Genome editing revolutionizes biomedical research and inspires new therapeutic approaches for diseases from HIV/AIDS to cancer to immune deficiencies. The 13th Herrenhausen symposium discussed current applications, developments in research and the challenges to translate these into the clinic.

Genome editing uses meganucleases, Zinc Finger (ZNF), so-called TALEN nucleases or the bacterial CRISPR/Cas system to insert, delete or replace DNA sequences in a living organism. These nucleases create double-strand breaks at precisely defined locations in the genome, which are then repaired by the cell's DNA repair mechanism. Depending on the conditions, it either stitches the loose ends together via non-homologous end joining (NHEJ) or replaces the damaged part through homologous directed repair (HDR) using the intact copy on the other chromosome as template. HDR can be exploited for genome editing by providing an appropriate donor DNA instead. Whereas ZNF and TALEN nucleases must be engineered to recognize a specific target sequence, CRISPR/Cas can be 'programmed' via a specific guide RNA (gRNA).

In practice, donor DNA, gRNA and the nuclease must be delivered to the cell; the nuclease can be delivered either as protein, DNA or messenger RNA. The nuclease then continues to cut their target DNA sequence until the cell's repair mechanism has removed the sequence through deletion, insertion or via HDR. Clinical applications involve either ex vivo manipulation of patients' own stem cells outside the body and re-injecting the corrected cells — which is standard practice in classical gene
therapy— or in vivo treatment by delivering nuclease and donor DNA to target cells, usually via Adeno-
associated viruses (AAV) or lentiviruses.

Toni Cathomen, in his keynote talk, gave an overview on the current state of gene and cell therapies
and the future impact of genome editing: whereas gene therapy provides an additional intact copy of
the mutated gene, genome editing can fix the error itself. Cathomen described various pre-clinical
developments to find cures for hemophilia, HIV or immune disorders and explained the major
challenges for genome editing in clinical use. These are in particular specificity to avoid off-target
editing elsewhere in the host genome, efficiency in terms of the number of cells that are correctly
edited, and delivery of the editing machinery to the correct cells.

Luigi Naldini described the success of gene therapy for SCID, which is now a safe and efficient cure
for this fatal immune deficiency. It uses ex vivo treatment of the patients’ blood stem cells to add an
intact copy of the mutated gene and re-injecting the corrected cells. Naldini’s group is now exploring
the use of genome editing to correct the gene, which still yields a lower percentage of ‘repaired’ cells
compared to gene therapy. Optimized procedures and stimulating blood stem cells prior to editing to
activate their DNA repair mechanism could improve efficiency.

Matthew Porteus and Daniel Bauer discussed the use of genome editing to cure sickle cell anemia.
Porteus’ group uses AAV to transport CRISPR/Cas, chemically modified gRNA and donor DNA into
blood stem cells to repair mutations in the ß-hemoglobin gene that cause the disease. Bauer takes a
different route by reactivating the γ-hemoglobin gene instead, which is usually shut down after birth.
Instead of the more challenging HDR to repair mutations, this requires only NHEJ to cleave a specific
genome sequences that keeps the gene silent.

Katherine High presented results of clinical trials to test the safety and efficacy of investigational
treatments for hemophilia and progressive vision loss. The latter uses AAV-mediated in vivo gene
transfer to the retinal pigment epithelial cells in affected individuals. This treatment for vision loss
involves injecting the vector with the repair machinery directly into the eye where it targets and repairs the cells that support the light-sensing cells.

Carl June and Isabelle Rivière demonstrated the use of gene editing for generating optimized CAR T cells – immune cells that are engineered to recognize and attack tumor cells. Rivière showed how gene editing could improve these cells’ efficiency against leukemia while June discussed generating CAR T cells that destroy solid tumors.

James Wilson’s experiments in mice to cure metabolic liver diseases by correcting gene defects with CRISPR/Cas showed that repairing only a small percentage of cells is sufficient to restore normal function. His work also unveiled that the efficiency of gene editing differs between adults and newborns, potentially owing to the fact that the newborn’s cells are rapidly proliferating and therefore more amenable to HDR.

Frank Buchholz explores gene editing to cure AIDS by destroying the quiescent HIV genomes in the human genome. His group has been exploring using recombinases instead of nucleases as these can excise longer DNA sequences from the genome. They engineered recombinases against HIV DNA and are testing these with HIV-infected blood stem cells.

Carlos Moraes explained how genome editing of mitochondrial DNA could be used to cure inherited mitochondrial diseases. Mitochondria, the cell’s energy-producing organelles, contain many copies of their own genome; it is therefore not necessary to repair gene defects but just destroy erroneous copies to restore proper function.

Preclinical developments and clinical trials of genome editing are not just pushing the boundaries of medical research but also challenging the regulation of drug development. Natalie Mount commented that regular and open interaction with regulatory agencies is therefore key for a successful transition of these Advanced Therapy Medicinal Products into the clinic.
A major issue for genome editing is to avoid off-target effects in other areas of the genome. One solution is to increase the nuclease’s specificity for the target sequence. Benjamin Kleinstiver presented his work on engineering Cas9 and the related Cpf1 nuclease to increase both target specificity and cutting efficiency.

Cutting is only the first step in gene editing, followed by NHEJ or HDR. Eric Hendrickson demonstrated that it generates quite different results depending on how the cell repairs DNA breaks. Understanding these mechanisms could help to further improve genome editing and gene therapy through the choice of appropriate gRNA and donor DNA.

Delivering the nuclease and donor DNA to the target cell is another major challenge for gene therapy. Thierry VandenDriessche uses tissue-specific promoter sequences to ensure that Cas is expressed only in specific cell types. Proof of principle experiments demonstrate a potential cure for myotonic dystrophy, a muscle-wasting disease, by repairing the gene defect in muscle cells.

Mark Kay’s group has engineered AAV that infect only specific cell types such as liver or nerve cells. As AAV integrates its DNA into the host cell genome, Kay uses a nuclease-free approach called gene ride to transfer a corrective DNA sequence into the target cell. One application is fixing a point mutation in liver cells that causes Crigler Najjar Syndrome, which leads to brain damage in infants.

Niren Murthy has developed a delivery system for the CRISP/Cas system based on gold nanoparticles. Experiments in mice models of Duchenne Muscular Dystrophy show some efficiency in correcting the dystrophin gene in muscle tissue with little off-target effects. Aditi Mehta presented her research on inhalable nanoparticles to deliver Cas9 and donor DNA in an attempt to target and destroy tumor cells in lung cancer.

As quiescent cells are more likely to use NHEJ whereas growing and dividing cells prefer HDR, Tony Gutschner has linked Cas activity to the cell cycle to improve the efficiency of genome engineering.
The longer CRISPR/Cas remains active in the cell, the higher the risk of off-target effects. Gianluca Petris’ group has thus developed a CRISPR/Cas9 system that self-destructs after it completed its mission.

Lukas Dow demonstrated the use of gene editing to model human disease in mice. They use CRISPR/Cas to cause mutations similar to those found in human colorectal cancer. Growing mutated stem cells into organoids — a three-dimensional organ bud with realistic micro-anatomy — allows them to study the effect of these mutations on cancer growth and metastasis.

Kosuke Yusa presented the application of gene editing for drug screening. His group uses CRISPR/Cas with a library of gRNAs to mutate cancer cells. Mutations that cause cell death could hint to genes essential for the cancer cell’s survival and thereby potential drug targets. His research has already yielded two such candidate genes for treating leukemia.

The discussion showed that many challenges remain to harness the full potential of gene editing. The choice of nuclease, target cells and DNA sequence, delivery vehicle and the mode of DNA repair determine efficiency and specificity, making it a personalized therapy. “Every patient gets a unique product,” Porteus remarked.

All academic titles have been omitted.

**AUTHOR**

Holger Breithaupt, embl, Science reporter

**FOR MORE INFORMATION VISIT**

ORGANIZATION
Christine Borowski, Nature Medicine, USA
Kevin Da Silva, Nature Medicine, USA
Markus Elsner, Nature Biotechnology, Germany
Alison Farrell, Nature Medicine, USA
Oliver Grewe, Volkswagen Foundation, Germany

SPEAKERS AND CHAIRS
Daniel Anderson, Massachusetts Institute of Technology, USA, Daniel Bauer, Harvard Medical School, USA, Frank Buchholz, Technische Universitäten Dresden, Germany, Toni Cathomen, Universitätsklinikum Freiburg, Germany, Lukas Dow, Weill Cornell Medical College, USA, Tony Gutschner, The University of Texas MD Anderson Cancer Center, USA, Eric Hendrickson, University of Minnesota, USA, Katherine High, University of Pennsylvania, USA, Carl June, University of Pennsylvania, USA, Mark Kay, Stanford School of Medicine, USA, Benjamin Kleinstiver, Harvard Medical School, USA, Carlos Moraes, University of Miami, USA, Natalie Mount, Cell and Gene Therapy Catapult, UK, Niren Murthy, University of California, Berkeley, USA, Luigi Naldini, Ospedale San Raffaele, Italy, Matt Porteus, Stanford University School of Medicine, USA, Isabelle Rivière, Memorial Sloan Kettering Cancer Center, USA, Thierry VandenDriessche, Free University of Brussels, Belgium, James Wilson, Perelman School of Medicine University of Pennsylvania, USA, Kosuke Yusa, Wellcome Trust Sanger Institute, UK

CONTACT
Volkswagen Foundation
Mareike Rüßmann, Event Management
Oliver Grewe, Funding Department

Kastanienallee 35
30519 Hannover, Germany
Phone: +49 (0)511 83 81 335
ruessmann@volkswagenstiftung.de