Advances in the CRISPR-Cas9 system and gene therapy

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 is a genome editing tool that helps scientists modify the DNA of living organisms selectively and precisely. The discovery of this system has led to changes in the approaches to gene therapy. In this article, I delve into the role of CRISPR-Cas9 in the development of treatment using gene therapy and the drawbacks of this system. Also, I discuss the role of medical writers in the dissemination of information and research on CRISPR-Cas9 and gene therapy.

Gene therapy involves the manipulation of genes or their expression inside cells to treat a disease or to stop its progress. Various mechanisms by which gene therapy can work include: replacing a mutated gene with a healthy copy of a gene; turning off a mutated gene or turning on a healthy gene; or correcting a mutated gene to treat a disease.[1]

Genome editing over the years

The eukaryotic genome is made of millions of base pairs (bps) of DNA. Breakthrough research in the 1980s showed that mammalian cells can incorporate exogenous DNA in their genomes through homologous recombination (the process of exchange of genetic material between two strands of DNA with very similar base sequences). However, the rate of integration is quite low. This rate is enhanced by the introduction of double-stranded breaks (DSBs) using endonucleases called meganucleases (enzymes that recognise a 14-40 bp DNA stretch). However, the specificity of meganucleases is a drawback. Furthermore, the DSBs are repaired through a non-homologous end joining (NHEJ) mechanism, which is error-prone and can delete or insert DNA sequences. The discovery of zinc-finger nucleases (ZFNs) furthered the field of genome editing. Each zinc finger module can identify 3 bps of DNA; thus, multiple zinc finger modules can be assembled to achieve higher binding specificity and increase the efficiency of homology-directed repair (HDR). Similarly, transcription activator-like effector nucleases (TALENs) can recognise single bp DNA and multiple TALENs can be put together for greater specificity. These discoveries have furthered our research in genome editing (Figure 1).[2]

The story of CRISPR-Cas system

Even though, ZFNs and TALENs increased editing efficiency, the targeting of various parts of the genome required cloning and expression of new sets of proteins, which proved to be a challenge. The third kind of genetic scissor used in genome editing is Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – Cas (CRISPR-associated) system, an RNA-protein complex, made of a single guide RNA (sgRNA) and Cas effector protein. CRISPR-Cas is an adaptive immune system found in prokaryotes that protects the bacteria from phage/viral infections. As the CRISPR name suggests, the short repeats of DNA are interspaced by spacer sequences which were found to belong to phages and viruses.[3] CRISPR-Cas systems are split into 2 classes (Class 1 has multi-unit effector molecule and Class 2 has a single effector molecule) and 6 types – types I to VI based on the Cas genes and the respective loci, and individual molecular mechanisms that lead to nucleic acid cleavage.[4,5] The most widely used CRISPR system is the type II CRISPR-Cas9 that belongs to *Streptococcus pyrogenes*.[2]

CRISPR-Cas9 was first identified in 1987 in *Escherichia coli* and, since then, various research groups have contributed to elucidating the mechanism, functions, and applications of the CRISPR-Cas9 system, culminating in Jennifer Doudna and Emmanuelle Charpentier being awarded the Nobel Prize in Chemistry in 2020 for their breakthrough work on CRISPR-Cas9.[6] Lander presents a great review of the scientific ecosystem that brought about these discoveries.[7]

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Advantages and challenges of using the CRISPR-Cas9 system

The CRISPR-Cas9 system has revolutionised the field of biology. These genetic scissors allow biochemists, cell biologists, geneticists, and molecular biologists to study the functions of various genes and their role in different diseases, gene expression regulation, and epigenetic modification. Apart from its function to study human and animal diseases, the CRISPR-Cas9 system has been used in plant biotechnology and agriculture to produce crops resistant to stress and diseases, with higher yields, etc. To target a specific DNA sequence using CRISPR-Cas9, only the sgRNA needs to be modified and no complex cloning and protein engineering is required as is the case with ZFNs and TALENs. There were still challenges with the CRISPR system as it produced DSBs that could be repaired either through NHEJ (which introduces unwanted genetic changes) or HDR. Thus, research has also focused on improving HDR rates by introducing changes in the Cas9 genes or by inhibiting the NHEJ pathway. Cas9 variants found in nature are large proteins making them difficult to package in various vectors for delivery. Therefore, scientists have also been working on finding smaller Cas9 variants in other bacterial species. Furthermore, there can be challenges such as lower on-target

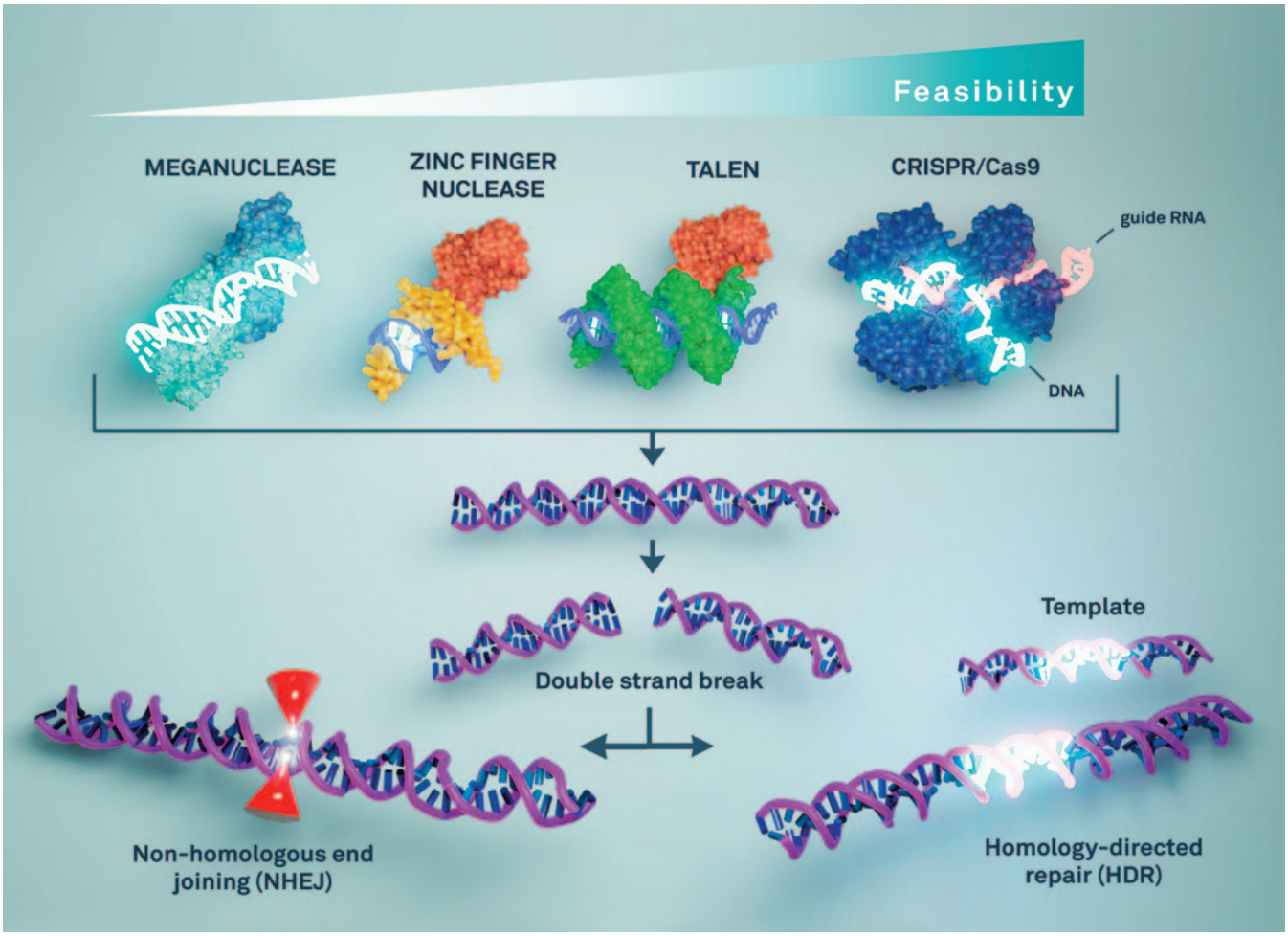

Figure 1. Major genome editing technologies

Feasibility here refers to the increased efficacy of targeted genome editing. Redrawn from Adli M. Nat Commun. 2018;9(1):1911.[2]

editing efficiency (on-target editing refers to genetic modifications at the sites intended), off-target editing (genetic modification at unintended sites) and mosaicism (two or more different genetic lines in a single organism).

More details on different CRISPR systems, and the engineered changes to these systems to make them more efficient for introduction into human cells, have been reviewed elsewhere and are beyond the scope of this article.[2-4, 8]

Delivery of CRISPR-Cas9

In vitro, plasmids or RNAs carrying CRISPR-Cas9 are delivered into cells with the use of transfection reagents.[9] However, this method cannot be used *in vivo*. Methods for *in vivo* delivery are discussed in the next section on gene therapy.

Gene therapy

Integrating and non-integrating vectors

Gene therapy can be *in vivo* (vector directly administered into patients) or *ex vivo* (vector delivered into cultured cells that are taken from patients and later administered back)[10]. Furthermore, the vector can be integrating (integrates into the genome) or non-integrating. This is a crucial factor when the therapy targets stem cells or mature post-mitotic cells. For stem cells, it is good to use integrating vectors, so that the corrected DNA passes on to the daughter cells. In cells that are no longer dividing, non-integrating vectors should do the job.[10]

The most used vectors in gene therapy and for CRISPR-Cas9 are adeno-associated viral (AAV) vectors (*in vivo*) and lentiviral vectors (*ex vivo*). Gene therapy does come with some risks, such as insertional mutagenesis with integrating vectors, immune responses to vectors that can be life-threatening, and excessive T-cell activation.[9,10] AAV is the most common choice, as it has low immunogenicity, thus reducing the likelihood of inflammatory response. For CRISPR-Cas9, special AAVs are constructed as the gene editing system exceeds the carrying capacity of AAVs. Lentiviral vectors have a larger carrying capacity, but can integrate randomly into the genome and, therefore, are not frequently used. In recent times, there has been research into new vectors. For example, baculovirus has a large carrying capacity and does not integrate into the genome and, thus, may be a safer alternative. Lipid-based nanocarriers and polymer-based nanoparticles are also considered promising tools for CRISPR-Cas9 delivery.[9]

Table 1. EMA-approved gene therapies

Trade name	Year of approval	Indications
Abecma	2021	Relapsed or refractory myeloma
Breyanzi	2022	Large B-cell lymphoma
Carvykti	2023	Relapsed or refractory myeloma
Hemgenix	2023	Haemophilia B (congenital factor IX deficiency)
Imlygic	2015	Local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma
Kymriah	2018	Relapsed or refractory follicular lymphoma
Libmeldy	2020	Metachromatic leukodystrophy
Luxturna	2018	Biallelic RPE65 mutation-associated retinal dystrophy
Roctavian	2022	Severe haemophilia A (congenital factor VIII deficiency with factor VIII activity <1 IU/dL)
Strimvelis	2016	Severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID)
Tecartus	2020	Relapsed or refractory mantle cell lymphoma
Upstaza	2022	Severe aromatic L-amino acid decarboxylase (AADC)
Yescarta	2018	Relapsed or refractory large B-cell lymphoma
Zolgensma	2020	Spinal muscular atropy (type I)

Gene therapy trials

Gene therapy clinical trials began in the 1990s and since then more than 3800 trials have either been performed or are currently ongoing.[11] The first gene therapy to be approved by the EMA, in 2012, was Glybera®, a treatment for hereditary lipoprotein lipase deficiency.[10] Since then, several gene therapies have been approved by both the EMA and the US FDA. As of September 2023, there are currently 14 EMA-approved gene therapies (Table 1).[12] The indications of gene therapies have ranged from ultra-rare and rare genetic diseases to various cancers.

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The use of CRISPR-Cas9 as a tool for gene therapy has also gained momentum in the last 5 years, with 42 trials in progress using CRISPR-Cas9 for gene therapy.[11] There are trials in progress (mostly Phase 1 but a few Phase 2/3) for: monogenic diseases, such as sickle-cell disease, β-thalassaemia, and hereditary amyloidosis; various types of cancers; and infectious diseases (like COVID-19). This year, 2023, is particularly significant for CRISPR-Cas9-based gene therapies as Vertex and CRISPR Therapeutics have submitted their *ex vivo* therapy (exacel) for sickle cell disease and β-thalassaemia, developed using CRISPR-Cas9, for approval in the US and EU. And in November, 2023, the Medicines and Healthcare products Regulatory Agency (MHRA) approved this therapy in the UK.[13]

Ethical conundrum of using the CRISPR-Cas9 system

Apart from potential complications like the possibility of limited on-target editing efficiency, off-target editing, and mosaicism, there are moral and ethical issues that need addressing. The most significant of these issues is the integration of the corrected gene into the genome and, thus, its transfer on to the progeny. Given there is uncertainty even in organisms in which CRISPR-Cas9 is used, it is unclear how the gene might react in the new genetic milieu of the offspring. Furthermore, a gene is very complex and interacts with various other genes and proteins in an organism and the environment to produce a phenotype. This phenomenon is not yet fully understood. Therefore, to modify a gene without clear knowledge of all the variables presents a moral and ethical conundrum.[14,15]

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While the benefits and risks are understood, the efficacy and safety of this technology is still not clear. Thus, the FDA, EMA, and other regulatory authorities have strict criteria for approval of these therapies and post-market surveillance requirements.

Role of medical writers

Advanced therapy medicinal products (ATMPs), including cell, gene, and RNA therapies, have been gaining momentum; six novel therapies were approved in 2022 and a higher number is expected to be approved in 2023. The field is still new and the challenges of submitting regulatory documents for these therapies are also unique. Medical writers with advanced degrees in genetics, cell biology, RNA biology, and similar fields will have an increasing role to play in the correct interpretation and dissemination of information on ATMPs in a simple and lucid manner.

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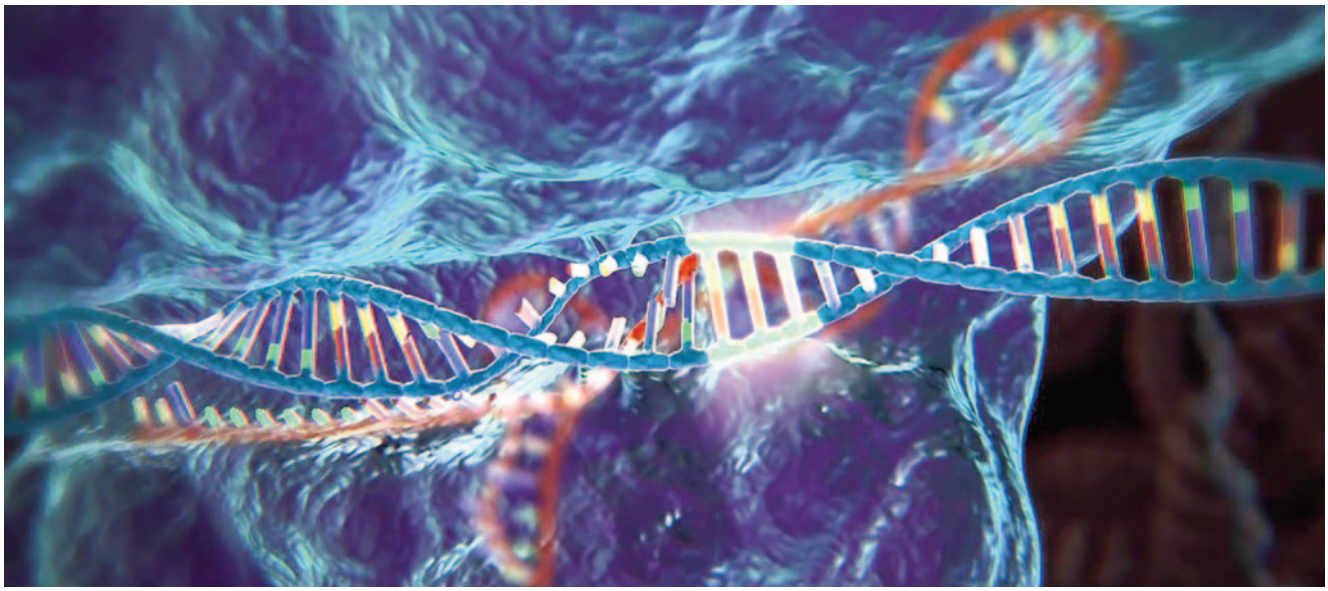
Disclaimers

The opinions expressed in this article are the author's own and are not necessarily shared by EMWA.



Disclosures and conflicts of interest

The author declares no conflicts of interest.

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