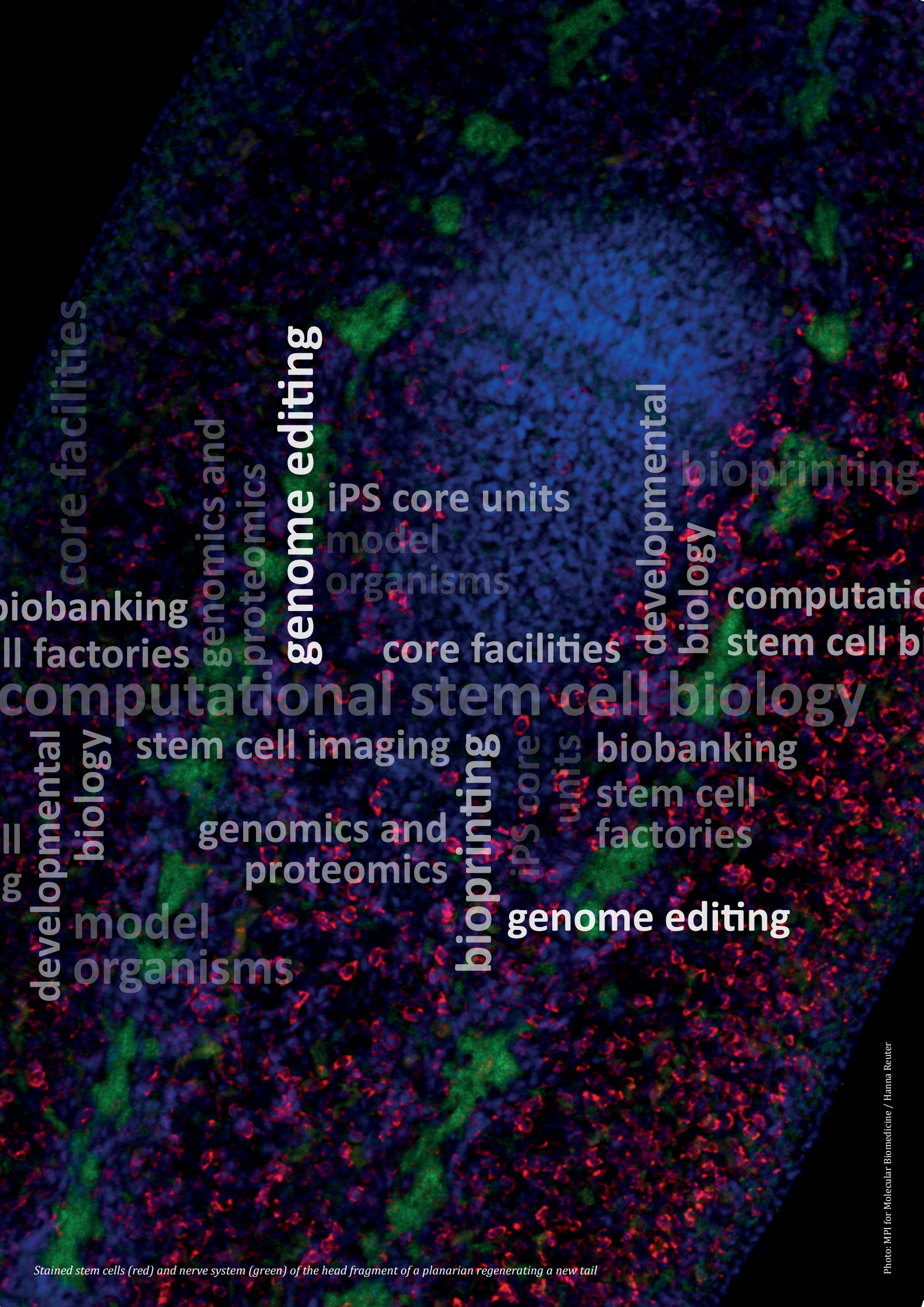


Stained stem cells (red) and nerve system (green) of the head fragment of a planarian regenerating a new tail

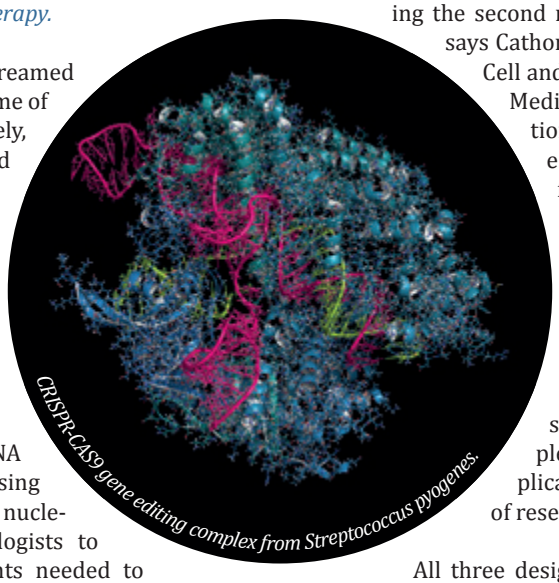
Photo: MPI for Molecular Biomedicine / Hanna Reuter

Genome Editing

Designer cuts in the genome

They've only been around for a few years, but designer nucleases have rapidly become indispensable tools for molecular biologists. These "molecular scissors" can be used to make quick, precise changes to the genome, opening up a wide range of possibilities for biotechnology and health research. The German stem cell community has been quick to adopt the new technology. "Genome editing" can be used to improve stem cell-based models of disease, while the proponents of molecular medicine are already dreaming of new, even safer routes to gene therapy.

Molecular biologists have long dreamed of being able to modify the genome of an organism quickly and precisely, but up to now they have lacked the necessary tools to do so. Instead, researchers wanting to introduce defined changes into a genome have for decades had to rely on the complicated and time-consuming process of homologous recombination in embryonic stem cells (ES cells).



CRISPR-CAS9 gene editing complex from Streptococcus pyogenes.

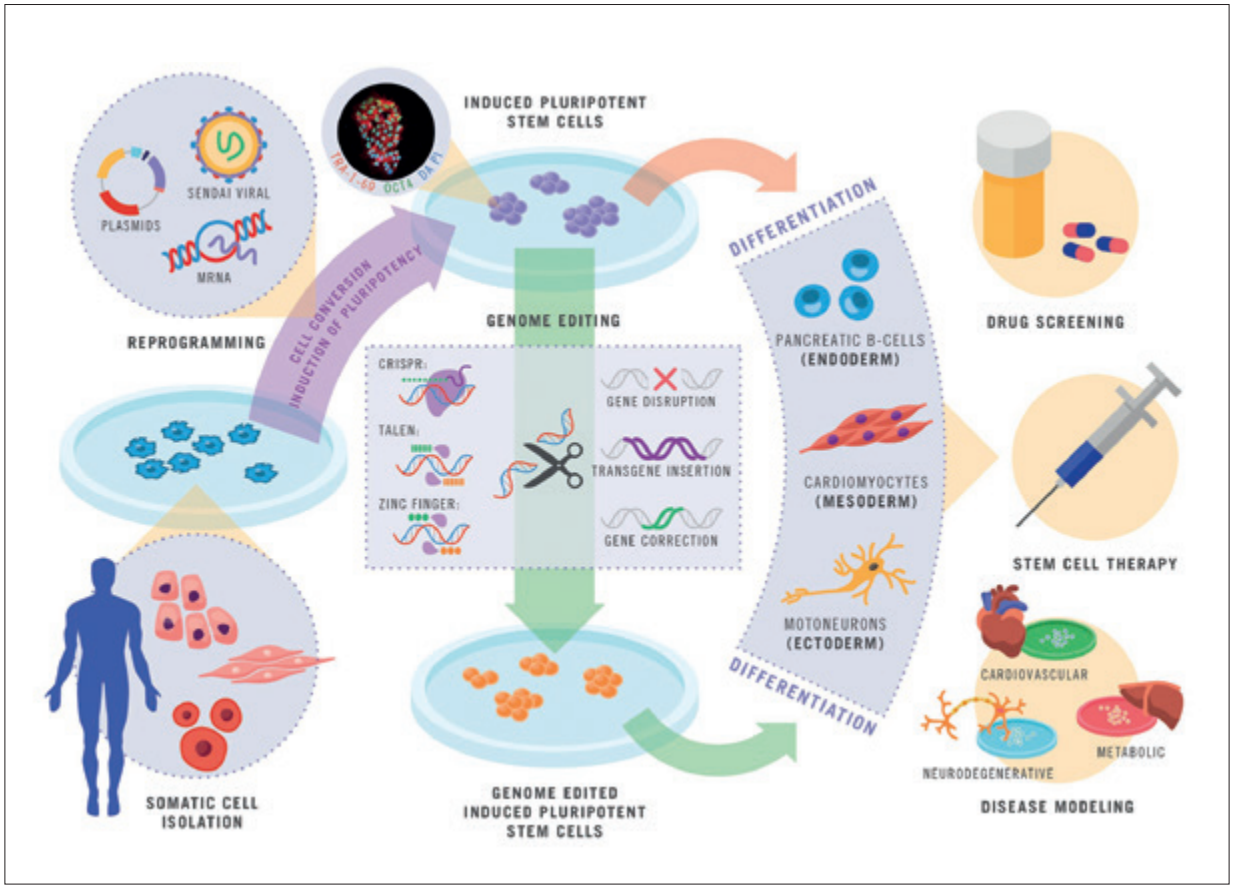
In recent years, the field of DNA technologies has been progressing in leaps and bounds. "Designer nucleases" have enabled biotechnologists to create the precision instruments needed to perform genome engineering. Using these newly discovered molecular scissors, it has become possible for the first time to alter the genome with absolute precision, to introduce mutations or whole DNA segments at a specific location, or even to correct such mutations. The precise manipulation and rearrangement of the DNA "text" in this way has come to be known as "genome editing".

Revolution in genetic engineering

This represents ground-breaking progress for many, including professor Toni Cathomen, Freiburg-based specialist in molecular medicine. "After restriction enzymes in the 1970s, there is no doubt that we are now experiencing the second revolution in gene engineering," says Cathomen, director of the Institute for Cell and Gene Therapy at the University Medical Center Freiburg. The revolution has taken place in three phases, which Cathomen has followed from the very beginning. Zinc finger nucleases (ZFNs) were developed about ten years ago, and TAL effector nucleases (TALENs) followed in 2011. "Then, in 2013, the really big breakthrough for the field came with the CRISPR-Cas system," says Cathomen. "Since it is simple, economical and universally applicable, it spread through the world of research like wildfire."

All three designer nuclease formats work on a similar principle. The protein molecules can be programmed to recognize a particular target sequence in the genetic material. The molecular scissors are activated at the targeted location in the genome and the cut leads to a double-strand break in the DNA. "This activates the repair

Photo: Fotolia / molekuul.be



Reprogramming and Genome Editing

Graphic: The Korean Association of Internal Medicine, Stanford University / Sebastian Diecke

response in the cell, which can then be used to bring about the intended modifications," explains Cathomen.

Simple and efficient

Because ZFNs and TALENs use purely protein-based DNA recognition systems, their engineering is relatively complicated and expensive. A degree of expertise in protein design is also called for. That is not the case with the CRISPR system. In this equivalent of an adaptive bacterial immune system, a special RNA molecule is all that is needed to focus the Cas9 enzyme onto a particular target. "The system can be engineered and introduced into cells using basic techniques available in any molecular biology laboratory," confirms Cathomen. "After one week, you can already expect a customized result with CRISPR-Cas," he adds. "It works extremely well."

Other researchers are also fascinated by this precision and speed. "It's quite amazing," says bioengineer Frank Buchholz of the medical faculty at TU Dresden. "This is a bacterial system that can be efficiently transferred to other cells, regardless of species." An additional benefit is that multiple locations in the genome can be processed at the same time. The prestigious publications that appear almost every week demonstrate the extent to which the new molecular scissors are fuelling the imagination of genetic researchers.

DFG Research Center for Regenerative Therapies Dresden (CRTD)

CRTD

At the DFG Research Center for Regenerative Therapies Dresden (CRTD), Cluster of Excellence at the TU Dresden scientists are seeking to understand the mechanisms of regeneration using model organisms to translate the results to man and to develop novel regenerative therapies for thus far incurable diseases. The center's major research areas are focused on hematology/immunology, diabetes, neurodegenerative diseases, bone regeneration and technology development. Currently, seven professors and ten group leaders are working at the CRTD. They are integrated into a network of over 80 member labs at 7 different institutions in Dresden. In addition, 21 partners from industry are supporting the research projects. The synergies in the network allow for a fast translation of results from basic research to clinical applications.

CRTD / DFG Research Center for Regenerative Therapies Dresden – Cluster of Excellence
Fetscherstraße 105
01307 Dresden
www.crt-dresden.de

The scope of application for genome editing is extremely broad. "Genetically modified organisms can now be produced relatively easily and quickly," says Buchholz. For example, it is possible in this way to develop better animal models for human diseases. He even sees CRIPR-Cas as a promising tool for functional high-throughput genomic screens as it allows the precise up- or down-regulation of gene activity.

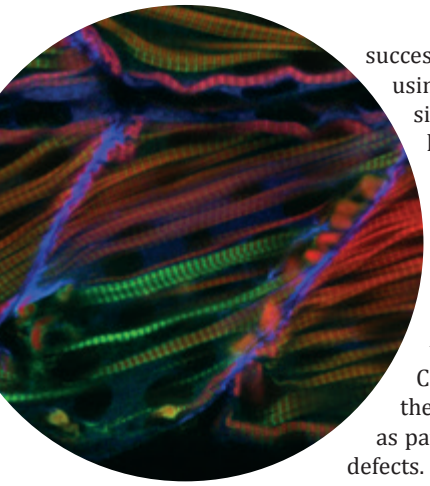
Controls for disease models

The German stem cell community has also been quick to adopt the designer nucleases. One of the great benefits in this area arises from combination with another new and powerful technology – that of cell reprogramming (see the chapter Stem cells from the factory, page 37). In this way, biomedical researchers can, for example, develop better "disease in a dish" models. "Either you introduce a mutation into the genome of induced pluripotent stem cells (iPS cells) of healthy individuals and investigate disease features in the cell types derived from them, or you use the nucleases to correct mutations in the iPS cells derived from patients," explains Toni Cathomen. An additional advantage of genome editing is the generation of what are called isogenic iPS cell lines: two lines that share an identical genotype with the single exception of the edited sequence. "These are the ideal controls, because they have the same genetic background," explains Christine Klein, a neurogeneticist at the Lübeck University Medical Center. This ensures that like is being compared with like. Klein is using genome editing in nerve cells derived from iPS cells for her research into the molecular causes of Parkinson's disease. This approach leads to significantly more robust models, even if a degree of biological and technical variability cannot be completely excluded with iPS cells.

Opportunities for gene therapy

The new super-scissors are also inspiring researchers to consider entirely new applications in personalized medicine. In gene therapy, for instance, the techniques of molecular biology are brought to bear in the treatment of hereditary gene defects. Until now, this has usually involved the compensation of a molecular defect by using gene ferries to introduce an additional, healthy copy of the gene into the patient.

The designer nucleases now enable researchers to work with surgical precision, correcting only the original mutation in a targeted fashion. Blood stem cells and T-cells are currently the focus of gene therapeutic strategies of this sort. Researchers in the U.S. have already conducted



successful early-stage clinical studies using zinc finger nucleases, the small size of which make them particularly well suited for clinical applications. These researchers succeeded in using genome editing to achieve a gene alteration in HIV patients that made them at least temporarily immune to the HIV virus in their bodies. Cathomen's team at the University Medical Center Freiburg is also working on therapies to cure HIV patients as well as patients with other chronic immune defects. And, he is convinced, "It is just a question of time before the most recent technology platforms, like TALENs and CRISPR, follow suit and find their way into clinical application."

Frank Buchholz' team is also working on an HIV therapy based on genome editing. He, however, is using a different group of high-tech tools called "tre-recombinases". "They cut viral genetic material out of infected cells very precisely, and they also glue the DNA strands back together accurately after the procedure," says Buchholz. This, he explains, makes the system very safe. Based on encouraging preclinical data, the researchers in Dresden are now working hard with their colleagues in Hamburg to test this therapeutic concept in early clinical studies.

Avoiding the misses

Despite their popularity, designer nucleases are not entirely error-free. They occasionally miss the target, and the molecular scalpels then make their cuts at other locations in the genome. These off-target effects must be excluded as far as possible if the technique is to be used for medical applications such as gene therapy. Based on studies performed in his lab, Toni Cathomen is reassuring on this point: "With the doses of TALENs we have used and with CRISPR-Cas, we have observed a high degree of cutting efficiency and scarcely any off-target effects."

Meanwhile, biotechnologists are tinkering with ways to make the nucleases even more reliable and user-friendly. One of the limitations that need to be addressed concerns the relatively large genetic blueprint of the Cas9 nuclease. This makes it difficult to pack as freight into gene ferries for delivery to living cells. However, there are already innovations here that promise to help solve the problem. The genetic engineering revolution is clearly moving ahead full steam.

Text: Philipp Graf

Editorial note:

*The fascinating possibilities in the techniques of genome editing, in particular by the CRISPR-Cas9 system, should facilitate the genetic tailoring of cell and tissue therapeutics in the future but raise far-reaching ethical issues. These methods would allow rapid intervention in the human germ line leading to the inheritance of both targeted and unwanted genetic alterations introduced into the genome. Accordingly, a group of leading scientists, including an inventor of the CRISPR system, published a statement in March 2015 in the journal Science warning against germline alterations in human beings and calling for a worldwide moratorium. They recommend that scientists should "strongly discourage, even in those countries with lax jurisdictions where it might be permitted, any attempts at germline genome modification for clinical application in humans, while societal, environmental, and ethical implications of such activity are discussed among scientific and governmental organizations." *The International Society for Stem Cell Research (ISSCR) also supports a moratorium on interventions in the human germline by genome engineering in reproductive medicine.*

**Original source:* Science *Journal: Baltimore et al. „A prudent path forward for genomic engineering and germline gene modification, 19 March 2015.*

Photo: MDC / Cecile Otten