HUMAN GENOME EDITING

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The rapidly evolving genome editing techniques are steadily moving from research laboratories to clinical practice. Fundamentally new methods of editing the genome of human embryos in the early stages of development have been developed. Tools for correction of genetic disorders in people of any age have also been created. In fact, the doctor is becoming a corrector of genetic instructions on construction and functioning of the human body. This review generalizes the data on the current state of genome editing techniques and existing approaches to applying them in clinical practice.

Keywords: genome editing, gene therapy, hereditary diseases, oncology, HIV

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РЕДАКТИРОВАНИЕ ГЕНОМА ЧЕЛОВЕКА

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Быстро развивающиеся технологии редактирования генома из научно-исследовательских лабораторий уверенно переходят в клиническую практику. Разработаны принципиально новые методы изменения генома человеческих эмбрионов на ранних стадиях развития. Создан инструментарий для исправления генетических нарушений у людей в любом возрасте. Врач, по сути, становится корректором генетической инструкции по построению и функционированию организма человека. В обзоре обобщены сведения о современном состоянии технологий редактирования генома и существующих подходах к их использованию в клинической практике.

Ключевые слова: редактирование генома, генотерапия, наследственные заболевания, онкология, ВИЧ

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Genome changes occur constantly in living organisms, determining the course of their evolution. Man started interfering in this process thousands of years ago, selecting successfully cultivated plants and producing breeds of pets. Genetic engineering, which emerged a little more than half a century ago, made it possible to create transgenic organisms: gene transfer between genomes or gene manipulation within a single genome. The idea of using DNA imported from the outside for treatment of human genetic diseases originated in the early 1970s [1]. In the 1980s, improvement in gene handling techniques and creation of eukaryotic vectors opened up a real opportunity for correction of human genetic material for therapeutic purposes. However, the first successful result was only reported in 1990 [2]. That same year, researchers used a retrovirus to inject a working adenosine deaminase (ADA) gene into the cells of four-year and nine-year old female patients with severe combined immunodeficiency. From 1993, gene therapy was regularly used to treat newborns with ADA deficiency, by delivering the gene into undifferentiated cells of the umbilical cord blood.

We live in the genomics era, and the term ‘genomic therapy’ is increasingly found in science literature today. Perhaps, the use of the terms ‘gene therapy’ and ‘genomic therapy’ needs to be clarified. Since terminology issues are not fundamental, these terms can either be considered as interchangeable or one can consider the term ‘genomic therapy’ as a variant of ‘gene therapy’ in which nuclear genome (chromosomal DNA) changes. The point is that gene therapy may not have anything to do with chromosome — the delivered gene may operate as an extrachromosomal element (plasmid) or can be injected in the form of messenger RNA (mRNA); moreover, mitochondrial DNA may be subjected to modification.

Between 1989 and 2016, over 2,300 clinical trials had been conducted worldwide [3]. To date, there are more or less effective approaches to gene therapy for treatment of over 50 genetically determined diseases in humans, including severe combined immunodeficiency [4], hemophilia [5, 6], hemoglobinopathies [7–13], cystic fibrosis [14,15], achromatopsia [16], Leber’s congenital amaurosis [17–19], epilepsy [20], osteoarthritis [21, 22], Parkinson’s disease [23–25], and a wide range of cancers [26–32].

For the past few years, with the emergence of qualitatively new directed genome change techniques (ZFNs, TALENs, CRISPR/Cas9), areas of applications for clinical trials of gene therapy drugs have skyrocketed in number like an avalanche. Thanks to the simplicity and accuracy of new techniques

deployed to introduce changes in the genomic DNA of eukaryotic cells, a new term 'genome editing' even arose — after all, DNA change may in the future be used not only for therapeutic purposes but also for less important tasks.

Genomic therapy can be applied for: treatment of hereditary (usually monogenic) diseases, treatment of diseases caused by somatic mutations (mostly cancer), and attempts to treat HIV infections by destroying the copies of the virus integrated into the genome or receptor genes allowing the virus to enter the cell. Genomic therapy is one of the variants of personalized medicine, when the approach used is selected individually to the patient's disease (and sometimes even to his genome).

Russia's Federal Law No. 86-FZ dated 5th July 1996 "On State Regulation in the Field of Genetic Engineering" defines gene therapy as a set of genetic engineering (biotechnological) and medical techniques aimed at making changes in the genetic apparatus of human somatic cells for the purpose of treating diseases. With the advent of Federal Law No. 180-FZ dated 23 June 2016 "On Biomedical Cell Products", the number of clinical trials of innovative gene therapy products in Russia is expected to increase.

Genome editing techniques

Although there are a variety of methods for directed change of complex eukaryotic genomes, only a few of the methods are currently used in practice:

a) non-break induced homologous recombination [33];

b) site-specific recombination (recombinase and transposase) [34, 35];

c) repair induced by site-specific nuclease, where the following are used as the nuclease:

– artificial (hybrid, designer) nucleases with zinc finger nucleases (ZFNs) [36–38],

– natural or hybrid endonucleases of gene conversion or meganucleases (homing endonucleases, HEs) [39],

– artificial (hybrid, designer) nucleases with transcription activator-like effector nucleases (TALENs) [40],

– natural RNA-guided nucleases (RGNs), in particular, clustered regularly interspaced short palindromic repeats / CRISPR-associated nuclease 9 (CRISPR / Cas9) with designer 'guide' RNA [41],

– a combination of various nucleases [42–44].

Fig. 1 shows a timeline indicating the evolvement of genome editing techniques [45].

To date, the most promising techniques are those based on the use of artificial (so-called hybrid or designer) site-specific nucleases: ZFNs, TALENs and CRISPR/Cas9 [46]. Although, the term 'hybrid' (or 'designer') nuclease was initially fully applied to 'protein' techniques ZFN and Talen, today the CRISPR/Cas9 technique can be confidently categorized under the same class since the RNA in this system is the designer component (similarly to the 'guide' blocks of the ZF or TALE domain) (fig. 2) [47].

In general, each of these genome editing tools consists of three components: 'guide' specific to DNA sequence (indicating where to cut), cutting DNA 'scissors' (endonuclease) and the actually introduced DNA sequence (not always necessary). Delivery into cells a 'genetic patch' (a DNA fragment to be substituted) is required in those cases where it is necessary to add or substitute a genomic fragment. However, in some cases, only part of the sequence needs to be removed. Table 1 shows the features of the most popular genome editing systems.

We use the 'guide' to indicate endonuclease, where a cut needs to be made in the DNA molecule. The cut is stitched usually through intracellular repair systems (such as double-stranded break repair or homologous recombination).

Since genome editing techniques — based on homologous recombination, recombinase and transposase — have been actively used in clinical practice for over 30 years and they are well described in literature (a Nobel Prize was even awarded in 2007 for homologous recombination techniques), we will not delve into details in this review. Rather we will elaborate on relatively new approaches based on hybrid nucleases and meganucleases, as well as directed double-stranded break repair.

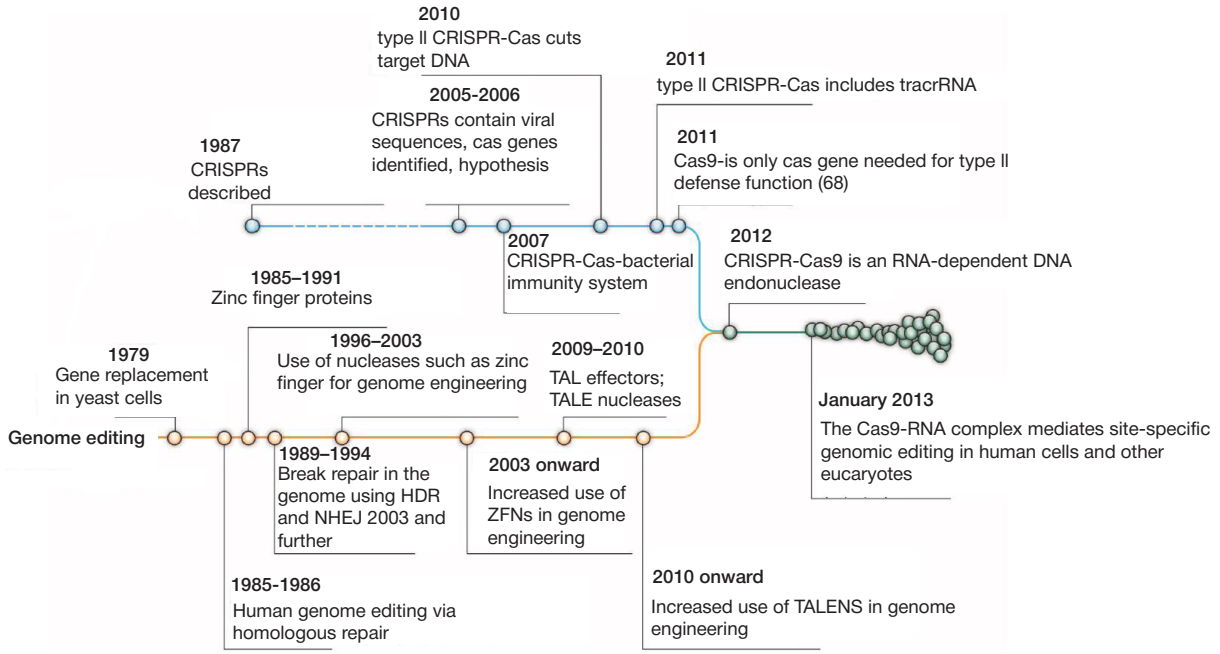

Fig. 1. Timeline indicating the evolvement of some genome editing systems (Doudna, Charpentier [45])

Guidance of nuclease using zinc fingers (ZFNs)

Small domains stabilized by one or more zinc ions and known as 'zinc fingers' were identified around the middle of the 1980s (for the first time as part of *Xenopus laevis* transcription factor TFIIIA). These domains can bind DNA, RNA, proteins and lipids effectively and quite specifically [48]. It turned out that a zinc finger specifically binds a triplet of nucleotides. If 3–6 zinc fingers with known specificity are combined into a single protein, sufficiently accurate recognition of DNA sequence in 9–18 base pairs can be achieved. Here, any endonuclease (the most commonly used is non-specific endonuclease and FokI catalytic domain from *Flavobacterium okeanokoites* which introduces a single-stranded break) is added to the zinc fingers, you get targeted endonuclease. To get a double-stranded break, it is necessary to create two such enzymes recognizing neighboring regions on opposite DNA strands (fig. 2).

Since the beginning of the 2000s and up till now, zinc-finger systems had been successfully used in a wide range of practical modifications of genomes both on plant and animal models, and on therapeutic approaches (table 2). The advantage of the method is the versatility of its nuclease targeting technique. The disadvantages include the relatively high complexity of genetic engineering assembly of the enzyme gene; the need to create two enzymes for each of the DNA strands; toxicity associated with lack of specificity of this type of systems [49]; risk of immunogenicity of foreign proteins [50]. In this regard, the use of zinc-finger systems is gradually being replaced by new approaches.

Hybrid meganucleases

In 2003, Epinat et al. proposed a genomic editing technique based on the so-called meganucleases [39] (fig. 2). Meganucleases were found in archaea, bacteria, phage, yeast, algae and some plants, and they are endodeoxyribonuclease — small proteins, mirror monomers or homodimers, characterized by a very long double-stranded DNA recognition site: from about 10 to 40 base pairs. Usually, a site of such length is seen only once in the genome or even never. For example, the I-SceI meganuclease recognition site, which is 18 base pairs in length, theoretically occurs once in the genome, exceeding the length of the human genome by 20 times. Typically, they form a part of introns or transposable elements of the genome. The biological function of meganucleases is unclear.

Representatives of the LAGLIDADG meganuclease family, found in the mitochondria and chloroplasts of unicellular eukaryotes, are the most widely used tool for genomic editing. Advantages of the technique involve the fairly high site-specificity and spontaneous dimer assembly. The disadvantage — high limitation on impact site selection.

TALEN (transcription activator-like effector nucleases) technique

The history of development of the TALEN system is connected with the study of the *Xanthomonas* bacteria genus. The reason for the long-term study of this group of bacteria was their pathogenic effects on crops, including tomatoes, peppers, rice and others. It was found that *Xanthomonas* secrets regulatory proteins — transcription activator-like effectors (TALE) — into the plant cell cytoplasm. These proteins increase the susceptibility of cells to the pathogen. Upon further study of the mechanisms of action of these proteins, they were found to be capable of binding to DNA and activating the expression of certain genes, mimicking host cell transcription factors [51, 52].

It was found that the TALE of a particular site in the DNA is recognized with the help of a series of small domains, each of which recognizes a single nucleotide in the site. Researchers figured out quite quickly the specificity of domains to specific nucleotides. This allowed to collect from them 'packs' precisely recognizing a specific sequence of bases in DNA.

Thus, the principle of use of TALEN system is similar to that of the system described above using triplet specific zinc-finger domains. The only difference is that nucleotide-specific domains (transcription activator analogs), connected in series by 12–20 pieces each are used as the 'guide', while the proven FokI catalytic domain is used as the nuclease. For double-stranded break, it is necessary to create two such enzymes (the target landing sites of 'guide' TALEs) that should be on the opposite DNA strands and separated by a site of about 20 base pairs (fig. 2). Advantages of the method: versatility of the nuclease guiding technique and versatility of the designer nuclease assembly technique. Disadvantages: high complexity of genetic engineering assembly of the enzyme gene and the need for creation of two enzymes for each of the DNA strands.

There are attempts to cross individual elements of different techniques. For example, hybrids of 'guide' TALEs and meganucleases (megaTALs) are described [42]. There are attempts to attach enzymes to meganucleases (by one way or another), which process (for example, destroy) the ends of the double-stranded break in order to strengthen the mutagenic effect of this break, and achieve other effects [43, 44].

In 2012, the Nature Methods journal named high-precision genome editing methods the methodical discovery of the year. TALEN was included in the methods.

CRISPR/Cas9 (nuclease associated with regularly interspaced short palindromic repeats) technique

The CRISPR/Cas9 system — proposed just a few years after TALEN — is a fundamentally different system when it comes to mechanism for guiding a nuclease to the target. The system differs from TALEN by the fact that as a 'guide', it uses not

Table 1. Composition of the components of the main enzyme of genome editing systems, and some features of the systems

Technique	Sequence	«Guide»	Scissors	Double-stranded break caused by:
ZFNs	Almost none	3–6 zinc finger protein domain	Non-specific endonuclease (for example, FokI)	Artificial heterodimer
HEs	Limited set of sites	Meganuclease	Meganuclease	Natural mirror monomer or homodimer
TALENs	Almost none	12–20 protein domains from transcription activator	Non-specific endonuclease sequence (for example, FokI)	Artificial heterodimer
RGNs (CRISPR/Cas9)	None	RNA of about 40 nucleotides in length	Cas9 nuclease	Artificial heterodimer

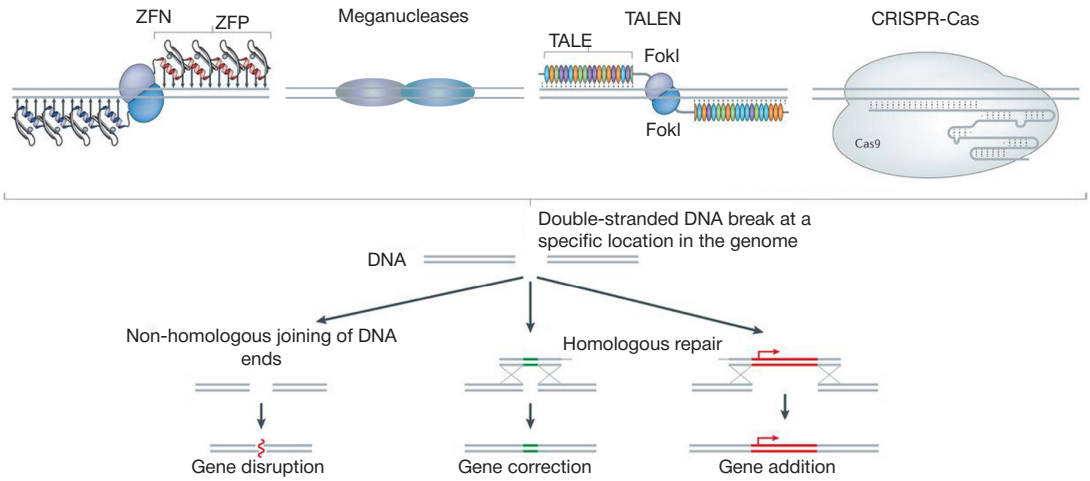

Fig. 2. Scheme of genomic editing based on zinc-finger hybrid nucleases, meganucleases, TALE hybrid nucleases and CRISPR/Cas9 (Yin et al., 2014 [47], as amended)

protein domains but RNA molecule (subgenomic RNA, sgRNA) of about 40 nucleotides in length, consisting of two parts: 'guide' crRNA and adaptor (trans-activating) tracrRNA. CRISPR elements were discovered in bacterial and archaeal genomes in the late eighties. It turned out that this is apeculiar bacterial 'immune system' element protecting the immune system against foreign DNA (such as bacteriophage penetration) by reading from complementary DNA phage of replicates of RNA molecules, which, in association with specific nuclease, disrupt the phage genome. Moreover, bacteria are able to remember the DNA sequences that infected their phages in order to continue to use them for reading 'guide' RNA [53].

It was also found that the sequence of these 'guide' RNAs can be changed, making them complementary to any DNA region without losing the nuclease activity of the Cas9 enzyme (fig. 2). Moreover, the RNA itself can be used as a genetic patch donor if the corresponding sequence is built in it [54].

At the moment, the CRISPR/Cas9 looks the most promising genome editing tool because it is versatile, fairly simple to apply and has high site specificity.

The method has several important advantages: versatility of the nuclease guiding method; there is no need for genetically engineered assembly of enzyme — only the 'guide' RNA changes; ability of Cas9 to cut both DNA chains; ability to integrate a genetic patch into the 'guide' RNA. Disadvantage: potential immunogenicity of a foreign protein.

Genomic therapy algorithms

Therapeutic uses of genome editing systems can be divided into three groups: 1) changing the genome of gamete/zygote/blastomeres for the purpose of obtaining a whole organism from one modified cell (fetal gene therapy); 2) changing the genome of individual somatic cells selected from the body for the purpose of subsequently returning the modified cells to the organism (somatic cell gene therapy); 3) changing the genome of individual groups (or all) of somatic cells in a multicellular organism directly (tissue somatic gene therapy).

The first two approaches involve manipulation of cell cultures in the lab (for which the broadest technology base is currently being developed). For the third approach, special systems (preferably tissue-specific systems) should be used for delivery of genetically engineered constructs into the body's cells.

Genetically engineered constructs

Hybrid nucleases and genetic patches (genetic material to be replaced) are typically delivered into the cell in the form of genetically engineered constructs from which the corresponding RNA and proteins are developed inside the cell. Variants of direct introduction into the mRNA cell are described, particularly for the CRISPR/Cas9 system [55].

A typical genetic construct for site-specific designer nuclease system contains a nuclear localization signal, an artificial guide unit (zinc fingers, TALE or 'guide' RNA), nuclease catalytic domain (for example FokI) and, if required, fragment to be replaced.

Gene delivery systems

Various viral and non-viral systems recognizing a large number of potential target tissues (skin, muscle, lung, brain, colon, spleen, liver, blood cells, and so on.) have been designed for delivery of 'therapeutic' genes or genetic constructs. The delivery system should ensure high efficiency of absorption of the genetic construct by the target cells, immunity to intracellular destruction during transportation to the nucleus and maintenance of necessary expression level.

Non-viral systems include direct introduction of DNA constructs into cells and tissues (e.g. electroporation), liposomes, cationic polymers, and others. Among viral systems, the most common are systems based on retroviruses, lentiviruses, adenoviruses, adeno-associated viruses and herpes simplex virus. Targeted delivery is determined by the presence of specific molecules (recognizable by target cell receptors) on the surface of viral particles or on the liposome's membrane. Such molecules could be viral capsid proteins, antibodies to surface cell antigens (inserted into the liposome's membrane), folic acid molecule (strongly captured by tumor cells), and others.

There are much attempts to use viral and non-viral delivery methods to deliver vectors with hybrid nucleases [56].

Genomic therapy of genetic disorders

As mentioned above, therapeutic approaches based on addition of genetic material into a cell with the help of viral vectors have been in use since the beginning of the 1990s.

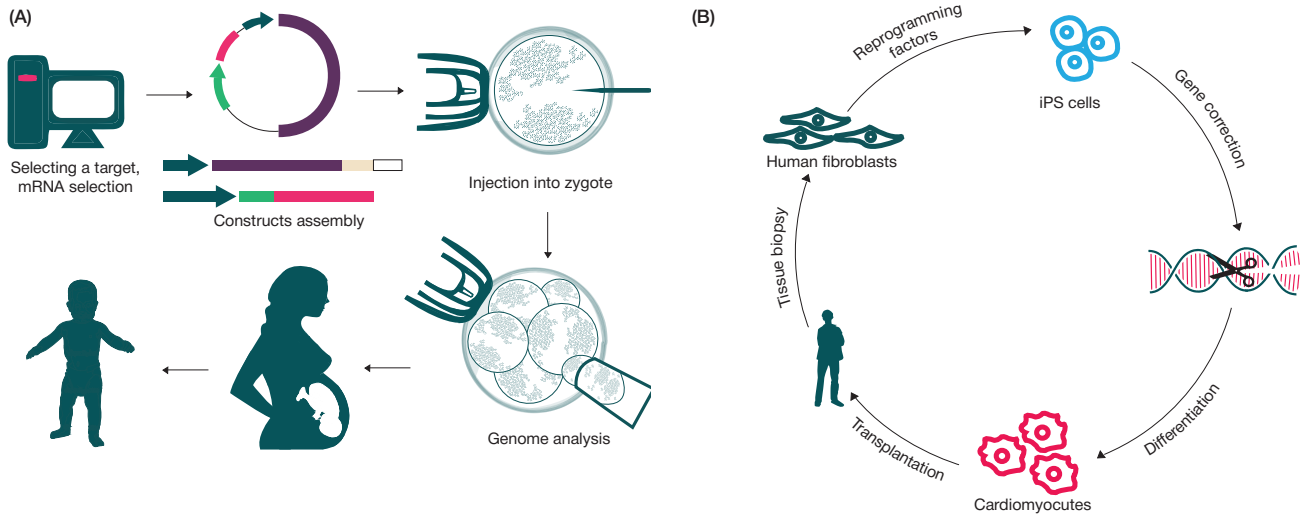

Fig. 3. Fetal **(A)** and somatic cell **(B)** gene therapy algorithms

These techniques restore synthesis of protein, whose gene is dysfunctional in both copies in the chromosome. However, changing or deleting DNA regions has long been an extremely complex and non-reproducible approach. With the advent of designer nucleases, researchers began to rapidly develop methods of directed DNA change directly in the chromosome structure. At present, there are treatment options fro retinitis pigmentosa, glaucoma, hemoglobinopathies, muscular dystrophies (table 2).

Fetal gene therapy is the most actively developing direction. In 2015-2016, many laboratories in the US, China, UK and several other countries, as well as some biotech companies, namely Ovascience (USA), Editas Medicine (USA), etc announced their plans for modification of human embryo genomes using CRISPR/Cas9-mediated gene editing techniques. If for a given pair of individuals, a potentially healthy genotype cannot be selected from 'natural' variants of embryos, genome editing methods aimed at adding/correcting a pathogenic allele in the zygote stage can be used.

Liang et al. published their work in April 2015, in which the CRISPR/Cas9 system was used at the zygote level to repair a mutant beta-globin gene. Of the 86 zygotes taken for experiment, only 4 cases were repaired [7].

Somatic gene therapy

"Chemotherapy will be obsolete within 20 years," said Prof Jeremy Farrer, head of the Wellcome Trust Sanger Institute. "We will look back in 20 years time and the blockbuster chemotherapy drugs that gave you all those nasty side effects will be a thing of the past and we will think 'gosh what an era that was'. Just as today, we are terrified by examples of electricity treatment at the beginning of the last century. Understanding humanity's genetic code is going to be fundamental to the medicine of the future. In rare congenital disease, in cancer and in infections, genomic insights are already transforming diagnosis and treatment" [57].

Designer nucleases can be used to effectively and accurately alter the DNA to correct any mutations. This opens up great opportunities for their use for correction of disorders that caused tumorigenesis [29, 30]. Variants for use of the CRISPR/Cas9 system for treatment of sarcoma and lung cancer have been proposed [31, 32]. For example, correction or deletion of mutant version of the *EGFR* gene using the CRISPR/Cas9 system delivered by virus has been proposed for lung cancer [32].

Antiviral therapy

HIV gene therapy

The fight against HIV is another direction of therapeutic use of hybrid nucleases. There are two directions in this fight: deleting HIV copies from the genome of the HIV carrier and altering the receptor genes through which the virus penetrates the T-lymphocytes, particularly the *CCR5* gene (table 2). By destroying the proviral DNA copies in the genome, it is theoretically possible to completely neutralize the virus and

Table 2. Examples of diseases treated using genomic editing based on designer nucleases

Area of application	Mechanism	Technique	Reference
Genetic eye dieseases	Gene disruption	TALENs, CRISPR/Cas9	[59–63]
Hemoglobinopathies (sickle cell anemia, β-thalassemia)	Insertion of working β-globin gene	ZFNs, TALENs, CRISPR/Cas9	[7–13]
Muscular dystrophy	Insertion of working dystrophin gene or deletion of bad exon in the existing gene	ZFNs, TALENs, CRISPR/Cas9	[64–71]
Oncology	Removal or correction of mutant gene variant	TALENs, CRISPR/Cas9	[29–32]
HIV	Cutting off DNA copies of virus from the human genome or deletion of receptor gene through which virus enters the cell	ZFNs, TALENs, CRISPR/Cas9	[72–82]
Hepatitis B virus	Virus genome destruction	CRISPR/Cas9	[58]
Genetic doping	Adding the desired gene allele	TALENs, CRISPR/Cas9	[83–87]
Reprogenetics	All kinds of changes	TALENs, CRISPR/Cas9	[83–87]

prevent it from reactivating in the patient's cells. Another approach — altering the receptor gene — does not allow the virus to infect the lymphocytes, thus leading to restoration of the patient's T cell population.

One of the problems in the development of gene-editing antiviral drugs is the ability of the virus to very quickly change sequence and thereby leave from the 'guide', specific to a particular sequence of the attack site. However, with proper legislative regulation of the release of modifications of gene therapy drugs, the release of new 'antivirus' can easily overtake the pace of HIV.

Fight against non-integrated viruses

The research world is also trying to apply genome editing systems to fight against viruses that do no integrate genetic material into the cellular genome. Their destruction mechanism is the same as in the case of HIV, but hybrid nuclease attacks free viral genome. The use of CRISPR/Cas9 to fight hepatitis B virus is described in [58].

Non-therapeutic genomic editing objectives

Genetic doping

Genetic doping is a variant of non-therapeutic use of genome editing to enhance athletic performance. It is no secret that maximum sports performance is largely determined by the individual's genetic component. Athletes from Kenya or Ethiopia almost always win marathons because genetically determined glucose metabolism pathway, which determines the ability to quickly run a marathon, is most developed in the African population of these countries.

At present, athletic success is linked with over 150 polymorphic positions in the DNA out of which 93 are associated with endurance and 62 with power load [91]. The spectrum of potential genes for effecting an influence by means of genomic editing is very wide: erythropoietin, insulin-like growth factor 1, human growth hormone, myostatin, endothelial growth factor, fibroblast growth factor, endorphins, enkephalins, cytoskeleton protein genes, etc. Approaches have already been developed for some of these genes, while clinical trials on introduction of specific alleles into the human genome have been carried out [85].

Reprogenetics

In classical interpretation, reprogenetics involves the selection of human embryos with certain properties from the resulting 'natural' variants. However, genome-editing technology helps to expand the features of the approach by creating variants that are impossible for a given pair of parents [88]. This raises a lot of ethical issues that mankind is yet to address [90].

Genomic editing: ethical and regulatory issues

Despite the fact that genome editing techniques via designer nucleases have enormous potential for creation of an effective therapy for patients suffering from genetic diseases, their use for therapeutic purposes is still in its infancy. In this regard, development of an ethical and legal framework that would ensure the effectiveness and safety of using genomic editing is an extremely important factor [92].

While developing such a framework, ethics committees and authorized government bodies need to establish and clarify aspects influencing the clinical implementation of genome editing techniques. These bodies should propose such a road map for development and implementation of genomic editing techniques that would allow to safely and quickly transfer the latest techniques into clinical practice.

Rapid development of innovative medical technologies does not allow the legislator to work on the legal framework for the use of these technologies in the same way as before. Presently, there is a change in the paradigm of legislative regulation on introduction of new medical technologies from research laboratory to the clinic. Globalization has made innovation to be spread around the world literally at the speed of light. New promising medical technology, no matter where it has been developed, is inevitably developed and primarily used in countries with more flexible and liberal laws. Such countries receive a head start on early introduction of innovative approaches, despite the risks involved in such approaches. Many legal restrictions on 'research-to-medicine' transition in certain countries do not make sense because technologies quickly spread to the rest of the world from 'research offshores', attracting clients to their territory.

From the very start, some countries try to prohibit the use of designer nucleases for human genome editing. However, Such countries are forced to quickly change position to avoid being at the tail of technological leaders. After a Chinese team announced in 2015 that it has carried out experiments on editing of genomes of human embryos through the CRISPR/Cas9 method, a group of British scientists were in February 2016 granted permission to genetically modify human embryos through CRISPR/Cas9 and related designer nuclease methods [93].

Public opinion amidst the introduction of techniques in separate jurisdictions is changing rapidly and starting to put pressure on their own legal regulation.

CONCLUSION

Genome editing methods created over the last few years are an improvement of gene therapy approaches existing at the end of the last century. However, it can be confidently argued that today the paradigm in the field of genomic medicine is shifting. The beginning of the second decade of the 21st century witnessed several technological breakthroughs with a strong synergistic effect – improvement in directed cell differentiation techniques, considerable reduction in cost, routine application of genome sequencers and creation of the described genome editing systems. All of these combined will inevitably give birth to new-quality personalized genomic medicine in the next 3–5 years. Directed genome alteration techniques will be a new tool for doctors.

References

1. Friedmann T, Roblin R. Gene Therapy for Human Genetic Disease? Science. 1972; 175 (4025): 949–55. doi:10.1126/science.175.4025.949.
2. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990 Aug 30; 323 (9): 570–8. doi:10.1056/NEJM199008303230904.
3. Gene Therapy Clinical Trials Worldwide Database [Internet]. John Wiley and Sons Ltd., provided by the Journal of Gene Medicine. c2016 – [updated 2016 Feb; cited 2016 Jun]. Available from: http://www.wiley.com/legacy/wileychi/genmed/clinical/.
4. De Ravin SS, Wu X, Moir S, Anaya-O'Brien S, Kwatemaa N, Little P, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med. 2016 Apr 20; 8 (335): 335ra57. doi: 10.1126/scitranslmed.aad8856.
5. Ward P, Walsh CE. Current and future prospects for hemophilia gene therapy. Expert Rev Hematol. 2016 May 26: 1–11. [Epub ahead of print].
6. Swystun LL, Lillicrap D. Gene Therapy for Coagulation Disorders. Circ Res. 2016 Apr 29; 118 (9): 1443–52. doi: 10.1161/CIRCRESAHA.115.307015.
7. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015 May; 6 (5): 363–72. doi: 10.1007/s13238-015-0153-5. Epub 2015 Apr 18.
8. Canver MC, Orkin SH. Customizing the genome as therapy for the β-hemoglobinopathies. Blood. 2016 May 26; 127 (21): 2536–45. doi: 10.1182/blood-2016-01-678128. Epub 2016 Apr 6.
9. Cottle RN, Lee CM, Bao G. Treating hemoglobinopathies using gene-correction approaches: promises and challenges. Hum Genet. 2016 Jun 17. [Epub ahead of print].
10. Tasan I, Jain S, Zhao H. Use of genome-editing tools to treat sickle cell disease. Hum Genet. 2016 Jun 1. [Epub ahead of print].
11. Osborn MJ, Belanto JJ, Tolar J, Voytas DF. Gene editing and its application for hematological diseases. Int J Hematol. 2016 May 27. [Epub ahead of print].
12. Yang Y, Zhang X, Yi L, Hou Z, Chen J, Kou X, et al. Naïve Induced Pluripotent Stem Cells Generated From β-Thalassemia Fibroblasts Allow Efficient Gene Correction With CRISPR/Cas9. Stem Cells Transl Med. 2016 Jan; 5 (1): 8–19. doi: 10.5966/sctm.2015-0157. Epub 2015 Dec 16. Erratum in: Stem Cells Transl Med. 2016 Feb; 5 (2): 267.
13. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. Hum Mol Genet. 2016 Jun 23. pii: ddw170. [Epub ahead of print].
14. Lee TW, Southern KW, Perry LA, Penny-Dimri JC, Aslam AA. Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease. Cochrane Database Syst Rev. 2016 Jun 17; 6: CD005599.
15. Alton EW, Armstrong DK, Ashby D, Bayfield KJ, Bilton D, Bloomfield EV, et al. Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015 Sep; 3 (9): 684–91. doi:10.1016/S2213-2600(15)00245-3.
16. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Sheibani N, Gurel Z, et al. Cone-Specific Promoters for Gene Therapy of Achromatopsia and Other Retinal Diseases. Hum Gene Ther. 2016 Jan; 27 (1): 72–82. doi: 10.1089/hum.2015.130.
17. Maguire AM, Simonelli F, Pierce EA, Pugh EN, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008; 358 (21): 2240–8. doi:10.1056/NEJMoa0802315.
18. Bainbridge JWB, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008; 358 (21): 2231–9. doi:10.1056/NEJMoa0802268.
19. Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, et al. Treatment of Leber Congenital Amaurosis Due to RPE65Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results of a Phase I Trial. Hum Gene Ther. 2008 Oct; 19 (10): 979–90. doi:10.1089/hum.2008.107.
20. Walker MC, Schorge S, Kullmann DM, Wykes RC, Heeroma JH, Mantoan L. Gene therapy in status epilepticus. Epilepsia. 2013 Sep; 54 Suppl 6: 43–5. doi:10.1111/epi.12275.
21. Giuncamp C, Pap T, Schedel J, Pap G, Moller-Ladner U, Gay RE, Gay S. Gene therapy in osteoarthritis. Joint Bone Spine. 2000; 67 (6): 570–1.
22. Evans CH, Gouze JN, Gouze E, Robbins PD, Ghivizzani SG. Osteoarthritis gene therapy. Gene Ther. 2004 Feb; 11 (4): 379–89. doi: 10.1038/sj.gt.3302196.
23. Carlsson T, Winkler C, Burger C, Muzyczka N, Mandel RJ, Cenci A, et al. Reversal of dyskinesias in an animal model of Parkinson's disease by continuous L-DOPA delivery using rAAV vectors. Brain. 2005 Mar; 128 (Pt 3): 559–69. doi: 10.1093/brain/awh374.
24. Forsayeth J, Bankiewicz KS, Aminoff MJ. Gene therapy for Parkinson's disease: Where are we now and where are we going? Expert Rev Neurother. 2010 Dec; 10 (12): 1839–45. doi: 10.1586/ern.10.161.
25. Palfi S, Gurruchaga, JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, et al. Long-term safety and tolerability of Pro Savin, a lentiviral vector-based gene therapy for Parkinson's disease: A dose escalation, open-label, phase 1/2 trial. Lancet. 2014 Mar 29; 383 (9923): 1138–46. doi: 10.1016/S0140-6736(13)61939-X.
26. Cross D, Burmester JK. Gene Therapy for Cancer Treatment: Past, Present and Future. Clin Med Res. 2006 Sep; 4 (3): 218–27.
27. Andersen JB, Thorgeirsson SS. A perspective on molecular therapy in cholangiocarcinoma: present status and future directions. Hepat Oncol. 2014 Jan 1; 1 (1): 143–57.
28. Amer MH. Gene therapy for cancer: present status and future perspective. Mol Cell Ther. 2014 Sep 10; 2: 27. doi: 10.1186/2052-8426-2-27.
29. Khan FA, Pandupuspitasari NS, Chun-Jie H, Ao Z, Jamal M, Zohaib A, et al. CRISPR/Cas9 therapeutics: a cure for cancer and other genetic diseases. Oncotarget. 2016 May 26. doi: 10.18632/oncotarget.9646. [Epub ahead of print].
30. White MK, Khalili K. CRISPR/Cas9 and cancer targets: future possibilities and present challenges. Oncotarget. 2016 Mar 15; 7 (11): 12305–17. doi: 10.18632/oncotarget.7104.
31. Liu T, Shen JK, Li Z, Choy E, Hornicek FJ, Duan Z. Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma. Cancer Lett. 2016 Apr 1; 373 (1): 109–18. doi: 10.1016/j.canlet.2016.01.030. Epub 2016 Jan 21.
32. Tang H, Shrager JB. CRISPR/Cas-mediated genome editing to treat EGFR-mutant lung cancer: a personalized molecular surgical therapy. EMBO Mol Med. 2016 Jan 8; 8 (2): 83–5. doi: 10.15252/emmm.201506006.
33. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Chapter 8.5: Gene Replacement and Transgenic Animals: DNA Is Transferred into Eukaryotic Cells in Various Ways. In: Molecular Cell Biology. 4th ed. New York: W. H. Freeman; 2002. ISBN 0-7167-3136-3.
34. O'Gorman S, Fox DT, Wahl GM. Recombinase-mediated gene activation and site-specific integration in mammalian cells. Science. 1991 Mar 15; 251 (4999): 1351–5. doi: 10.1126/science.1900642.
35. Karow M, Calos MP. The therapeutic potential of phiC31 integrase as a gene therapy system. Expert Opin Biol Ther. 2011 Oct; 11 (10): 1287–96. doi:10.1517/14712598.2011.601293.
36. Pabo CO, Peisach E, Grant RA. Design and Selection of Novel Cys2His2 Zinc Finger Proteins. Annu. Rev Biochem. 2001; 70: 313–40.
37. Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, et al. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell. 2008 Jul 25; 31 (2): 294–301. doi: 10.1016/j.molcel.2008.06.016.
38. Carroll D. Genome engineering with zinc-finger nucleases.

Genetics. 2011 Aug; 188 (4): 773–82. doi: 10.1534/genetics.111.131433.
39. Epinat JC, Arnould S, Chames P, Rochaix P, Desfontaines D, Puzin C, et al. A novel engineered meganuclease induces homologous recombination in yeast and mammalian cells. Nucleic Acids Res. 2003 Jun 1; 31 (11): 2952–62. doi: 10.1093/nar/gkg375.
40. Boch J. TALEs of genome targeting. Nat Biotechnol. 2011 Feb; 29 (2): 135–6. doi: 10.1038/nbt.1767.
41. Mali P, Esvelt KM, Church GM. Cas9 as a versatile tool for engineering biology. Nat Methods. 2013 Oct; 10 (10): 957–63. doi: 10.1038/nmeth.2649.
42. Boissel S, Jarjour J, Astrakhan A, Adey A, Gouble A, Duchateau P, et al. megaTALs: a rare-cleaving nuclease architecture for therapeutic genome engineering. Nucleic Acids Res. 2014 Feb; 42 (4): 2591–601. doi: 10.1093/nar/gkt1224.
43. Certo MT, Gwiazda KS, Kuhar R, Sather B, Curinga G, Mandt T, et al. Coupling endonucleases with DNA end-processing enzymes to drive gene disruption. Nat Methods. 2012 Oct; 9 (10): 973–5. doi: 10.1038/nmeth.2177.
44. Delacôte F, Perez C, Guyot V, Duhamel M, Rochon C, Ollivier N, et al. High frequency targeted mutagenesis using engineered endonucleases and DNA-end processing enzymes. PLoS One. 2013; 8 (1): e53217. doi: 10.1371/journal.pone.0053217.
45. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28; 346 (6213): 1258096. doi: 10.1126/science.1258096
46. Nemudriy AA, Valetdinova KR, Medvedev SP, Zakiyan SM. Sistemy redaktirovaniya genomov TALEN i CRISPR/Cas — instrumenty otkrytiy. Acta naturae. 2014; vol. 6, 3 (22): 20–41.
47. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet. 2014 Aug; 15 (8): 541–55. doi: 10.1038/nrg3763. Epub 2014 Jul 15.
48. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A. 1996 Feb 6; 93 (3): 1156–60.
49. Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing Off-Target Cleavage Specificities of Zinc Finger Nucleases by in Vitro Selection. Nat Methods, 2011 Aug 7; 8 (9): 765–70. doi: 10.1038/nmeth.1670.
50. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH, et al. Targeted Genome Editing Across Species Using ZFNs and TALENs. Science. 2011 Jul 15; 333 (6040): 307. doi: 10.1126/science.1207773.
51. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors. Science. 2009 Dec 11; 326 (5959): 1509–12. doi: 10.1126/science.1178811.
52. Moscou MJ, Bogdanove AJ. A Simple Cipher Governs DNA Recognition by TAL Effectors. Science. 2009 Dec 11; 326 (5959): 1501. Bibcode:2009Sci...326.1501M. doi:10.1126/science.1178817. PMID 19933106.
53. Horvath P, Barrangou R (January 2010). "CRISPR/Cas, the immune system of bacteria and archaea". Science 327 (5962): 167–70. doi: 10.1126/Science.1179555.
54. Swarts DC, Mosterd C, van Passel MW, Brouns SJ. CRISPR interference directs strand specific spacer acquisition. PloS One. 2012; 7 (4): e35888. doi: 10.1371/journal.pone.0035888.
55. Boroviak K, Doe B, Banerjee R, Yang F, Bradley A. Chromosome engineering in zygotes with CRISPR/Cas9. Genesis. 2016 Feb; 54 (2): 78–85. doi: 10.1002/dvg.22915. Epub 2016 Jan 25.
56. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016 Mar; 34 (3): 328–33. doi: 10.1038/nbt.3471. Epub 2016 Feb 1.
57. End of chemotherapy within 20 years as pioneering DNA project launched. The Daily Telegraph, 01.08.2014
58. Zhu W, Xie K, Xu Y, Wang L, Chen K, Zhang L, Fang J. CRISPR/Cas9 produces anti-hepatitis B virus effect in hepatoma cells and transgenic mouse. Virus Res. 2016 Jun 2; 217: 125–32. doi: 10.1016/j.virusres.2016.04.003. Epub 2016 Apr 2.
59. Hung SS, McCaughey T, Swann O, Pébay A, Hewitt AW. Genome engineering in ophthalmology: Application of CRISPR/Cas to the treatment of eye disease. Prog Retin Eye Res. 2016 Jun; 53; 1–20. doi: 10.1016/j.preteyeres.2016.05.001. [Epub ahead of print].
60. Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells. Sci Rep. 2016 Jan 27; 6:19969. doi: 10.1038/srep19969.
61. Nafissi N, Foldvari M. Neuroprotective therapies in glaucoma: II. Genetic nanotechnology tools. Front Neurosci. 2015 Oct 14; 9: 355. doi: 10.3389/fnins.2015.00355.
62. Li Y, Chan L, Nguyen HV, Tsang SH. Personalized Medicine: Cell and Gene Therapy Based on Patient-Specific iPSC-Derived Retinal Pigment Epithelium Cells. Adv Exp Med Biol. 2016; 854: 549–55. doi: 10.1007/978-3-319-17121-0_73
63. Wu WH, Tsai YT, Justus S, Lee T, Zhang L, Lin CS, et al. CRISPR repair reveals causative mutation in a preclinical model of retinitis pigmentosa. Mol Ther. 2016 May 20. doi: 10.1038/mt.2016.107. [Epub ahead of print].
64. Mendell JR, Rodino-Klapac LR. Duchenne muscular dystrophy: CRISPR/Cas9 treatment. Cell Res. 2016 May; 26 (5): 513–4. doi: 10.1038/cr.2016.28. Epub 2016 Mar 1.
65. Himeda CL, Jones TI, Jones PL. Scalpel or Straitjacket: CRISPR/Cas9 Approaches for Muscular Dystrophies. Trends Pharmacol Sci. 2016 Apr; 37 (4): 249–51. doi: 10.1016/j.tips.2016.02.001. Epub 2016 Feb 22.
66. Iyombe-Engembe JP, Ouellet DL, Barbeau X, Rousseau J, Chapdelaine P, Lagüe P, Tremblay JP. Efficient Restoration of the Dystrophin Gene Reading Frame and Protein Structure in DMD Myoblasts Using the CinDel Method. Mol Ther Nucleic Acids. 2016 Jan 26; 5: e283. doi: 10.1038/mtna.2015.58.
67. Maggio I, Stefanucci L, Janssen JM, Liu J, Chen X, Mouly V, Gonçalves MA. Selection-free gene repair after adenoviral vector transduction of designer nucleases: rescue of dystrophin synthesis in DMD muscle cell populations. Nucleic Acids Res. 2016 Feb 18; 44 (3): 1449–70. doi: 10.1093/nar/gkv1540. Epub 2016 Jan 13.
68. Kawecka K, Theodoulides M, Hasoglu Y, Jarmin S, Kymalainen H, Le-Heron A, et al. Adeno-Associated Virus (AAV) Mediated Dystrophin Gene Transfer Studies and Exon Skipping Strategies for Duchenne Muscular Dystrophy (DMD). Curr Gene Ther. 2015; 15 (4): 395–415.
69. Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, Gersbach CA. Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nat Commun. 2015 Feb 18; 6: 6244. doi: 10.1038/ncomms7244.
70. Ousterout DG, Kabadi AM, Thakore PI, Perez-Pinera P, Brown MT, Majoros WH, et al. Correction of dystrophin expression in cells from Duchenne muscular dystrophy patients through genomic excision of exon 51 by zinc finger nucleases. Mol Ther. 2015 Mar; 23 (3): 523–32. doi: 10.1038/mt.2014.234. Epub 2014 Dec 10.
71. Maggio I, Chen X, Gonçalves MA. The emerging role of viral vectors as vehicles for DMD gene editing. Genome Med. 2016 May 23; 8 (1): 59. doi: 10.1186/s13073-016-0316-x.
72. Reardon S. Gene-editing method tackles HIV in first clinical test. Nature. 2014 Mar 05. doi: 10.1038/nature.2014.14813.
73. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV. N Engl J Med. 2014 Mar 6; 370 (10): 901–10. doi: 10.1056/NEJMoa1300662.
74. Kaminski R, Bella R, Yin C, Otte J, Ferrante P, Gendelman HE, et al. Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene Ther. 2016 May 19. doi: 10.1038/gt.2016.41. [Epub ahead of print].
75. Ueda S, Ebina H, Kanemura Y, Misawa N, Koyanagi Y. Insufficient anti-HIV-1 potency of the CRISPR/Cas9 system for full viral replication. Microbiol Immunol. 2016 Jun 9. doi: 10.1111/1348-0421.12395. [Epub ahead of print].
76. Stone D, Niyonzima N, Jerome KR. Genome editing and the next generation of antiviral therapy. Hum Genet. 2016 Jun 8. [Epub ahead of print].
77. Pernet O, Yadav SS, An DS. Stem cell-based therapies for

HIV/AIDS. Adv Drug Deliv Rev. 2016 May 2. pii: S0169–409X(16)30137–5. doi: 10.1016/j.addr.2016.04.027. [Epub ahead of print].
78. Kim M, Siliciano RF. Genome editing for clinical HIV isolates. Nat Biotechnol. 2016 Apr 7; 34 (4): 388–9. doi: 10.1038/nbt.3531.
79. Wang CX, Cannon PM. The clinical applications of genome editing in HIV. Blood. 2016 May 26; 127 (21): 2546–52. doi: 10.1182/blood-2016-01-678144. Epub 2016 Apr 6.
80. Wang Z, Pan Q, Gendron P, Zhu W, Guo F, Cen S, et al. CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape. Cell Rep. 2016 Apr 19; 15 (3): 481–9. doi: 10.1016/j.celrep.2016.03.042. Epub 2016 Apr 7.
81. Wang G, Zhao N, Berkhout B, Das AT. CRISPR-Cas9 Can Inhibit HIV-1 Replication but NHEJ Repair Facilitates Virus Escape. Mol Ther. 2016 Mar; 24 (3): 522–6. doi: 10.1038/mt.2016.24. Epub 2016 Jan 22.
82. Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Yet al. Elimination of HIV-1 Genomes from Human T-lymphoid Cells by CRISPR/Cas9 Gene Editing. Sci Rep. 2016 Mar 4; 6: 22555. doi: 10.1038/srep22555.
83. Momaya A, Fawal M, Estes R. Performance-enhancing substances in sports: a review of the literature. Sports Med. 2015 Apr; 45 (4): 517–31. doi: 10.1007/s40279-015-0308-9.
84. World Anti-Doping Agency. The World Anti-Doping Code: The 2015 Prohibited List of International Standards. Montreal (Quebec); 2014 Sep 20. 10 p.
85. Adis Insight [Internet]. Cham (Switzerland): Springer International Publishing AG; [cited 2016 Jun]. Drug Profile: Erythropoietin gene therapy — Oxford BioMedica. Available from: http://adisinsight.springer.com/drugs/800017416.
86. Barry P. Finding the golden genes. Science News. 2008 Jul 18.
87. Miah A. Genetically Modified Athletes: Biomedical Ethics, Gene Doping and Sport. London; New York: Routledge; 2004. 208 p.
88. Regalado A. Engineering the Perfect Baby. MIT Technology Review. 2015 Mar 5.
89. Parens E, Knowles LP. Reprogenetics and public policy. Reflections and recommendations. Hastings Cent Rep. 2003 Jul–Aug; 33 (4): S1–24.
90. Pray L. Embryo screening and the ethics of human genetic engineering. Nature Education. 2008; 1 (1): 207.
91. Ahmetov II, Egorova ES, Gabdrakhmanova LJ, Fedotovskaya ON. Genes and Athletic Performance: An Update. Med Sport Sci. 2016; 61: 41–54. doi: 10.1159/000445240. Epub 2016 Jun 10.
92. Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. A prudent path forward for genomic engineering and germline gene modification. Science. 2015 Apr 3; 348 (6230): 36–3. doi: 10.1126/science.aab1028.
93. Knapton S. British scientists granted permission to genetically modify human embryos. The Daily Telegraph. 2016 Feb 1.

Литература

1. Friedmann T, Roblin R. Gene Therapy for Human Genetic Disease? Science. 1972; 175 (4025): 949–55. doi:10.1126/science.175.4025.949.
2. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990 Aug 30; 323 (9): 570–8. doi:10.1056/NEJM199008303230904.
3. Gene Therapy Clinical Trials Worldwide Database [Интернет]. John Wiley and Sons Ltd., provided by the Journal of Gene Medicine. c2016 – [обновлено: февраль 2016 г.; дата обращения: июнь 2016 г.]. Доступно по ссылке: http://www.wiley.com/legacy/wileychi/genmed/clinical/.
4. De Ravin SS, Wu X, Moir S, Anaya-O'Brien S, Kwatemaa N, Little P, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med. 2016 Apr 20; 8 (335): 335ra57. doi: 10.1126/scitranslmed.aad8856.
5. Ward P, Walsh CE. Current and future prospects for hemophilia gene therapy. Expert Rev Hematol. 2016 May 26: 1–11. [Epub ahead of print].
6. Swystun LL, Lillicrap D. Gene Therapy for Coagulation Disorders. Circ Res. 2016 Apr 29; 118 (9): 1443–52. doi: 10.1161/CIRCRESAHA.115.307015.
7. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015 May; 6 (5): 363–72. doi: 10.1007/s13238-015-0153-5. Epub 2015 Apr 18.
8. Canver MC, Orkin SH. Customizing the genome as therapy for the β-hemoglobinopathies. Blood. 2016 May 26; 127 (21): 2536–45. doi: 10.1182/blood-2016-01-678128. Epub 2016 Apr 6.
9. Cottle RN, Lee CM, Bao G. Treating hemoglobinopathies using gene-correction approaches: promises and challenges. Hum Genet. 2016 Jun 17. [Epub ahead of print].
10. Tasan I, Jain S, Zhao H. Use of genome-editing tools to treat sickle cell disease. Hum Genet. 2016 Jun 1. [Epub ahead of print].
11. Osborn MJ, Belanto JJ, Tolar J, Voytas DF. Gene editing and its application for hematological diseases. Int J Hematol. 2016 May 27. [Epub ahead of print].
12. Yang Y, Zhang X, Yi L, Hou Z, Chen J, Kou X, et al. Naïve Induced Pluripotent Stem Cells Generated From β-Thalassemia Fibroblasts Allow Efficient Gene Correction With CRISPR/Cas9. Stem Cells Transl Med. 2016 Jan; 5 (1): 8–19. doi: 10.5966/sctm.2015-0157. Epub 2015 Dec 16. Erratum in: Stem Cells Transl Med. 2016 Feb; 5 (2): 267.
13. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. Hum Mol Genet. 2016 Jun 23. pii: ddw170. [Epub ahead of print].
14. Lee TW, Southern KW, Perry LA, Penny-Dimri JC, Aslam AA. Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease. Cochrane Database Syst Rev. 2016 Jun 17; 6: CD005599.
15. Alton EW, Armstrong DK, Ashby D, Bayfield KJ, Bilton D, Bloomfield EV, et al. Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015 Sep; 3 (9): 684–91. doi:10.1016/S2213-2600(15)00245-3.
16. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Sheibani N, Gurel Z, et al. Cone-Specific Promoters for Gene Therapy of Achromatopsia and Other Retinal Diseases. Hum Gene Ther. 2016 Jan; 27 (1): 72–82. doi: 10.1089/hum.2015.130.
17. Maguire AM, Simonelli F, Pierce EA, Pugh EN, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008; 358 (21): 2240–8. doi:10.1056/NEJMoa0802315.
18. Bainbridge JWB, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008; 358 (21): 2231–9. doi:10.1056/NEJMoa0802268.
19. Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, et al. Treatment of Leber Congenital Amaurosis Due to RPE65Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results of a Phase I Trial. Hum Gene Ther. 2008 Oct; 19 (10): 979–90. doi:10.1089/hum.2008.107.
20. Walker MC, Schorge S, Kullmann DM, Wykes RC, Heeroma JH, Mantoan L. Gene therapy in status epilepticus. Epilepsia. 2013 Sep; 54 Suppl 6: 43–5. doi:10.1111/epi.12275.
21. Giuncamp C, Pap T, Schedel J, Pap G, Moller-Ladner U, Gay RE, Gay S. Gene therapy in osteoarthritis. Joint Bone Spine. 2000; 67 (6): 570–1.
22. Evans CH, Gouze JN, Gouze E, Robbins PD, Ghivizzani SG. Osteoarthritis gene therapy. Gene Ther. 2004 Feb; 11 (4): 379–89. doi: 10.1038/sj.gt.3302196.
23. Carlsson T, Winkler C, Burger C, Muzyczka N, Mandel RJ,

Cenci A, et al. Reversal of dyskinesias in an animal model of Parkinson's disease by continuous L-DOPA delivery using rAAV vectors. Brain. 2005 Mar; 128 (Pt 3): 559–69. doi: 10.1093/brain/awh374.
24. Forsayeth J, Bankiewicz KS, Aminoff MJ. Gene therapy for Parkinson's disease: Where are we now and where are we going? Expert Rev Neurother. 2010 Dec; 10 (12): 1839–45. doi: 10.1586/ern.10.161.
25. Palfi S, Gurruchaga, JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, et al. Long-term safety and tolerability of Pro Savin, a lentiviral vector-based gene therapy for Parkinson's disease: A dose escalation, open-label, phase 1/2 trial. Lancet. 2014 Mar 29; 383 (9923): 1138–46. doi: 10.1016/S0140-6736(13)61939-X.
26. Cross D, Burmester JK. Gene Therapy for Cancer Treatment: Past, Present and Future. Clin Med Res. 2006 Sep; 4 (3): 218–27.
27. Andersen JB, Thorgeirsson SS. A perspective on molecular therapy in cholangiocarcinoma: present status and future directions. Hepat Oncol. 2014 Jan 1; 1 (1): 143–57.
28. Amer MH. Gene therapy for cancer: present status and future perspective. Mol Cell Ther. 2014 Sep 10; 2: 27. doi: 10.1186/2052-8426-2-27.
29. Khan FA, Pandupuspitasari NS, Chun-Jie H, Ao Z, Jamal M, Zohaib A, et al. CRISPR/Cas9 therapeutics: a cure for cancer and other genetic diseases. Oncotarget. 2016 May 26. doi: 10.18632/oncotarget.9646. [Epub ahead of print].
30. White MK, Khalili K. CRISPR/Cas9 and cancer targets: future possibilities and present challenges. Oncotarget. 2016 Mar 15; 7 (11): 12305–17. doi: 10.18632/oncotarget.7104.
31. Liu T, Shen JK, Li Z, Choy E, Hornicek FJ, Duan Z. Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma. Cancer Lett. 2016 Apr 1; 373 (1): 109–18. doi: 10.1016/j.canlet.2016.01.030. Epub 2016 Jan 21.
32. Tang H, Shrager JB. CRISPR/Cas-mediated genome editing to treat EGFR-mutant lung cancer: a personalized molecular surgical therapy. EMBO Mol Med. 2016 Jan 8; 8 (2): 83–5. doi: 10.15252/emmm.201506006.
33. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Chapter 8.5: Gene Replacement and Transgenic Animals: DNA Is Transferred into Eukaryotic Cells in Various Ways. In: Molecular Cell Biology. 4th ed. New York: W. H. Freeman; 2002. ISBN 0-7167-3136-3.
34. O'Gorman S, Fox DT, Wahl GM. Recombinase-mediated gene activation and site-specific integration in mammalian cells. Science. 1991 Mar 15; 251 (4999): 1351–5. doi: 10.1126/science.1900642.
35. Karow M, Calos MP. The therapeutic potential of phiC31 integrase as a gene therapy system. Expert Opin Biol Ther. 2011 Oct; 11 (10): 1287–96. doi:10.1517/14712598.2011.601293.
36. Pabo CO, Peisach E, Grant RA. Design and Selection of Novel Cys2His2 Zinc Finger Proteins. Annu. Rev Biochem. 2001; 70: 313–40.
37. Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, et al. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell. 2008 Jul 25; 31 (2): 294–301. doi: 10.1016/j.molcel.2008.06.016.
38. Carroll D. Genome engineering with zinc-finger nucleases. Genetics. 2011 Aug; 188 (4): 773–82. doi: 10.1534/genetics.111.131433.
39. Epinat JC, Arnould S, Chames P, Rochaix P, Desfontaines D, Puzin C, et al. A novel engineered meganuclease induces homologous recombination in yeast and mammalian cells. Nucleic Acids Res. 2003 Jun 1; 31 (11): 2952–62. doi: 10.1093/nar/gkg375.
40. Boch J. TALEs of genome targeting. Nat Biotechnol. 2011 Feb; 29 (2): 135–6. doi: 10.1038/nbt.1767.
41. Mali P, Esvelt KM, Church GM. Cas9 as a versatile tool for engineering biology. Nat Methods. 2013 Oct; 10 (10): 957–63. doi: 10.1038/nmeth.2649.
42. Boissel S, Jarjour J, Astrakhan A, Adey A, Gouble A, Duchateau P, et al. megaTALs: a rare-cleaving nuclease architecture for therapeutic genome engineering. Nucleic Acids Res. 2014 Feb; 42 (4): 2591–601. doi: 10.1093/nar/gkt1224.
43. Certo MT, Gwiazda KS, Kuhar R, Sather B, Curinga G, Mandt T, et al. Coupling endonucleases with DNA end-processing enzymes to drive gene disruption. Nat Methods. 2012 Oct; 9 (10): 973–5. doi: 10.1038/nmeth.2177.
44. Delacôte F, Perez C, Guyot V, Duhamel M, Rochon C, Ollivier N, et al. High frequency targeted mutagenesis using engineered endonucleases and DNA-end processing enzymes. PLoS One. 2013; 8 (1): e53217. doi: 10.1371/journal.pone.0053217.
45. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28; 346 (6213): 1258096. doi: 10.1126/science.1258096
46. Немудрый А. А., Валетдинова К. Р., Медведев С. П., Закиян С. М. Системы редактирования геномов TALEN и CRISPR/Cas — инструменты открытий. Acta naturae. 2014; т. 6, 3 (22): 20–41.
47. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet. 2014 Aug; 15 (8): 541–55. doi: 10.1038/nrg3763. Epub 2014 Jul 15.
48. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A. 1996 Feb 6; 93 (3): 1156–60.
49. Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing Off-Target Cleavage Specificities of Zinc Finger Nucleases by in Vitro Selection. Nat Methods, 2011 Aug 7; 8 (9): 765–70. doi: 10.1038/nmeth.1670.
50. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH, et al. Targeted Genome Editing Across Species Using ZFNs and TALENs. Science. 2011 Jul 15; 333 (6040): 307. doi: 10.1126/science.1207773.
51. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors. Science. 2009 Dec 11; 326 (5959): 1509–12. doi: 10.1126/science.1178811.
52. Moscou MJ, Bogdanove AJ. A Simple Cipher Governs DNA Recognition by TAL Effectors. Science. 2009 Dec 11; 326 (5959): 1501. Bibcode:2009Sci...326.1501M. doi:10.1126/science.1178817. PMID 19933106.
53. Horvath P, Barrangou R (January 2010). "CRISPR/Cas, the immune system of bacteria and archaea". Science 327 (5962): 167–70. doi: 10.1126/Science.1179555.
54. Swarts DC, Mosterd C, van Passel MW, Brouns SJ. CRISPR interference directs strand specific spacer acquisition. PloS One. 2012; 7 (4): e35888. doi: 10.1371/journal.pone.0035888.
55. Boroviak K, Doe B, Banerjee R, Yang F, Bradley A. Chromosome engineering in zygotes with CRISPR/Cas9. Genesis. 2016 Feb; 54 (2): 78–85. doi: 10.1002/dvg.22915. Epub 2016 Jan 25.
56. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016 Mar; 34 (3): 328–33. doi: 10.1038/nbt.3471. Epub 2016 Feb 1.
57. End of chemotherapy within 20 years as pioneering DNA project launched. The Daily Telegraph, 01.08.2014
58. Zhu W, Xie K, Xu Y, Wang L, Chen K, Zhang L, Fang J. CRISPR/Cas9 produces anti-hepatitis B virus effect in hepatoma cells and transgenic mouse. Virus Res. 2016 Jun 2; 217: 125–32. doi: 10.1016/j.virusres.2016.04.003. Epub 2016 Apr 2.
59. Hung SS, McCaughey T, Swann O, Pébay A, Hewitt AW. Genome engineering in ophthalmology: Application of CRISPR/Cas to the treatment of eye disease. Prog Retin Eye Res. 2016 Jun; 53; 1–20. doi: 10.1016/j.preteyeres.2016.05.001. [Epub ahead of print].
60. Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells. Sci Rep. 2016 Jan 27; 6:19969. doi: 10.1038/srep19969.
61. Nafissi N, Foldvari M. Neuroprotective therapies in glaucoma: II. Genetic nanotechnology tools. Front Neurosci. 2015 Oct 14; 9: 355. doi: 10.3389/fnins.2015.00355.
62. Li Y, Chan L, Nguyen HV, Tsang SH. Personalized Medicine: Cell and Gene Therapy Based on Patient-Specific iPSC-Derived Retinal Pigment Epithelium Cells. Adv Exp Med Biol. 2016; 854: 549–55. doi: 10.1007/978-3-319-17121-0_73

63. Wu WH, Tsai YT, Justus S, Lee T, Zhang L, Lin CS, et al. CRISPR repair reveals causative mutation in a preclinical model of retinitis pigmentosa. Mol Ther. 2016 May 20. doi: 10.1038/mt.2016.107. [Epub ahead of print].
64. Mendell JR, Rodino-Klapac LR. Duchenne muscular dystrophy: CRISPR/Cas9 treatment. Cell Res. 2016 May; 26 (5): 513–4. doi: 10.1038/cr.2016.28. Epub 2016 Mar 1.
65. Himeda CL, Jones TI, Jones PL. Scalpel or Straitjacket: CRISPR/Cas9 Approaches for Muscular Dystrophies. Trends Pharmacol Sci. 2016 Apr; 37 (4): 249–51. doi: 10.1016/j.tips.2016.02.001. Epub 2016 Feb 22.
66. Iyombe-Engembe JP, Ouellet DL, Barbeau X, Rousseau J, Chapdelaine P, Lagüe P, Tremblay JP. Efficient Restoration of the Dystrophin Gene Reading Frame and Protein Structure in DMD Myoblasts Using the CinDel Method. Mol Ther Nucleic Acids. 2016 Jan 26; 5: e283. doi: 10.1038/mtna.2015.58.
67. Maggio I, Stefanucci L, Janssen JM, Liu J, Chen X, Mouly V, Gonçalves MA. Selection-free gene repair after adenoviral vector transduction of designer nucleases: rescue of dystrophin synthesis in DMD muscle cell populations. Nucleic Acids Res. 2016 Feb 18; 44 (3): 1449–70. doi: 10.1093/nar/gkv1540. Epub 2016 Jan 13.
68. Kawecka K, Theodoulides M, Hasoglu Y, Jarmin S, Kymalainen H, Le-Heron A, et al. Adeno-Associated Virus (AAV) Mediated Dystrophin Gene Transfer Studies and Exon Skipping Strategies for Duchenne Muscular Dystrophy (DMD). Curr Gene Ther. 2015; 15 (4): 395–415.
69. Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, Gersbach CA. Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nat Commun. 2015 Feb 18; 6: 6244. doi: 10.1038/ncomms7244.
70. Ousterout DG, Kabadi AM, Thakore PI, Perez-Pinera P, Brown MT, Majoros WH, et al. Correction of dystrophin expression in cells from Duchenne muscular dystrophy patients through genomic excision of exon 51 by zinc finger nucleases. Mol Ther. 2015 Mar; 23 (3): 523–32. doi: 10.1038/mt.2014.234. Epub 2014 Dec 10.
71. Maggio I, Chen X, Gonçalves MA. The emerging role of viral vectors as vehicles for DMD gene editing. Genome Med. 2016 May 23; 8 (1): 59. doi: 10.1186/s13073-016-0316-x.
72. Reardon S. Gene-editing method tackles HIV in first clinical test. Nature. 2014 Mar 05. doi: 10.1038/nature.2014.14813.
73. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV. N Engl J Med. 2014 Mar 6; 370 (10): 901–10. doi: 10.1056/NEJMoa1300662.
74. Kaminski R, Bella R, Yin C, Otte J, Ferrante P, Gendelman HE, et al. Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene Ther. 2016 May 19. doi: 10.1038/gt.2016.41. [Epub ahead of print].
75. Ueda S, Ebina H, Kanemura Y, Misawa N, Koyanagi Y. Insufficient anti-HIV-1 potency of the CRISPR/Cas9 system for full viral replication. Microbiol Immunol. 2016 Jun 9. doi: 10.1111/1348-0421.12395. [Epub ahead of print].
76. Stone D, Niyonzima N, Jerome KR. Genome editing and the next generation of antiviral therapy. Hum Genet. 2016 Jun 8. [Epub ahead of print].
77. Pernet O, Yadav SS, An DS. Stem cell-based therapies for HIV/AIDS. Adv Drug Deliv Rev. 2016 May 2. pii: S0169–409X(16)30137–5. doi: 10.1016/j.addr.2016.04.027. [Epub ahead of print].
78. Kim M, Siliciano RF. Genome editing for clinical HIV isolates. Nat Biotechnol. 2016 Apr 7; 34 (4): 388–9. doi: 10.1038/nbt.3531.
79. Wang CX, Cannon PM. The clinical applications of genome editing in HIV. Blood. 2016 May 26; 127 (21): 2546–52. doi: 10.1182/blood-2016-01-678144. Epub 2016 Apr 6.
80. Wang Z, Pan Q, Gendron P, Zhu W, Guo F, Cen S, et al. CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape. Cell Rep. 2016 Apr 19; 15 (3): 481–9. doi: 10.1016/j.celrep.2016.03.042. Epub 2016 Apr 7.
81. Wang G, Zhao N, Berkhout B, Das AT. CRISPR-Cas9 Can Inhibit HIV-1 Replication but NHEJ Repair Facilitates Virus Escape. Mol Ther. 2016 Mar; 24 (3): 522–6. doi: 10.1038/mt.2016.24. Epub 2016 Jan 22.
82. Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Yet al. Elimination of HIV-1 Genomes from Human T-lymphoid Cells by CRISPR/Cas9 Gene Editing. Sci Rep. 2016 Mar 4; 6: 22555. doi: 10.1038/srep22555.
83. Momaya A, Fawal M, Estes R. Performance-enhancing substances in sports: a review of the literature. Sports Med. 2015 Apr; 45 (4): 517–31. doi: 10.1007/s40279-015-0308-9.
84. World Anti-Doping Agency. The World Anti-Doping Code: The 2015 Prohibited List of International Standards. Montreal (Quebec); 2014 Sep 20. 10 p.
85. Adis Insight [Интернет]. Cham (Switzerland): Springer International Publishing AG; [дата обращения: июнь 2016 г.]. Drug Profile: Erythropoietin gene therapy — Oxford BioMedica. Доступно по ссылке: http://adisinsight.springer.com/drugs/800017416.
86. Barry P. Finding the golden genes. Science News. 2008 Jul 18.
87. Miah A. Genetically Modified Athletes: Biomedical Ethics, Gene Doping and Sport. London; New York: Routledge; 2004. 208 p.
88. Regalado A. Engineering the Perfect Baby. MIT Technology Review. 2015 Mar 5.
89. Parens E, Knowles LP. Reprogenetics and public policy. Reflections and recommendations. Hastings Cent Rep. 2003 Jul–Aug; 33 (4): S1–24.
90. Pray L. Embryo screening and the ethics of human genetic engineering. Nature Education. 2008; 1 (1): 207.
91. Ahmetov II, Egorova ES, Gabdrakhmanova LJ, Fedotovskaya ON. Genes and Athletic Performance: An Update. Med Sport Sci. 2016; 61: 41–54. doi: 10.1159/000445240. Epub 2016 Jun 10.
92. Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. A prudent path forward for genomic engineering and germline gene modification. Science. 2015 Apr 3; 348 (6230): 36–3. doi: 10.1126/science.aab1028.
93. Knapton S. British scientists granted permission to genetically modify human embryos. The Daily Telegraph. 2016 Feb 1.