides an opportunity to insert sequences of choice into a specified position. One strategy being explored to accommodate almost any mutation is to insert the protein-coding sequence of *CFTR* (a cDNA) upstream of the mutations so that the modified gene would produce functional *CFTR* before it reached mutations, regardless of their location in the gene, yet still maintaining endogenous regulation of the gene

DOI: 10.1002/ppul.23791

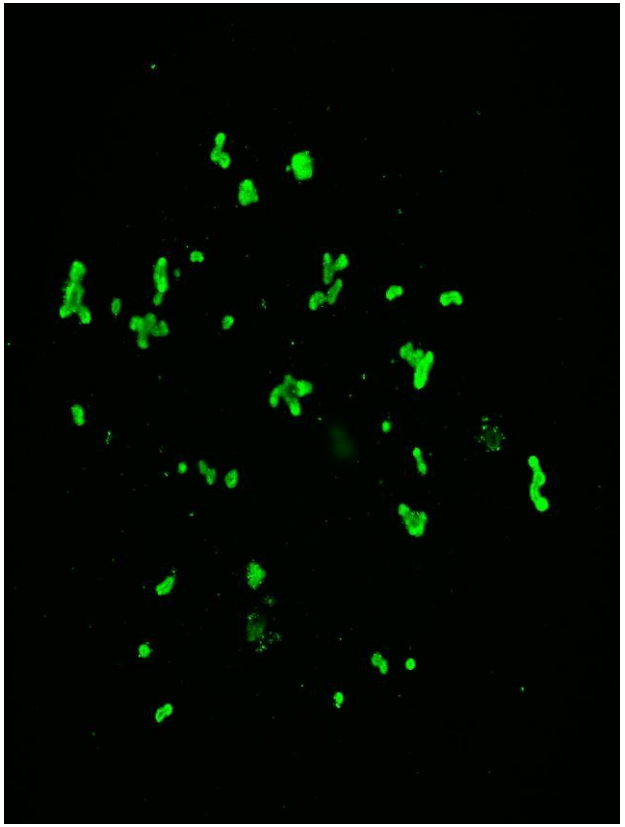
Organoidy jako *ex vivo* CFTR biomarkers („CF Avataři“)



Non-CF



CF - F508del/F508del



CF - F508del/F508del
Ivacaftor+lumacaftor

Současný stav personalizované léčby CF (2020)

1) Jedna (drahá), ale vysoce účinná léčiva pro CF mutace (CFTR modulátory)
3) Není lék pro nonsense mutace / delece + inserce
4) Mnoho pacientů se vzácnými mutacemi nebylo testováno na CFTR modulaci
5) Trojitá kombinovaná terapie?
6) CRISPR-Cas9 je problematický a GT1 stále není zavedena

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

19 March, 2015

THE ISSCR STATEMENT ON HUMAN GERMLINE GENOME MODIFICATION

The International Society for Stem Cell Research calls for a moratorium on attempts at clinical application of nuclear genome editing of the human germ line to enable more extensive scientific analysis of the potential risks of genome editing and broader public discussion of the societal and ethical implications.

Genome editing, the purposeful modification of the DNA sequence in a cell, has played an essential role in biomedical research for several decades, allowing scientists to investigate disease and develop new medical treatments. After many years of foundational research on gene transfer into mammalian cells, numerous clinical trials are currently underway that employ genome editing approaches in somatic (non-reproductive) cells and represent promising strategies for correcting inherited immune deficiencies or treating cancer.

Technologies used to introduce changes into the DNA sequence of cells have advanced rapidly, making genome editing increasingly simple. For example, zinc finger nuclease-, TALEN- and CRISPR-Cas9- based technologies are being used by researchers around the world to introduce or correct mutations in gene sequences in a wide range of cell types. Genome editing is feasible, not just in the somatic cells of an adult organism, but also in early embryos, as well as the gametes (sperm and egg) that carry the inheritable, germline DNA. Research involving germline nuclear genome editing has been performed to date in many organisms, including mice and monkeys, and applications to human embryos are possible.

Any consideration of applying nuclear genome editing to the human germ line in clinical practice raises significant ethical, societal and safety considerations. Current genome editing technologies carry risks of unintended genome damage, in addition to unknown consequences. These are of much greater concern in the context of the germ line, where, unlike changes in subsets of somatic cells, genetic changes in the germ line alter the genetic makeup of every cell in the body and are passed down to future generations. Moreover, consensus is lacking on what, if any, therapeutic applications of germ line genome modification might be permissible. For example, some argue that the ability to eradicate disease justifies attempts at therapeutic editing of the human germ line, while others emphasize the difficulty of drawing clear distinctions between applications in human disease and attempts at human enhancement.

The ISSCR calls for a moratorium on attempts to apply nuclear genome editing of the human germ line in clinical practice. Scientists currently lack an adequate understanding of the safety and potential long term risks of germline genome modification. Moreover, the ISSCR asserts that a deeper and more rigorous deliberation on the ethical, legal and societal implications of any attempts at modifying the human germ line is essential if its clinical practice is ever to be sanctioned.

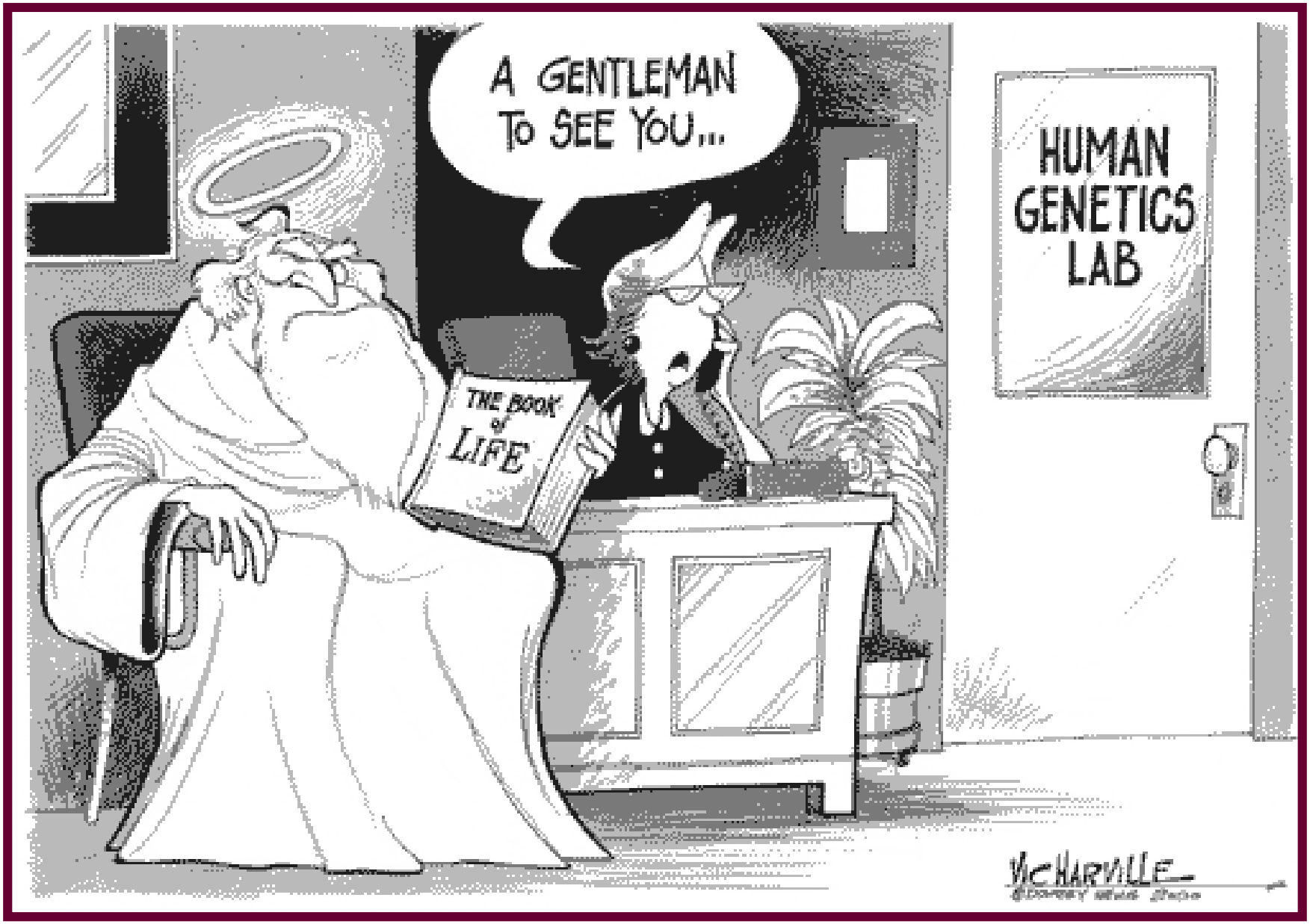
In calling for the above moratorium, the ISSCR is not taking a position on the clinical testing of mitochondrial replacement therapy, a form of germline modification that entails replacing the mitochondria (found outside the nucleus) in the eggs of women at risk of transmitting certain devastating diseases to their children. Mitochondrial replacement therapy does not entail direct modification to the nuclear genome and depends upon distinct technologies. Thoughtful scientific and ethical discussions of this technology have recently occurred in the United Kingdom and are underway in the United States and elsewhere in the world. The ISSCR applauds these current efforts as a model for deliberations on germline nuclear genome editing technologies.

At this time, the ISSCR supports in vitro laboratory research, performed under proper ethical oversight, to enhance basic knowledge and to better understand the safety issues associated with human genome editing technologies, including their potential for application in somatic tissues. The ISSCR also calls for broad public and international dialogue on the capabilities and limitations of these technologies and on the implications of their application to the human germ line. The ISSCR is committed to playing a role in catalyzing both the scientific and broader ethical deliberations about germline genome editing.

About the ISSCR

The International Society for Stem Cell Research (ISSCR) is an independent, nonprofit organization established to promote and foster the exchange and dissemination of information and ideas relating to stem cells, to encourage the general field of research involving stem cells and to promote professional and public education in all areas of stem cell research and application.

http://www.isscr.org/docs/default-source/policy-documents/isscr-statement-on-human-germline-genome-modification.pdf?sfvrsn=0


A GENTLEMAN TO SEE YOU...
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