CRISPR-Cas9: From a bacterial immune system to genome-edited human cells in clinical trials

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ABSTRACT
The adaptive bacterial immune system CRISPR-Cas is revolutionizing all fields of life science and has opened up new frontiers toward personalised medicine. Since the elucidation of the molecular mechanism of Cas9 from Streptococcus pyogenes in 2012 and its development as a genomic engineering tool, genetic modifications in more than 40 species have been performed, over 290 patents have been filed worldwide and the first clinical trials using CRISPR-Cas-modified T-cells have recently been started in China and in the US.

In this review we summarise current design developments, novel Cas systems and their antagonists, present and potential future applications as well as the ongoing debate on ethical issues, which has arisen through the CRISPR-Cas technology.

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Introduction

In the late 1980s, unusually spaced, homologous sequence structures were noticed in the E. coli genome. In 2002 they were named clustered regulatory interspaced short palindromic repeat (CRISPR) arrays following the discovery of similar genomic structures in other prokaryotes and identification of common features of these sequences. It was found that the sequences in the CRISPR arrays originated mainly from bacteriophages (reviewed in) and that they are part of an acquired bacterial immune system. In the past, CRISPR arrays were exploited for strain differentiation between pathogenic bacteria. Throughout the following years, more and more features of the CRISPR loci were unravelled: the connection of CRISPR arrays to the CRISPR associated (Cas) genes and trans-activating CRISPR RNAs (tracrRNA) encoded nearby (reviewed in), the ability of some Cas proteins to cleave double-stranded DNA, as well as the mode of Cas9 DNA cleavage using CRISPR RNA (crRNA) and tracrRNA. In 2014, Cas9 from Streptococcus pyogenes (SpCas9) was shown to be able to cleave genomic DNA in human cells site-specifically, directed by the sequence of a single-guide RNA (sgRNA), an artificial fusion product of a crRNA and a tracrRNA. This confirmed its potential applicability for genome engineering. By now, more than 800 publications report the application of the S. pyogenes CRISPR-Cas9 system for site-specific genome modifications, gene knockouts or replacements, gene expression control and functional genome screenings in over 40 different species including plants, microbes (reviewed in) and animals. Furthermore, other CRISPR-Cas systems have been discovered in bacteria and archaea, with different specificities and properties. In addition to the variety of CRISPR-Cas systems, several anti-CRISPR proteins were identified to be encoded in mobile genetic elements and bacteriophages. These mediate horizontal gene transfer despite the immunity conveyed by CRISPR-Cas systems.

Here we provide a summary of different CRISPR-Cas systems and CRISPR-Cas antagonists known to date. We report how they have been modified as tools for genome engineering and point out their current and possible future applications. This powerful...
technology can be easily used for the generation of genetically modified organisms, and can potentially be applied in medicine and in germline engineering. Therefore, numerous patents have been filed and an ongoing debate about associated risks and ethical implications has been started. These will be addressed, as well as the recently started first clinical trials with CRISPR-Cas-modified T-cells to target cancer cells.

**General function and classes of CRISPR-Cas systems**

How do CRISPR-Cas systems mediate immunity in bacteria and archaea? In the adaptation step the sequence information of invading nucleic acids is stored in the bacterial or archaeal genome on the CRISPR locus as so-called protospacer, thus creating a memory of past infections (Fig. 1A). Following transcription of the CRISPR locus and processing of the resulting pre-crRNAs into mature crRNAs, the crRNAs are bound by the Cas endonuclease. The thereby activated effector nuclease complex is able to recognize reoccurring invading nucleic acids due to base complementarity between crRNA and target strand. Crucial for Cas-mediated degradation is the presence of a specific 2–5 nucleobase sequence on the target nucleic acid, the protospacer adjacent motif (PAM), which prevents the endonuclease from self-targeting (reviewed in19).

CRISPR-Cas systems can be grouped into 2 classes and further divided into 6 subtypes, based on the architecture of the CRISPR loci, the presence of different Cas genes, the composition and processing of the crRNA, as well as the properties and function of the Cas endonuclease (Fig. 1B). To date, CRISPR-Cas systems have been found in about 50% of all bacterial and 95% of archaeal genomes, although different species possess different CRISPR-Cas subtypes.20 The effector nucleases of class 1 systems are multi-protein complexes (type I, III, IV). In contrast, in class 2 CRISPR-Cas systems (type II, V, VI) a single protein endonuclease is responsible for cleavage of the target nucleic acid.21 Class 2 endonucleases, such as Cas9 (type II), Cpf1 (type V) and C2c2 (type VI) (Fig. 1), are of particular interest for biotechnological and medical applications. They differ, for instance, in the PAM-sequences they recognize as well as in their cleavage properties. While Cas9 endonucleases create blunt ends in double-stranded DNA, the nuclease Cpf1 from *Francisella novicida* cleaves double-stranded DNA generating 5'-overhangs. In contrast, C2c2 from *Leptotrichia shahii* was shown to be an RNA-guided RNA endonuclease.15-17 Additionally,
Cas9 homologs from different species show variation in efficiency and specificity. Such distinct characteristics could prove suitable for different future applications discussed below.

**Anti-CRISPR systems**

The constant evolutionary arms race between the bacterial defense and their parasites (phages) is reflected in the existence of anti-CRISPR systems. Despite the presence of the powerful adaptive immunity provided by CRISPR-Cas, these anti-CRISPR systems permit horizontal gene transfer, which is the major driving force of bacterial evolution. Anti-CRISPR systems have been found in bacteriophages and parasitic mobile genetic elements and were shown to encode small, proteinogenic CRISPR-antagonists. These proteins comprise of less than 200 amino acids, bind directly to the effector nuclease, and hence block degradation of the target nucleic acids. In the future, anti-CRISPR proteins could be used to control genome editing by CRISPR-Cas, thus decreasing the number of off-target edits and increase the safety of the technique discussed below.

**Development of the CRISPR-Cas9 system as a genomic engineering tool**

To date, the class 2 Cas9 protein from *S. pyogenes* is the best-characterized effector nuclease. In nature, 2 RNAs are necessary for Cas9 activation: a tracrRNA and a crRNA. Cas9 recognizes the specific tertiary structure of the crRNA-tracrRNA complex. These 2 RNAs can be artificially combined to a single guide RNA (sgRNA) through a tetra-loop, hence reducing the system to 2 components, which facilitates the application of CRISPR-Cas9 (Fig. 2).

By alteration of the sgRNA sequence SpCas9 can be activated, programmed and located to a desired specific genomic position. However, the modification efficiency is dependent on the organism and the cell type, as well as on the sgRNA-stability and the accessibility of the target sequence in the genome. The latter is strongly influenced by the chromosomal context, due to the presence of nucleosomes and epigenetic modifications.

By now, the CRISPR-Cas9 technology is established as a general genome editing tool, with more than 800 published articles describing its use in various cell types and organisms, including yeast, fungi, plants, mammals and human zygotes. (reviewed in14,27). Its possible applications appear to be nearly unlimited: For one, the function of genes, their regulation as well as the interplay of whole gene and protein networks can be elucidated by investigating specific deletions, insertions or modifications of DNA sequences. Furthermore, a cleavage-incompetent Cas9 (dCas9) can be exploited to perform transcriptional activation or repression (CRISPR interference reviewed in13) or to deliver effector proteins, such as transcriptional regulators, DNA methylases, or fluorescent markers to distinct genomic locations. This can provide insights into function, architecture and spatial-temporal dynamics of genomic elements. In addition, resistant plants and model organisms for complex genetic diseases can now be efficiently generated within only a few months instead of years. Moreover, in the future it might be possible to cure viral diseases by targeting viral DNAs (i.e. from human immunodeficiency virus (HIV) or human papilloma virus (HPV)), as well as to correct genetic mutations with CRISPR-Cas9. Due to these prospects, at least 290 patents have been filed worldwide in the last 5 years, covering methods such as genome editing, gene conversion or transcriptional modulation, as well as genetically modified organisms and medical treatments including immunotherapy (Espacenet search28 on 24 January 2017).

**Challenges and ethics of the CRISPR-Cas9 technology**

However, questions regarding the safety and risk of CRISPR-modified organisms as well as the usage of the technology itself remain. For instance, CRISPR-Cas-modified plants and animals are currently bypassing legislation of biotech products in many countries, since they often do not contain any heterologous genetic material, i.e., from plant pests. Thus, the US Department of Agriculture recently decided that CRISPR-edited crops, such as the anti-browning mushroom and a waxy maize can be cultivated and sold without oversight. Other countries as well as the European Union are still debating about a reasonable consensus. An update of the regulations for genetically modified products is long overdue and a revision of the system has been started.

For medical applications, the CRISPR-Cas9 technology promises personalised medicine with, to date, unseen precision. In addition, it also seems to be able
to bridge the gap between the genetic information and the treatment of diseases. However, the CRISPR-Cas9 technology still has a long way to go before it can be applied in personalized medicine. Major issues that need to be resolved are the efficiency of on-target genome editing, as well as cleavage and mutations at unintended, near-cognate genomic sites (off-target effects), as this is a safety issue for potential therapeutic applications. For example, the frequently low modification efficiency leads to a mosaic genotype (chimeras). Hence, a large number of cells need to be treated and methods have to be developed to identify and select the desired cells.

SpCas9 tolerates up to 5 mismatches (reviewed in) which is not acceptable for therapeutic use. Consequently, it was shown that off-target effects are exacerbated by prolonged activity and excessive SpCas9. Additionally, on-target mutagenesis by non-homologous end-joining (NHEJ) could, for example, unintentionally convert sickle cell disease into β thalassemia. Moreover, the genetic background in which a disease mutation exists is usually unknown and might have partly adapted to carrying that mutation. Thus, correcting the pathogenic mutation could have unanticipated consequences and the long-term effects of such interventions are not known. Furthermore, editing of the genome counteracts natural selection in populations and diversity of human variants. Here the consequences are not predictable, since variant alleles may have important advantages in situations that cannot be anticipated yet. All these factors have to be considered when opting for full control over the technique and for safe applications, i.e., in the case of gene drives. Here, desired genes propagate throughout natural populations by non-Mendelian, forced inheritance, to restrict disease vectors such as mosquitoes. Once introduced in the environment such gene drives are difficult to restrain and could have unpredictable ecological repercussions.

By now, the SpCas9 efficiency could be increased and off-target effects could be limited by introducing mutations into SpCas9, which enforce more stringent Watson-Crick base pairing between the RNA guide and the target DNA strand. A number of sequencing and computational methods to evaluate genome editing outcomes have already been developed. In addition, it was shown that using an optimised sgRNA or multiple different sgRNAs to knock out a single target gene leads to an improved on-target
editing efficiency (reviewed in[34]). Moreover, by stabilization of the sgRNA or crRNA through chemical modifications such as methylation and fluorination of the 2’-hydroxyl, or usage of a phosphothioate backbone, a strategy previously used to stabilize small interfering RNAs in gene silencing, resulted in a significant increase of SpCas9 mediated on-target and decrease of off-target genome editing in human cells[39,40]. Furthermore, methods to control the genome editing activity by Cas9 are currently tested, which for example allow to specifically activate SpCas9 through stimuli such as light and drugs, or to genetically encode anti-CRISPRs[18,41,42]. These efforts are pointing into the right direction. However, despite the improvements of the SpCas9 technology, new methods are still needed to improve the safety of the CRISPR technology.

Not only safety issues, but also numerous ethical questions remain to be answered before attempting to edit the human genome or even the germline. If the technology could be used safely in the future, it would be expensive and hence only rich people and the richest societies might have access. In addition, no boundary of acceptable biological traits for editing is defined. Initially, only the most severe genetic diseases, such as cystic fibrosis, muscle dystrophy or hemophilia, might be treated. But is it acceptable to also remove mutations that could cause late onset or manageable diseases in combinations with other factors, such as Alzheimer’s disease and diabetes? Safety issues due to misuse and premature clinical applications will remain. Furthermore, genome modifications of fertilized eggs or embryos are passed on to every single cell in an organism, including the germline. Researchers and stakeholders have already called for a moratorium on the modification of the human germline. They call for transparency and an open, international discussion, to fully consider all possible issues, engaging a wide variety of experts, such as clinicians, scientists, lawyers, ethicists, as well as the general public.[43–45] Such open discussions are needed, particularly when taking into account that in China and the US the first clinical trials with CRISPR-Cas9 engineered autologous T-cells are on the way. Here T-cells are taken from a cancer patient and their T-cell receptors are modified such that they target and kill the tumor cells, followed by reinjection into the patient.[46]

In summary, CRISPR-Cas is one of the most revolutionising technologies of the last decades, both in science and society. Its potential applications and benefits will have to be carefully examined and weighed against the safety issues and ethical concerns that still have to be addressed. This should be done by the broad public and not by scientists or politicians alone to establish broad acceptance for the technology.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J. Bacteriol 1987; 169:5429-33; PMID:3316184; https://doi.org/10.1128/jb.169.12.5429-5433.1987
[2] Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. Mol. Microbiol 2002; 43:1565-75; PMID:11952905; https://doi.org/10.1046/j.1365-2958.2002.02839.x
[3] Mojica FJ, Diez-Villasenor C, Soria E, Juez G. Biological significance of a family of regularly spaced repeats in the genomes of archaea, bacteria and mitochondria. Mol. Microbiol 2000; 36:244-6; PMID:10760181; https://doi.org/10.1128/jb.169.12.5429-5433.1987
[4] Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindromic repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 2005; 151:2551-61; https://doi.org/10.1099/mic.0.28048-0
[5] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. Science 2007; 315:1709-12; PMID:17379808; https://doi.org/10.1126/science.1138140
[6] Groenen PM, Bunschoten AE, van Soolingen D, van Embden JD. Nature of DNA polymorphism in the direct repeat cluster of Mycobacterium tuberculosis; application for strain differentiation by a novel typing method. Mol. Microbiol 1993; 10:1057-65; PMID:7934856; https://doi.org/10.1111/j.1365-2958.1993.tb00976.x
[7] Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology 2005; 151:653-63; https://doi.org/10.1099/mic.0.27437-0
[8] Westra ER, Swarts DC, Staals RH, Jore MM, Brouns SJ, van der Oost J. The CRISPRs, they are a-changin’: how prokaryotes generate adaptive immunity. Annu. Rev. Genet 2012; 46:311-39; PMID:23145983; https://doi.org/10.1146/annurev-genet-110711-155447

[9] Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadán AH, Moineau S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 2010; 468:67-71; PMID:21048762; https://doi.org/10.1038/nature09523

[10] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012; 337:816-21; PMID:22745249; https://doi.org/10.1126/science.1225829

[11] Sakuma T, Nishikawa A, Kume S, Chayama K, Yamamoto T. Multiplex genome engineering in human cells using all-in-one CRISPR/Cas9 vector system. Sci. Rep. 2014; 4:5400; PMID:24954249; https://doi.org/10.1038/srep05400

[12] Noman A, Aqeed M, He S. CRISPR-Cas9: tool for qualitative and quantitative plant genome editing. Front. Plant Sci 2016; 7:1740; PMID:27917188; https://doi.org/10.3389/fpls.2016.01740

[13] Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Noman A, Aqeel M, He S. CRISPR-Cas9: tool for qualitative and quantitative plant genome editing. Nat Methods 2013; 10:1116-21; PMID:24076762; https://doi.org/10.1038/nmeth.2681

[14] Bondy-Denomy J, Garcia B, Strum S, Du M, Rollins MF, Hidalgo-Reyes Y, Lee J, Edraki A, Shah M, Sontheimer EJ, Maxwell KL, Davidson AR. Multiple mechanisms for CRISPR-Cas inhibition by anti-CRISPR proteins. Nature 2015; 526:136-9; PMID:26414091; https://doi.org/10.1038/nature15254

[15] Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell 2015; 6:363-72; PMID:25894090; https://doi.org/10.1007/s13238-015-0153-5

[16] Shmakov S, Abudayye O, Makarova KS, Wolf YI, Gootenberg JS, Semenova E, Minakhin L, Joung J, Konermann S, Severinov K, et al. Discovery and functional characterization of diverse Class 2 CRISPR-Cas systems. Mol. Cell. 2015; 60:385-97; PMID:26593719; https://doi.org/10.1016/j.molcel.2015.10.008

[17] Abudayye OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DB, Shmakov S, Makarova KS, Semenova E, Minakhin L, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 2016; 353:aaf5573; PMID:27256883

[18] Fonfara I, Richter H, Bratovic M, Le Rhun A, Charpentier E. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature 2016; 532:517-21; PMID:27096362; https://doi.org/10.1038/nature17945

[19] Pawluk A, Amrani N, Zhang Y, Garcia B, Hidalgo-Reyes Y, Lee J, Edraki A, Shah M, Sontheimer EJ, Maxwell KL, et al. Naturally occurring off-switches for CRISPR-Cas9. Cell 2016; 167:1829-38 e9; PMID:27984730; https://doi.org/10.1016/j.cell.2016.11.017

[20] Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A. 2012 Sep 25; 109(39):E2579-E2586.

[21] Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, Barrangou R, Brouns SJ, Charpentier E, Haft DH et al. An updated evolutionary classification of CRISPR-Cas systems. Nat. Rev. Microbiol 2015; 13:722-36; PMID:26411297; https://doi.org/10.1038/nrmicro3569

[22] Mohanraju P, Makarova KS, Zetsche B, Zhang F, Koonin EV, van der Oost J. Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems. Science 2016; 353:aad5147; PMID:27493190

[23] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern crispr AND (txt cas OR txt cas9).

[24] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[25] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[26] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[27] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[28] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[29] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[30] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."

[31] Espacenet: free access to the database of over 90 million patents, https://worldwide.espacenet.com, search pattern "txt = crispr AND (txt = cas OR txt = cas9)."
[32] Bolukbasi MF, Gupta A, Wolfe SA. Creating and evaluating accurate CRISPR-Cas9 scalpels for genomic surgery. Nat. Methods 2016; 13:41-50; PMID:26716561; https://doi.org/10.1038/nmeth.3684

[33] Pattanayak V, Lin S, Guilinger JP, Ma E, Doudna JA, Liu DR. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. Nat. Biotechnol 2013; 31:839-43; PMID:23934178; https://doi.org/10.1038/nbt.2673

[34] Nowak CM, Lawson S, Zerez M, Bleris L. Guide RNA engineering for versatile Cas9 functionality. Nucleic Acids Res 2016; 44:9555-64; PMID:27733506

[35] Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 2014; 24:1012-9; PMID:24696461; https://doi.org/10.1101/gr.171322.113

[36] Caplan AL, Parent B, Shen M, Plunkett C. No time to waste-the ethical challenges created by CRISPR: CRISPR/Cas, being an efficient, simple, and cheap technology to edit the genome of any organism, raises many ethical and regulatory issues beyond the use to manipulate human germ line cells. EMBO Rep 2015; 16:1421-6; PMID:26450575; https://doi.org/10.15252/embr.201541337

[37] Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science 2016; 351:84-8; PMID:26628643; https://doi.org/10.1126/science.aad5227

[38] Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V, Wyvekens N, Khayter C, Iafrate AJ, Le LP, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat. Biotechnol 2015; 33:187-97; PMID:25513782; https://doi.org/10.1038/nbt.3117

[39] Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, Steinfeld I, Lunstad BD, Kaiser RJ, Wilkens AB, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat. Biotechnol 2015; 33:39-50; PMID:26121415; https://doi.org/10.1038/nbt.3290

[40] Rahdar M, McMahon MA, Prakash TP, Swayze EE, Bennett CF, Cleveland DW. Synthetic CRISPR RNA-Cas9-guided genome editing in human cells. Proc. Natl. Acad. Sci. USA 2015; 112:E7110-7.

[41] Nihongaki Y, Kawano F, Nakajima T, Sato M. Photoactivatable CRISPR-Cas9 for optogenetic genome editing. Nat. Biotechnol 2015; 33:755-60; PMID:26076431; https://doi.org/10.1038/nbt.3245

[42] Nunez JK, Harrington LB, Doudna JA. Chemical and biophysical modulation of Cas9 for tunable genome engineering. ACS Chem. Biol 2016; 11:681-8; PMID:26857072; https://doi.org/10.1021/acschembio.5b01019

[43] Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, Corn JE, Daley GQ, Doudna JA, Fenner M, et al. Biotechnology. A prudent path forward for genomic engineering and germline gene modification. Science 2015; 348:36-8.

[44] Bosley KS, Botchan M, Bredenoord AL, Carroll D, Charo RA, Charpentier E, Cohen R, Corn J, Doudna J, Feng G, et al. CRISPR germline engineering - the community speaks. Nat. Biotechnol. 2015; 33:478-86; PMID:25965754; https://doi.org/10.1038/nbt.3227

[45] Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don’t edit the human germ line. Nature 2015; 519:410-1; PMID:25965754; https://doi.org/10.1038/nbt.3227

[46] Cyranoski D. CRISPR gene-editing tested in a person for the first time. Nature 2016; 539:479; PMID:27882996; https://doi.org/10.1038/nature.2016.20988